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AUTHENTICATION

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

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GROWER SUMMARY

Headline

- Increased levels of ‘rust’, an unpredictable physiological disorder of daffodils that degrades cut-flower quality and makes some produce unmarketable, was associated with high soil water content in the months before flowering.

Background

The physiological disorder known as daffodil ‘rust’ degrades stem quality and can make affected cut-flowers unmarketable. With commercial daffodil growing in the UK more dependent on the sales of cut-flowers than of bulbs, it is vital to avoid anything that might harm customers’ perception of product quality or damage the reputation of this important UK cut-flower crop.

Starting in the early-1990s UK daffodil growers became concerned about rust-like lesions appearing on daffodil stems. The symptoms occurred sporadically, sometimes presenting as a few insignificant spots, but in more serious cases the lesions were numerous and could form coalescing areas of damage that disfigured stems sufficiently to cause downgrading. In the worst cases stems showed transverse cracking and became brittle and unmarketable.

Despite rust’s convincingly disease-like lesions, initial testing apparently failed to reveal a pathogen associated with them and the condition was classed as a physiological disorder or abiotic disease. It soon came to be referred to as ‘physiological rust’ or, more recently, ‘stem rust’. For simplicity, this report refers to daffodil rust’ or simply ‘rust’.

Different severity of 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).

To gauge the extent and economic cost of rust, AHDB Horticulture organised surveys of daffodil growers in 2002 and 2003 and again in 2011 to 2013. The survey findings were summarised as part of this project, and confirmed that rust has continued to cause commercially significant losses that justify research into its cause and management. The surveys also revealed the ideas circulating in the industry about the cause of rust: suspected predisposing conditions often involved rapid changes in temperature, alternating cold and warm periods and adverse weather. The responses told us that growers had submitted affected plant samples for diagnostic examination, as well as soil and plant samples from affected and 'healthy' crops for the analysis of nutrients. No pathogenic or nutritional cause was found, though neither should be ruled out entirely because sampling has been opportunistic and non-replicated - more structured sampling could have provided more robust conclusions.

'Chocolate spot' is another physiological disorder of daffodils and has parallels with rust, though it has not been known to result in any serious damage. As with rust, pathological and nutritional causes were ruled out, and an association with increasing ambient temperatures has been suggested. Several physiological disorders of other horticultural crops are characterised by the appearance of brown or black spotting and have been linked with adverse environmental conditions. Apple leaf spot and drop, for example, has been associated with dry, hot summer weather and a sudden change in temperature, while lettuce dry (or marginal) tip-burn has been linked to water stress when transpiration exceeds water uptake, promoted by sudden checks in growth such as low temperature.

In the light of this evidence daffodil rust could be a physiological disorder brought about by adverse environmental conditions. The objective of AHDB Horticulture project BOF 76 was to test the proposition that the soil-water environment (soil structure, water availability, soil temperature, nutritional status, and so on) affects the development of rust. One practical way of assessing such effects is to monitor rust development and environmental factors and search for associations between them. Therefore, plots of the rust-susceptible daffodil cultivar 'Golden Ducat' were planted in ten commercial daffodil fields at varied locations through west Cornwall, the region where (at the time) it seemed crops were most prone to the disorder. This scheme should maximise the likelihood that rust, despite its unpredictability, would occur naturally in at least some of the test locations.

Since the project would supply a structured set of samples of rust- and non-rust-affected plants, the opportunity was used to examine further the pathological and nutritional theories of rust. Samples of plants were taken for plant clinic examination for fungal and bacterial pathogens, for sequencing viral RNA, and for the analysis of macro- and micro-nutrients, and soil samples from all sites were also taken for the determination of nutrient concentrations.

This report and summary cover both the surveys of growers and the results of field and analytical work over the first two years of the test crops, 2012 to 2014. Although this is the final report on project BOF 76, the project has been granted a year's extension (BOF 76a) so that observations can be extended to cover the whole period of typical three-year-down daffodil growing as practised in the UK.

Summary

Surveys of daffodil growers

In 2002, 68% of respondents reported having seen rust on their crops during the previous five years, and this figure rose to 86% by 2011, cited as the 'worst rust year so far'. In 2002 36% of respondents had seen some product downgraded, while 25% could not supply their preferred customer or had product that was unmarketable; in 2011 the corresponding figures had increased to 71 and 57%. For both survey periods the loss of turnover due to rust was estimated at between 0 and 3% in a ‘good year’ but up to 15% in a ‘bad year’.

Many cultivars were reported as being subject to rust, with the important cultivars ‘Golden Ducat’ and ‘Mando’ cited a disproportionate number of times in reports. ‘Carlton’, ‘Kerensa’ and ‘Tamara’ were other significant cultivars apparently prone to being affected. Crops were reported to have shown rust symptoms in their first-, second- and subsequent crop-years.

Field and related work

In autumn 2012, 50kg-plots (comprising about 1,000 bulbs) of the rust-prone daffodil cultivar ‘Golden Ducat’ were planted in bulb fields at ten sites across west Cornwall, the locations being chosen to represent different situations, topographies, soil types, etc. From west to east the locations were Kelynack, St Buryan, Tregiffian, Rosevidney, Roseworthy, Bodilly, Mawla, Penventon, Fourburrow and Goonhavern. At each site a weather station was set up, logging soil water content (SWC) at three depths, soil temperature, air temperature, RH and precipitation. This would enable the subsequent levels of rust to be studied in relation to soil-water and other features of the sites, in order to identify associations between these factors and the incidence and severity of rust.

Each year the main assessments of crop and rust development were made about two weeks before the flower-picking stage (early- to mid-February), around the flower-picking stage (late-February to early-March), and about two weeks after the flower-picking stage (though flowers were not picked in order that their development could be followed) (late-March to early-April).

- In spring 2013 no characteristic rust lesions were seen on stems at the pre-picking stage, with the exception of Tregiffian where two small rust lesions were seen on each of two stems. At the flower-picking stage only infrequent, small, isolated rust lesions were found, and at only five sites, though at Tregiffian ten stems bore single lesions or several lesions. At the post-picking assessment larger numbers of small rust lesions were seen, differing in severity between one or two small spots or occasionally streaks per stem (at Goonhavern) to individual small spots and groups of up to about 15 small spots (at Tregiffian), and rust incidence varied from <10 affected stems/plot (Goonhavern) to more than 50% of stems (Tregiffian). By the post-picking stage rust severity scores reached 0 to 2 (out of 5), incidence scores 0 to 4 (out of 5) and the number of stems per plot with rust 0 to 144 (out of ca 1,000 stems).
- In spring 2014, rust lesions had increased in severity and incidence. At the pre-picking

stage very low levels of rust (up to six stems affected per plot) were seen at four sites (Kelynack, St Buryan, Tregiffian and Bodilly) and none at the remaining sites. There had been a marked increase in rust by the flower-picking stage, with some rust found on all sites and incidence varying from two to 68 stems per plot, the most affected sites being Tregiffian and Fourburrow. At the post-picking stage all sites had substantial rust, with most or all stems affected at seven sites (all stems at Tregiffian and Bodilly). Tregiffian exhibited the most severe rust symptoms, with some stem-cracking observed. By the post-picking stage rust severity scores reached 2 to 3, incidence scores 4 to 5, and the number of stems per plot with rust 168 to 1,000.

- At the start of the project it was expected that affected crops or sites would either be free of rust, or would exhibit rust at the severity and incidence typical of affected commercial crops. None of the project assessments found a commercially-significant level of rust, with the possible exception of the minor stem-cracking observed after picking at Tregiffian in 2014. On examination it soon became evident that rust often occurs at much lower, trace levels that would probably go unnoticed in a commercial situation, and that much development of rust would have occurred only after cropping, again probably going unnoticed. This may change our understanding of rust, from a disorder thought to flare-up around flowering time as a result of some recent stimulus, to one that may exist at an insidious level much of the time - at lease in susceptible cultivars - as a result of some on-going condition.
- Inconspicuous lengthways pitting or blistering of stems or pale or yellowing spots were observed at all sites. Observations suggested these were an early stage in the development of rust lesions.
- Rust-like spots or streaks were also found on leaves at all sites and were most obvious later in the growing season.

Relationships between levels of rust, soil water content and other factors

Rust was mostly seen in the test plots at lower levels of severity and incidence, and later in the season, than would be of concern in commercial production. In both years of the study, however, rust levels varied substantially enough between the ten sites to justify their examination for relationships or correlations with SWC and other site-, soil-, weather- and husbandry-related factors. The examination of SWC and weather factors was facilitated by the data logged in the months leading up to flowering each year. Information on other factors – elevation, soil type, soil and plant levels of major nutrients and trace elements, previous cropping and so on – were obtained by examination, sampling and analysis or from the growers. This work concentrated on the expression of rust as the number of stems per plot with rust post-picking, since this showed more variability than severity and incidence scores and better reflected the increasing development of rust lesions through the flowering season. After preliminary data assessment, the possible relationships of rust incidence with these factors were examined (where appropriate) through regression analysis. The most striking result was a relationship between rust levels and SWC.

SWC revealed substantial differences between sites whether measured at individual depths in the soil (0-10, 10-20 and 20-30cm) or averaged across depths. In the first crop-year, analysis of SWC and rust levels showed that three of the four sites with the highest incidence of rust were associated with the highest SWC – Tregiffian, St Buryan and Rosevidney, the exception being Penventon (which is steeply sloping). In making these assessments the measure of SWC used was an accumulation of SWC over the five months before flower picking (since the start of data logging). To identify any critical period of high SWC, the data were examined over shorter periods of months or weeks. This showed that a high SWC in November and December was most closely related to high levels of rust at flowering, while high SWC in January, February and March were less so.

Analysis of SWC for the second crop-year was facilitated by the longer run of logged data available. Rust levels at the picking and post-picking stage in 2014 were examined in relation to the cumulated SWC data from April 2013 to March 2014. There was a strong tendency for high rust levels to fall at the wetter end of the SWC scale and for low levels to fall at the drier end, though all sites did not conform exactly using rust scores and counts. This effect was seen at all three sensor depths but was clearest in the 30cm readings, perhaps because these would have reflected water-logging above the deeper impervious layer. In order to see the effects of SWC over other periods, rust levels were plotted against SWC in the periods March 2014 to April 2014, February 2014 to April 2014, January 2014 to April 2014… and so on back to the period November 2012 to April 2014. For all these periods the results were consistent – except for a slightly weaker effect for the one-month period March 2014 to April 2014 - and mirrored those described above, a strong tendency for higher rust scores at the wetter sites and lower rust scores at the drier end. This consistency suggests the effect of SWC on rust levels relates to an ongoing process – perhaps climate-related – rather than to a particular short-term effect.

The main results for factors other than SWC are described below.

- Soil and air temperatures and RH were uniform across the ten sites in both crop-years.
- Geographical data – longitude, latitude, altitude and distance from the sea in the prevailing wind direction (which would affect the amount of salt-laden air) – failed to

show any associations with rust levels in either year.

- Soil structural factors – Visual Soil Structure Quality Assessment, ADAS soil texture assessment, soil depth and the proportions of clay, silt, sand and stone particles - also failed to show any association with the levels of rust in either year.
- There was evidence for increasing rust incidence with increasing top-soil organic matter, with R^2 = 0.6841 (tested only in 2014). This finding should be treated with caution until it can be confirmed.
- Before bulb planting, fertilisers were applied at most sites, generally P and K, but also N at Roseworthy and organic fertiliser at Penventon. At seven sites brassicas (which leave high N residues) were the previous crop. There was no evidence that high or low levels of rust in either year were associated with the type of fertiliser applied or previous cropping.
- Because of continuing wet weather in autumn 2012 when the plots were planted, planting was delayed at three sites, which might have affected plant growth. However, there was no association between planting date and rust levels in either year.
- There was no evidence for associations between levels of rust in either year and the concentrations of soil N, P, K or Mg or soil pH measured in the same crop-year.
- With four exceptions, there was no evidence for associations between levels of rust in either year and trace element concentrations in the soil measured in the same crop-year. The exceptions were that in 2014 increasing concentrations of calcium and of sulphate appeared to be associated with increased rust incidence, while increasing concentrations of manganese appeared to diminish rust incidence; however, these findings should be treated with caution until confirmed.
- There was no evidence for associations between levels of rust and the current concentrations of major nutrients or trace elements in the leaves (tested only in 2014).

Fungal and bacterial disease diagnostics

No evidence of bacterial infection was seen in any of the samples during microscopic observation. In some samples the presence of Ramularia sporophores and conidia was very obvious, but no other fungi were noted. Microscopic observation of cleared and stained tissues did not provide any insights in the cause of the rust symptoms. A Stemphylium species was the most consistently isolated fungus, being isolated from samples of eight out of the ten sites. The two samples where Stemphylium was not isolated had either predominantly or exclusively 'streak' symptoms rather than typical rust spots. Stemphylium was also obvious during humid box incubation in five out of the ten samples. Ramularia and Botrytis spp. were also isolated from some samples.

Financial Benefits

On the basis of information provided by growers, rust can result in a 3% average annual loss of revenue from cut-flowers (spread across all years), or losses of 10% in one year in three (with negligible losses in the intervening years). A 3% annual loss is estimated to amount to about £0.7m annually to UK growers, or around £2.3m every three years. These are direct monetary losses resulting from reduced flower yields and downgraded or unmarketable product. Such losses might be largely eliminated if this project and its extension lead to the provision of solutions for the rust problem and the development of strategies for rust avoidance or risk management.

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

Action Points

- A third year of results is desirable to confirm the link between sites with high SWC and high rust levels. Nevertheless, forward cropping plans might begin to take account of possible avoidance strategies, primarily not planting rust-prone daffodil cultivars in poorly drained sites.

SCIENCE SECTION

Introduction

Daffodil rust – a problem in contemporary cut-flower production

The physiological disorder now commonly called daffodil rust degrades stem quality, making some cut-flowers unmarketable. With contemporary commercial daffodil growing in the UK more dependent on the sales of cut-flowers than of bulbs, it is vital to avoid any issue that could harm customers' perception of product quality. However, the cause of daffodil rust is unknown.

From the early-1990s the minutes of the AHDB Horticulture Bulbs and Outdoor Flowers (BOF) Panel recorded the concerns of bulb growers over rust-like lesions being found on daffodil stems. The severity of the symptoms varied from a few insignificant spots, through larger groups of mildly disfiguring lesions, to prominent rusty lesions along much of an increasingly brittle stem. Similar lesions were seen on leaves, though attracting less attention because it is the cut-flowers that are the marketed commodity. References to the problem were initially sporadic, but soon increased, with some reports of batches that were so severely affected as to be unmarketable. The condition soon became known as 'physiological rust' and was regarded as a physiological disorder (non-parasitic disorder or abiotic disease), since 'rust' did not appear to be associated with a pathogen. More recently the term 'stem rust' has been adopted, though this ignores its foliar expression. For convenience, for the rest of this report the disorder will be referred to simply as 'daffodil rust'.

In anecdotal information circulating in the industry daffodil rust was reported on many cultivars in both Cornwall and eastern England. Daffodil rust was observed on crops in their second and subsequent years of growth, but there were differences of opinion as to the occurrence of daffodil rust on first-year crops, though this may have been simply due to a general lack of observation of first-year crops, from which flowers are not usually picked. Daffodil rust does not appear to have been observed in daffodils forced in glasshouses. Perhaps the most predictable feature of rust is its unpredictability. As would be expected, growers developed their own theories about the conditions that led to the appearance of these lesions, such as the "crop grows too fast after a cold frosty spell", or that it was seen mainly in waterlogged areas.

In 2002, prior to considering an experimental approach, the AHDB Horticulture BOF Panel instigated a survey of growers to establish the economic importance of daffodil rust, to seek possible relationships between cultural practices and the prevalence of daffodil rust, and to collect the industry's theories about the factors that predispose crops to the condition. The survey was repeated in 2003. In all 62 responses were received, and they confirmed the view that daffodil rust was leading to a substantial loss of quality and output. The survey was repeated in 2011, 2012 and 2013 and, although the response rate was much lower than in the earlier surveys, the daffodil acreage represented by the respondents was nevertheless substantial: the replies confirmed there had been no diminution of the negative economic effect of daffodil rust on cut-flower production. The data from all five years' questionnaires were summarised as part of the present project, and were included in the previous report on this project (BOF 76 Annual Report 2013); to make this Final Report a complete account of the project, they have also been appended to this report as Appendix 2.

Daffodil rust appears to have been the subject of little, if any, systematic research, although there have been sporadic individual attempts at disease diagnosis or correlation with soil or plant nutrient levels. These investigations uncovered no causal organism, nor any link to high or low levels of major nutrients or trace elements, and some examples are given in Appendix 2. The lack of a causal pathogen or nutritional imbalance for daffodil rust should dissuade growers from applying unnecessary fungicides or additional fertilisers in an attempt to treat the disorder. Daffodil rust does not appear to have been described in key advisory literature or research reviews in either the UK (e.g. Rees, 1992), the Netherlands (e.g. Langeslag, 1990; van Aartrijk et al., 1995) or the USA (e.g. Gould & Byther, 1979; Chastagner & Byther, 1985; De Hertogh & le Nard, 1993). Literature searches were conducted by the author, using CABI's 'Horticultural Abstracts' database covering world horticultural R&D from 1973 to date, and no references to daffodil rust were found; this may imply that daffodil rust is not a problem in other producer countries.

Possible causes of daffodil rust

Fungal disease

It should be stated that a true (fungal) rust disease is found on daffodils, though not (as far as is know) in the UK. In the Netherlands daffodils can be infected by the rust Aecidium narcissi spreading from the reed-grass Phalaris arundinacea which is used as a covering material to protect bulbs and shoots from frost and wind-blown sand (van Aartrijk et al., 1995). Covering materials are not used on daffodil crops in the UK. Apart from that, reports of true rust pathogens on daffodils are very rare, though Puccinia schroeteri, P. narcissi and Coleosporium narcissi have been seen (Moore et al., 1979; Chastagner & Byther, 1985). The diagnosis of a true rust infection should be relatively easy. Finding a pathogen associated with daffodil rust has been more difficult.

Some parallels can be drawn between daffodil rust and another physiological disorder of daffodils, chocolate spot (which is unconnected with chocolate spot of beans caused by Botrytis fabae). Chocolate spot of daffodils has been known to growers and advisors for much longer than rust, though it does not appear to have caused any serious concerns and so has been judged of little economic or other consequence. The symptoms consist of chocolate-coloured spots or streaks that appear sporadically on leaves. In the different circumstances of 30 years ago it would have been relatively easy to find a professional who might undertake such investigational work as part of an advisory role, and it appears that some effort was expended in looking for a causal organism, 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."

For daffodil rust there seems to have been no study comparable to the one on chocolate spot just described. It is known, however, that from time to time growers and advisors have sampled affected stems for examination at plant clinics, and some examples are included in Appendix 2. No primary pathogen appears to have been isolated from these samples, though it is unfortunate that samples seem to have been taken in an opportunistic way rather than as part of any structured programme. As it is difficult to prove a negative, there is lingering doubt about entirely dismissing a pathogenic cause for rust (or, for that matter, daffodil chocolate spot). Attempting to carry out systematic isolations from stems affected by rust would seem to be justified, and in this project 'rusty' and 'healthy' samples from the test sites were examined by a plant pathologist.

Viral disease

In the previous paragraph it was suggested that a viral cause of daffodil rust is unlikely. In Scotland "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 [my italics] or silver streak" (Kamerman et al., 1975). These reports that chocolate spot may be found in 'virus-free' and 'apparently healthy' daffodils 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 (Diseases and pests of bulb crops), include a condition called blad bruin (brown leaf). A translation from the second edition follows, this being almost identical to that in the first edition except for the later addition of the sentence 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 in the Netherlands 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 (Paul van Leeuwen, personal communication, 2015). These communications did not raise the issue of daffodil rust, but it is tempting to wonder whether a parallel situation – with a combined viral and environmental cause - might exist with both daffodil disorders.

Many other physiological disorders occur on horticultural crops (e.g. see Swain, 1985), and often show as distinct areas of necrotic tissue, 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).

As part of the current project, in 2014 tissue samples from stems with and without rust lesions were frozen in liquid nitrogen and stored at -70°C at Warwick Crop Centre (WCC), University of Warwick. These samples will be used to screen for viral RNA, work which is now underway as part of a project extension, BOF 76a (2014-2015).

Other pathogens

Diseases caused by bacteria and mycoplasma-like organisms are rare in daffodils (Hanks, 2013, p.44), so could be considered unlikely causes of daffodil rust.

Nutritional issues

If the pathogen theory of daffodil rust is rejected, it seems reasonable to consider next a nutritional cause, since nutritional issues are known to be important in many physiological disorders. Deficiencies could be caused directly by the low level 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). Several physiological disorders that are characterised by the production of darker brown or black spots on the surface or internally have been related to boron and (or) calcium deficiency (e.g. bitter pit of apples, internal rust spots of apple and 'five o'clock shadow' of carrot) (Swain, 1985). Similarities to other disorders related to nutrient deficiencies led to the suggestion (Andrew Tompsett, personal communication) that daffodil rust might result from boron deficiency, though no clear benefit was apparently observed in boron spray trials. When Ruamrungsri *et al*. (1996) reported the results of growing daffodil 'Garden Giant' in hydroponic culture with various nutrients omitted, the only physiological disorders reported were water-soaked areas near the base of the leaf when boron was omitted, and root-tip browning and death when calcium was missing.

It is known that occasional nutrient analysis of soil and tissue samples from daffodils with and without rust has been carried out for growers, in much the same way as disease diagnostics have. No association between nutrient levels and the development of daffodil rust has been reported. In spring 2003, for example, soil and tissue samples were taken from examples of healthy and rusty daffodil crops grown by a Lincolnshire grower and analysed under the auspices of AHDB Horticulture (AHDB Horticulture BOF Panel, personal communication, 2003). These results (described in Appendix 2) showed no clear trend that might indicate a link between nutrient levels and rust. As part of the current project a full set of plant and soil samples was taken from each site for the determination of major nutrient and trace element concentrations.

Following analysis at WCC of nutrients in plants and soil of rusty and healthy daffodils, John Hammond (personal communication, 2010) suggested that daffodil rust may not directly be a result of deficient nutrient levels, but “more an issue with rooting ability or development which is restricting uptake of nutrients overall, e.g. soil structure, which can explain why some crops grow out [of the daffodil rust symptoms] and the differences between cultivars”.

Adverse environmental conditions

Some physiological disorders characterised by the production of darker brown or black spotting have also been related to adverse environmental conditions (Swain, 1985). For example, dry hot summer weather and a sudden change in temperature have been implicated in 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. Rejecting a pathological cause for daffodil chocolate spot, Moore *et al*. (1979) suggested that the appearance of the spots is associated with increasing ambient temperatures, while in the Netherlands virus infection was linked with sunny weather as predisposing daffodils to chocolate spot (Aartrijk *et al.,* 1995).

As well as rust and chocolate spot, daffodils can show a number of physiological disorders of flower development, and although little is know of their causes, temperature and adverse water relations have often been implicated here too (Moore *et al*., 1979; Rees, 1972).

- Bud death can occur shortly before spathe-splitting and flower opening, resulting in a dried-out bud on a stem, resembling a ‘drumstick’. This is particularly a problem of double-flowered cultivars when forced under glass, and ‘Golden Ducat’ – the test cultivar for the present project – is very prone to it, even when field-grown. This disorder was investigated in ‘Golden Ducat’ and other double cultivars in AHDB Horticulture project BOF 27 (Hanks, 1992). Dissections of the stem and bud during development in the glasshouse suggested the plants were unable to translocate sufficient water to support the rapid growth of the particularly dense bud tissues. But misting or applying calcium, growth retardant or fungicides did not prevent bud death.
- Bud death in Narcissus poeticus ‘Flore Pleno’ (‘Tamar Double White’) is a specific instance of the preceding disorder. This cultivar was formerly a speciality crop of the Tamar Valley and studied intensively (Tompsett, 1972; Rees, 1972; Moore et al., 1979). Growing on warm slopes, extreme temperatures, hot, dry growing seasons, wet autumns and adverse water relations with a poor root system were all suggested as

predisposing factors. However, irrigation, mulching and shading treatments were unsuccessful in preventing bud death.

- ‘Bullhead’ is a physiological disorder resulting in abnormal flowers and abnormal flower opening in another double cultivar, ‘Cheerfulness’ (Tompsett, 1979; Moore et al., 1979). High temperatures were also linked with this disorder.

A possible link between daffodil rust, soil temperature and water availability was investigated in AHDB Horticulture project BOF 62 (Fellows & Hanks, 2007). Pot-grown bulbs of six cultivars were placed in a polyethylene/mesh ‘thermo-gradient tunnel’ at Warwick HRI, Wellesbourne (Wurr *et al.,* 1996). This facility maintained a temperature gradient from *ca* 4°C above outside temperature at the warm end to *ca* 0.5°C above outside temperature at the cool end. The treatments consisted of high, medium and low temperatures (plants placed at the warm or cool end of the tunnel or at the mid-point), combined with four levels of soil moisture from near-dry to very wet (42, 56, 69 and 82% of maximum water capacity, respectively). However, there was no development of daffodil rust symptoms in any of the treatments, so the hypothesis that conditions linked to temperature and water availability cause the disorder through the disturbance of normal water relations could not be tested. The range of treatments used in this project may have been too mild.

Research approach

The continuing unpredictable losses and down-grading of cut-flowers due to rust encouraged AHDB Horticulture to commission the present project in 2012. Ultimately the aim of research would be to discover the cause of daffodil rust and propose strategies for reducing, eliminating or avoiding it. The project aims specifically to test the proposition that rust develops as a consequence of adverse soil/water relations. Despite the failure of the earlier treatments (BOF 62) to provoke rust development using a matrix of temperature and SWC treatments, it is possible that the treatments in that project were simply not sufficiently extreme to do so. A different and more strategic approach was taken in the present project, which involved maximising the chance of rust occurring in experimental plots. Bulbs of a stock of the rust-prone cultivar ‘Golden Ducat’ were planted on ten diverse sites across west Cornwall to provide a range of natural soil, water and other environmental conditions, and variations in husbandry, that might lead to the development of rust to different extents across the sites. Soil, water and other environmental variables were monitored and regression analysis used to identify any relationship between rust development and specific conditions. The emphasis on soil and water conditions was not seen as excluding making use of the opportunity of a multi-site experiment to carry out systematic soil and plant nutrient analysis and disease diagnostics to test further the possible pathological and nutritional origins of the disorder.

This Final Report on project BOF 76 covers the field and associated work over the first two years of the test crops following planting in autumn 2012. It also includes (as Appendix 2) the results and discussion of the surveys of growers carried out in 2003-2004 and 2011-2013, along with the results of a flower trader's analysis of sendings that was contemporaneous with the field-work (spring 2013 and 2014). Since project BOF 76 was granted an extension (BOF 76a) to cover the third year of the test crop (autumn 2014 to summer 2015), this is still a work in progress. As three-year-down growing is a norm in UK daffodil growing, this gives the opportunity to study a typical commercial crop cycle. And, as in any study dependent on weather or 'the season', the extension enables better account to be taken of these vagaries, so hopefully achieving a more realistic result.

Materials and methods

Objective

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

Bulb material

A suitable stock of narcissus cv ‘Golden Ducat’ was sourced following consultation with the AHDB Horticulture Industry Representative and others. Two hundred and fifty kg of bulbs of each of two grades, 10-12 and 12-14cm circumference were obtained. The bulbs had received standard hot-water treatment. Twenty-five kg of each grade were allocated for planting at each of the ten sites. Since 25kg of bulbs is equivalent to *ca* 610 and 425 bulbs of the smaller and larger grades, respectively, each plot consists of *ca* 1,000 bulbs.

Sites

Following discussion with bulb growers about suitable sites, ten commercial daffodil fields were selected, taking into account the requirement to locate them throughout west Cornwall and to include varied soil types, topography and husbandry. The names used for the ten sites reflected current usage locally and were not necessarily definitive or unique. The site locations are shown in Figure 1, with photographic examples in Figure 2. Site locations, topography and aspect are described in Table 1. The sites are listed in west-to-east order throughout the report.

Information supplied by the growers on pre-planting soil analysis are provided in Table 2, and on previous cropping, fertiliser and lime applications and dates of bulb planting in Table 3. No subsequent soil analyses or fertiliser applications have been carried out by the growers at these sites during the course of the project. 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.

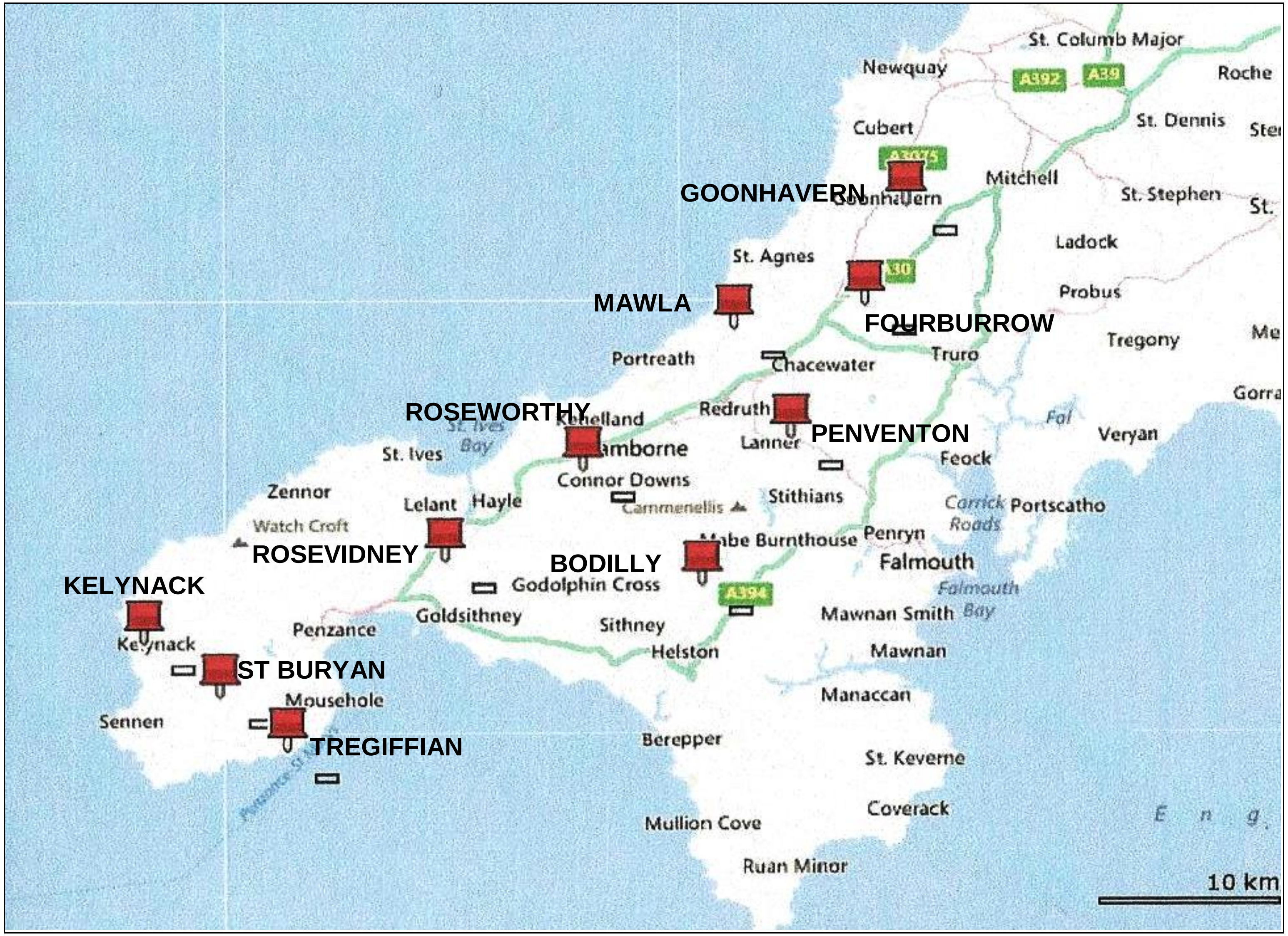


Figure 1. The locations of the ten experimental plots of 'Golden Ducat', indicated by red pins and place names in block capitals.

Figure 2. Some of the varied locations of the 'Golden Ducat' plots.

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

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). End of outside rows oriented E-W. Water can easily pool in furrows here exacerbated by adjacent heavy compaction from vehicle movements. No shelter except nearby hedgerows.
B St Buryan	SW 40491 26646	50.083135	-5.628959	121	Slightly undulating site, plot at lower end of field sloping gently to E. End of middle row oriented E-W. Furrows 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). 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. End of outside headland rows oriented SW-NE. Water can pool in furrows here. Sheltered on two sides by hedgerows and trees.
E Roseworthy	SW 61155 38796	50.200885	-5.348260	77	At higher side of site sloping gently N. Water can pool in furrows here. Open, exposed site away from hedgerows. 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 pool in furrows here. Some broken shelter (trees and buildings) to W. 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. 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 some pooling seen in furrows of plot. Broken shelter (hedgerow) to NW. End of middle rows oriented SE-NW.
I	Fourburrow	SW 77193 47636	50.286518	-5.129086	96	SW-facing steep slope, large, very exposed site. 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. End of outside rows oriented E-W. 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	na	na	na	na
F	Bodilly	5.9	3	1	2
G	Mawla	7.3	4	3	2
H	Penventon[1]	-	-	-	-
I	Fourburrow	na	na	na	na
J	Goonhavern	5.4	0	1	2

[1] Soil analysis not done at this site
na = information not available

Table 3. Previous cropping, and fertilizer and lime application for the ten sites.

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	na	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
na = information not available

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

Site reference and name		Soil texture [1]	Soil texture [2]	VSS QA [3]	Observations on soil surface state, horizons (see Figure 8) 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, now capped. 0-30 to 0-50cm: uniform, friable dark brown soil with small stones, becoming increasingly compacted >30 to >50cm: yellow, sandy and stonier >50cm: stony, not easily penetrable Water liable to pool 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 drain 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 >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.	Denbigh 2 (541k)

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 loam	Silty clay loam	1.00	Surface: very liberally strewn with quartz or blue-grey angular stones up 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.	Denbigh 2 (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) 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.

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

Bulb planting and subsequent husbandry at the sites followed each grower's usual practice. However, it was requested that flowers were not picked but left *in situ* to allow the full development of any daffodil rust symptoms for assessment, so the trial plots were marked (e.g. with high-visibility tape) to emphasise this requirement. (This was broadly successful, though a better protocol might be to arrange for the plots to be surrounded by varieties that flower substantially earlier or later than 'Golden Ducat'.) The markers also served to draw tractor drivers' attention to the location of the plots and their monitoring stations (see below), though at Kelynack one-half of one ridge, and at Mawla *ca* 1m at one end of each ridge, were damaged by vehicles 'cutting corners' (these damaged portions were not assessed and counts from the remaining plot were scaled-up to take account of these losses). At St Buryan one end of each ridge appeared to be planted with a different bulb stock and these areas too were excluded from assessments. Each grower was asked to provide details of sprays applied and other field operations for reference purposes.

Environmental monitoring

After planting the bulbs an 'Active Irrigation Scheduling' monitoring station, with added air temperature and humidity sensors, was set up in the plot at each site by Plantsystems[1] who continued to monitor and maintain them. Each station included a sensor to measure each of the following: percentage SWC at 0-10, 10-20 and 20-30cm depth, soil temperature at 15cm

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

depth, rainfall, air temperature and RH (Figure 3). The soil sensors were inserted in the ridge centre, while air temperature and humidity sensors were positioned 20 to 30cm above the ridge tops, roughly corresponding to mid-canopy height for the fully-grown crops. The measure 'percentage SWC' is equivalent to the amount of water in mm per 100mm depth (1% = 1mm of water in 100mm of soil), and could also be expressed as the average across the three depths (i.e. mm of water in 100mm of soil) or the total of the three depths (i.e. mm of water in 300mm of soil). All measurements were logged at 15-minute intervals, accumulated on Plantsystems' data-base, and checked and downloaded at appropriate intervals for analysis.

Figure 3. Plantsystems (Agrovista) 'Active Irrigation Scheduling' monitoring station on site.

Soil and plant sampling and analysis

Soil sampling and examination techniques were guided by 'RB209', the standard text (Defra and Welsh Assembly Government, 2010). For consistency, all regular soil samples were taken from half-way up the ridges.

<u>Autumn 2012</u>

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 to 20cm deep was taken by bulking at least ten cores from across a whole plot. Samples were analysed for the concentrations of major nutrients (nitrate-N, P, K and Mg) at WCC.
- Using a 90cm soil auger (2.5cm diameter), soil samples were taken from 0 to 30, 30 to

60 and (as far as practical) 60 to 90cm deep. The maximum practical auger depth was 45 to 50cm except at Roseworthy where it was 60cm. Soil from each layer was examined. A bulked sample of three to five 0 to 30cm cores were 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 principal layer was examined and described (a) by assessment of soil texture by hand (following 'RB209') and (b) using a 'Visual Soil Structure Quality Assessment' (VSSQA) which defines soil structure as an easily quantifiable score from 1 (friable) to 5 (very compact) (Ball et al., undated).

Spring 2013

On 26-28 March 2013, after the bulk of the winter rain and well after any fertilisers had been applied, soil samples were taken at each site using the methods previously described.

- Samples 0 to 20cm-deep were analysed for pH and the concentrations of major nutrients (N, P, K and Mg) and trace elements (Al, Ca, Fe, Mn and Na) by WCC.
- Samples were taken from as deep as practical (to 45cm at Fourburrow, Roseworthy, Mawla and Goonhavern and to 50cm at the other sites) and fresh samples analysed for total mineral N (NO_3-N and NH_4-N) by WCC.

Spring 2014

On 11–15 April 2014, by which date reasonable levels of rust were apparent on some plots, soil and leaf samples were collected from each site.

- Soil samples 0 to 20cm-deep were analysed for pH, top-soil organic matter and the available concentrations (or total concentration in the case of Al) of major nutrients (P, K and Mg) and trace elements (SO_4, Ca, Na, Fe, Mn, Cu, Zn, B, Mo and Al) by Natural Resource Management (NRM).
- Soil samples were taken from as deep as practical (to 45cm at Fourburrow, Roseworthy, Mawla and Goonhavern and to 50cm at the other sites) and fresh samples analysed for total mineral N (NO_3-N and NH_4-N) by WCC.
- Leaf samples consisted of ten groups of leaves per plot, excised at soil level and wiped free of soil. They were analysed for total concentrations of N, P, K, Mg, S, Ca, Na, Fe, Mn, Cu, Zn, B, Mo and Al by NRM. Leaves, rather than stems, were sampled since they would be expected to represent the current overall nutrient status of the plant more fairly.

Standard analytical methods were used, though due to failed equipment there was an unavoidable change of analytical laboratories in 2014. This meant that some changes in analytical methods, notably for Al, Fe and Na, where WCC had used cat-ion exchange with barium chloride, and NRM used DTPA-extraction (chelation) for Al and Fe and ammonium nitrate-extraction for Na. Where this occurs it is noted under 'Results', and in these cases comparisons should be made only between sites only within a year, not between years.

Fungal and bacterial disease diagnostics

On 5–7 April 2014 stem samples were collected from each site for disease diagnostics. At each site six stems with typical rust lesions, and – where available – three stems with no rust lesions were selected, avoiding stems with white mould symptoms. At Tregiffian, Bodilly, Fourburrow and Goonhavern most or all plants were affected by rust and so no rust-free stems could be obtained. Stems were cut at ground level and the remains of the flower removed before placing each sample of stems top-first into polythene bags to isolate each sample and allow them to be stood in water for transport to the laboratory. At each site three whole plants with rust symptoms on stems were also sampled and used for observations on the further development of rust lesions. The samples were taken to Plant Health Solutions, Warwick, for examination.

Microscopy

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

Surface slivers of tissue pieces containing symptoms 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.

Incubation in humid box

To encourage further development of symptoms and (or) sporulation of any fungi present, sections of tissues containing lesions, both surface-sterilised and non-surface-sterilised, were placed in humid boxes and incubated at RT for up to four weeks.

Isolations

For surface sterilisation, tissues pieces (*ca* 2cm^2) containing lesions were placed in a solution of 0.3% chlorine (prepared using 'Presept' tablets) for 30–60 seconds, blotted on paper towel, rinsed in sterile de-ionised water, and blotted dry.

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

For the isolation of fungi sections of tissue containing lesions were excised with a sterile scalpel and placed on the surface of two agar media, Cornmeal Agar (CMA) and Potato Dextrose Agar (PDA) in 9cm Petri dishes. Up to four pieces per sample were placed in a single Petri dish. The two media were selected on the basis that they may select for and encourage or limit sporulation of different types of fungi (CMA is relatively nutrient-poor, PDA is nutrient-rich). Plates were incubated for 7–14d at 20°C in the dark. Representatives of the different fungal 'types' were sub-cultured to fresh plates of PDA and CMA and examined microscopically.

Sampling for viral RNA analysis

Also on 5–7 April 2014 further stem samples were collected from each site – three per site with rust lesions and (where present) three per site without rust lesions – for the determination of viral RNA. After transport to WCC, *ca* 2cm-long pieces of stem were excised, cut into five or six smaller sections, frozen in liquid nitrogen, and stored in individually labelled vials at -70°C. For sites where very uniform lengths of stem with and without rust lesions could be identified – Kelynack, Mawla and Penventon - these pieces were taken from about mid-way along the stems. For the remaining sites, where stems were not considered uniform in regard to rust and other lesions, the pieces were taken from parts of the stem that included, or were free of, rust lesions (and were also free of other types of lesions). The analysis of these samples will take place at WCC in 2015 under the project extension (BOF 76a).

Crop and rust assessments

Each year plots were routinely assessed at three crop stages based around flower-picking, the time when the appearance of rust is most commercially relevant: pre-picking, picking and post-picking. The assessment dates were 7–8 February, 27 February–1 March and 26–28 March 2013 and 12–14 February, 4–6 March and 5–7 April 2014.

At each assessment the minimum, most usual and maximum crop growth stages (GS) were recorded, including stem and foliage heights where applicable (Table 5). At a later stage in the project the root profile of fully-grown crops will be examined.

Table 5. A scale of growth stages (GS) for daffodils[1]

Period	GS	Description	Notes
Unplanted bulb (GS 0)	0.1	'Dormant' bulb in storage	Bulbs would normally be planted at GS 0.1 or 0.2
	0.2	Root initial development evident close to the surface of the bulb	
	0.3	Shoot and/or roots emerging from stored bulb	Apply only to stored bulbs
	0.4	Bulb becoming desiccated with loss of skin, emerging roots or shoots becoming moribund	
	0.5	Bulb shrivelled, light in weight, or rotted	
Planted bulb (GS 1)	1.1	No clear emergence of shoot and/or roots	
	1.2	Roots and/or shoot emerging, <1cm in length	
	1.3	Roots and shoot elongating	
	1.4	Shoot tip close to soil surface	
Emergence (GS 2)	2.1	First shoots starting to emerge	Foliage height nominally 0
	2.2	Shoots elongating, but no buds obviously visible	Record foliage height (and stem height for 2.3 and 2.4) [2]
	2.3	Shoots elongating, tips of flower buds visible without pulling shoots apart	
	2.4	Full length of buds visible ('upright pencils')	
Anthesis (GS 3) [3]	3.1	Flower buds still 'upright pencils' with no colour showing, but becoming clear of the foliage; flower cropping could have begun if a very tight stage is required and stem length is adequate	Record foliage and stem heights
	3.2	Flower buds are 'fat pencils' with no colour showing, flower cropping should have begun	Record stem height
	3.3	Pedicels bending and/or spathes splitting, colour may be showing; a very late picking stage	
	3.4	Pedicels fully 'goose-necked' but flowers not open	This stage may pass quickly and variably
	3.5	Flowers (or florets) starting to open	
	3.6	Flowers fully open	For multi-headed types, 50% of florets open, senescing or senescent
	3.7	Flowers at least starting to senesce (petal tips dying) but not fully senescent	
	3.8	Flowers (or florets) fully senescent, leaves still fully green and upright	
Post-flowering (GS 4)	4.1	Leaves still fully green, but at least some leaves starting to bend to ground	
	4.2	As 4.1, but some leaves bending conspicuously and at least some leaves with senescent (yellowing and dying) tips	
	4.3	Most leaves almost flat, with general incidence of senescence at the leaf ends	

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

[1] Avoid the following when recording: plot or row ends; obvious rogues, off-types and atypically damaged/diseased plants; late flowers from lateral bulbs; and the most advanced plants if these are about 1% or less of the total.
[2] Record shoot height from the point of emergence from the soil to the uppermost tip of foliage, and stem height from the point of emergence from the soil to the topmost tip if the bud, spathe or flower.
[3] If flowers cropped and no remnants left to estimate exact GS, record as '3.C' (cropped).

To assess rust levels, all emerged stems in a plot were checked individually for the presence and extent of lesions. The total number of stems per plot with any rust was recorded. In assessing the stems it was useful to record the nature of any lesions present, e.g. "one or two small spots per stem", "several groups of larger lesions per stem", etc. From these observations, rust incidence and severity scores, each of 0 to 5, were recorded (see

Table **6** and Figure 4). A range of lesions is shown in Figure 5.

In some instances it was not appropriate to assess the whole plot, e.g. when part of the plot had been damaged by tractor movements, in which case the remainder of the plot was assessed and rust incidence was scaled-up to the equivalent of a full plot. Similarly, at some sites some stems had been lost before recording, e.g. by wind damage or picking, so the total number of stems remaining was recorded and the number with rust was scaled-up.

A note was made of possibly related lesions, such as areas of 'blistering' or 'pitting' that came to be considered as putative early-stage lesions. These putative lesions were not included in counts or scores, but in some cases were recorded in their own right. Foliage was quickly checked for the presence and nature of any rust lesions. On 1–2 June 2014 leaf rust was recorded in its own right, using 'clump-by-clump' (rather than individual leaf) assessments.

The general occurrence of pest, disease, disorder and other problems was noted.

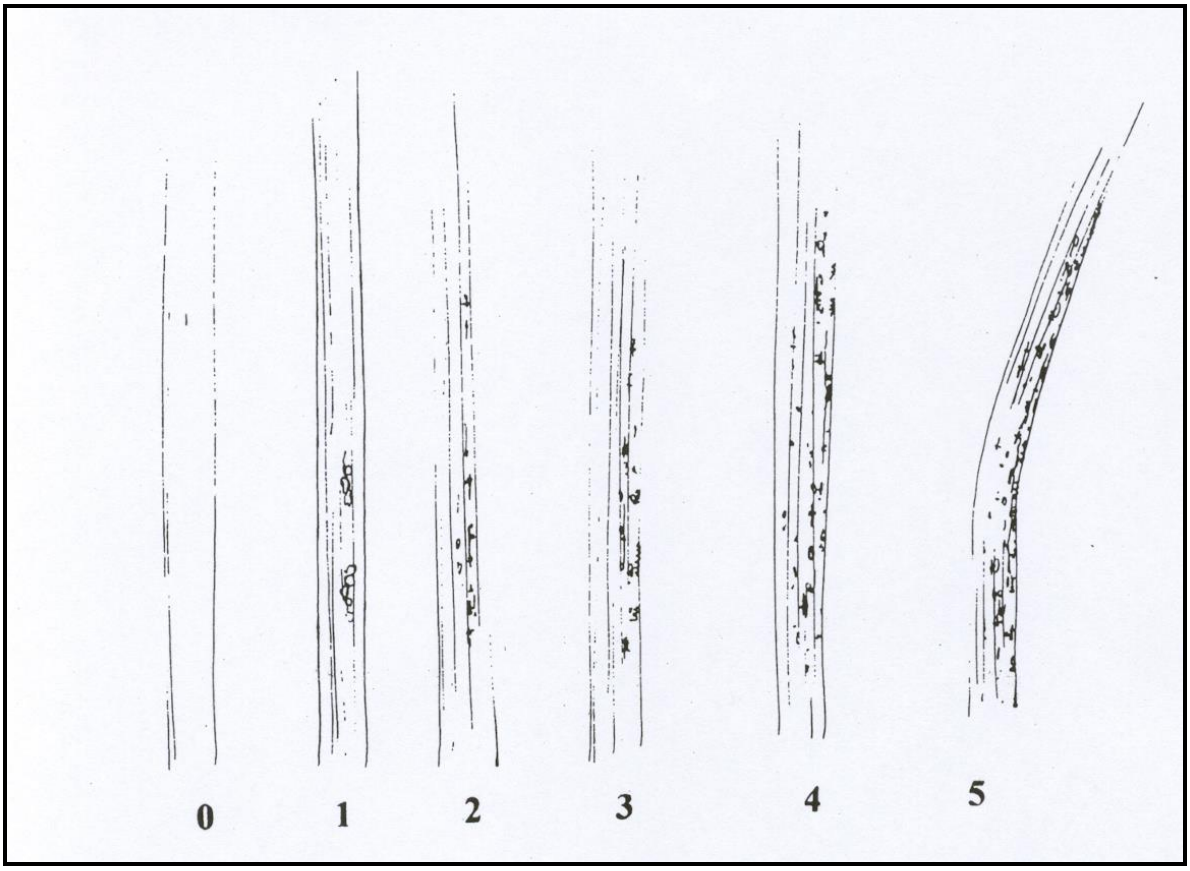


Figure 4. Daffodil rust lesions showing severity scores of 0 to 5 (by courtesy of Andrew Tompsett).

Figure 5. 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).

Table 6. Rust severity and incidence scores used in plot assessment.

Severity	Scores	Incidence
None seen	0	None seen
Slight markings or blistering that may not be rust-coloured, or typical rust spots but one or two small spots only, almost unnoticeable	1	Up to 1% of stems affected
Sparse but typical lesions; no commercial significance, but worth watching	2	Up to 5% of stems affected
Moderate lesions that are becoming disfiguring; commercially might lead to down-grading	3	Up to 10% of stems affected
Severe daffodil rust, some cracks, very disfiguring; flowers un-marketable	4	Up to 50% of stems affected
Very severe daffodil rust with very obvious cracking and stem bending; flowers un-marketable	5	Up to 100% of stems affected

Relationships between levels of rust, SWC and other factors

In preliminary data assessments in Year 1 and 2 the potential relationships at each site of the severity and incidence of rust with SWC and weather data was assessed using graphical summaries in the form of box-and-whisker plots, which provided convenient visualisation of the means and ranges of the many values. Using the data collected at 15-minute intervals for the factor and period being considered (say, air temperature during December 2012), the central thick line in the plots (see Figure 51 as an example) indicates the median value, the box covers values falling in the second and third quartiles, and the 'whiskers' extend to minimum and maximum values excluding any extreme values (or 'outliers', defined as those which fall outside a value of 1.5 × the inter-quartile range and shown on the plots by the circles). A box-and-whisker plot covering a one-month period is therefore derived from almost 3,000 data points at each site for any one factor.

In addition to analysing SWC and the weather data using the box-and-whisker approach, logistic regression plots or simple scatter plots were used, where appropriate, to search for potential relationships between the level of rust and other factors (geographical, soil, nutritional and husbandry factors). In these cases the regression coefficient (R^2) is quoted, a statistic that lies between -1 and 1, where 1 (or -1) means there is a perfect correlation between the two factors and 0 means there is no correlation; conventionally a substantial correlation might be accepted where R^2>0.6 (or <-0.6), say. Where logistic regression was used the regression coefficient quoted is McFadden's pseudo-R^2 which approximates the regular R^2 but is quoted as a positive value between 0 and 1. However, since not all factors of interest were easily quantifiable – for example, previous fertiliser practice or soil texture – in some cases simple histograms are presented as an aid to visualisation of the results.

Results

Soil analysis

Soil texture and particle size analysis

At the start of the project in autumn 2012 particle size analysis (Figure 6 and Figure 7) showed that the soil at all ten sites contained <20% v/v clay but 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 loams) and 30 to 50% sand at the remaining six sites (sandy silt loams). The soil textures quoted by the growers were also recorded (Table 4), but these differed somewhat and may not have been derived through using standard ADAS methods.

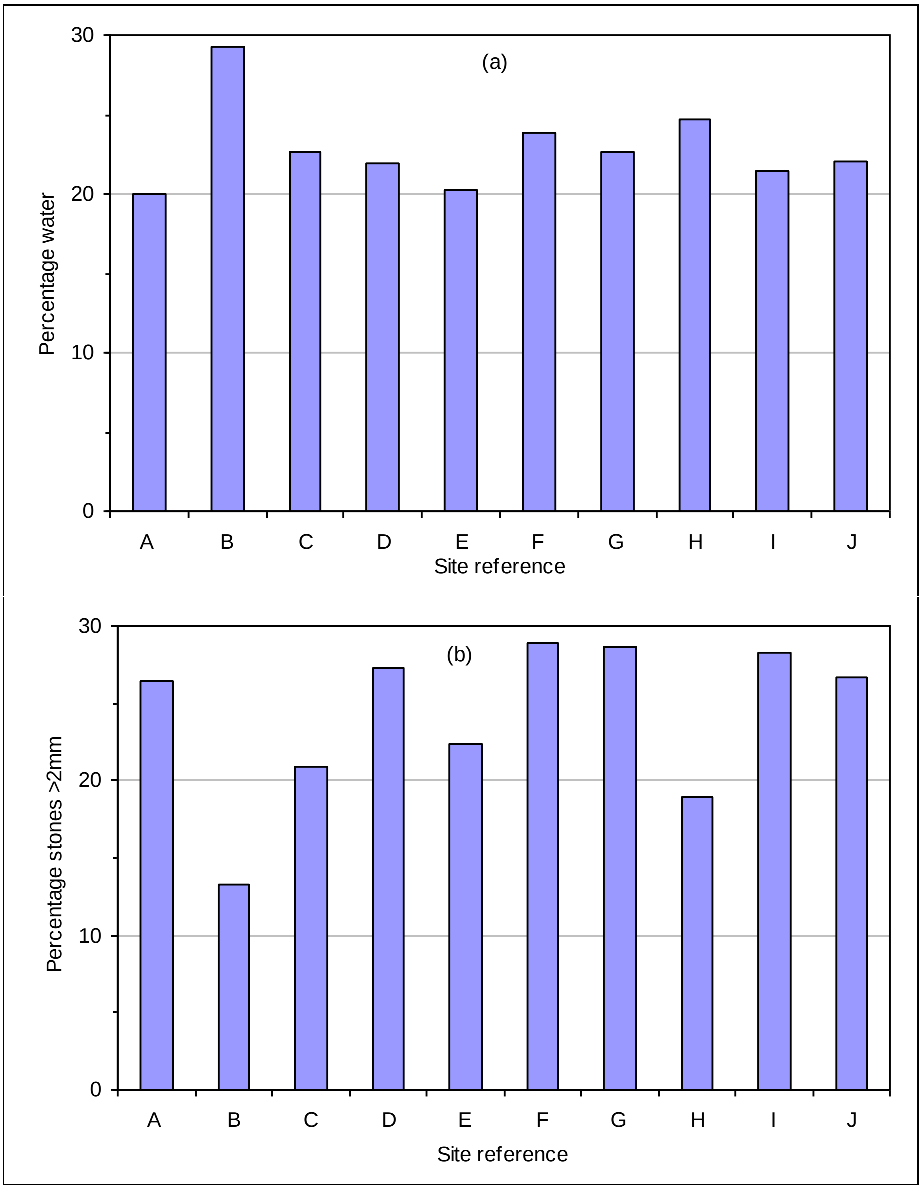
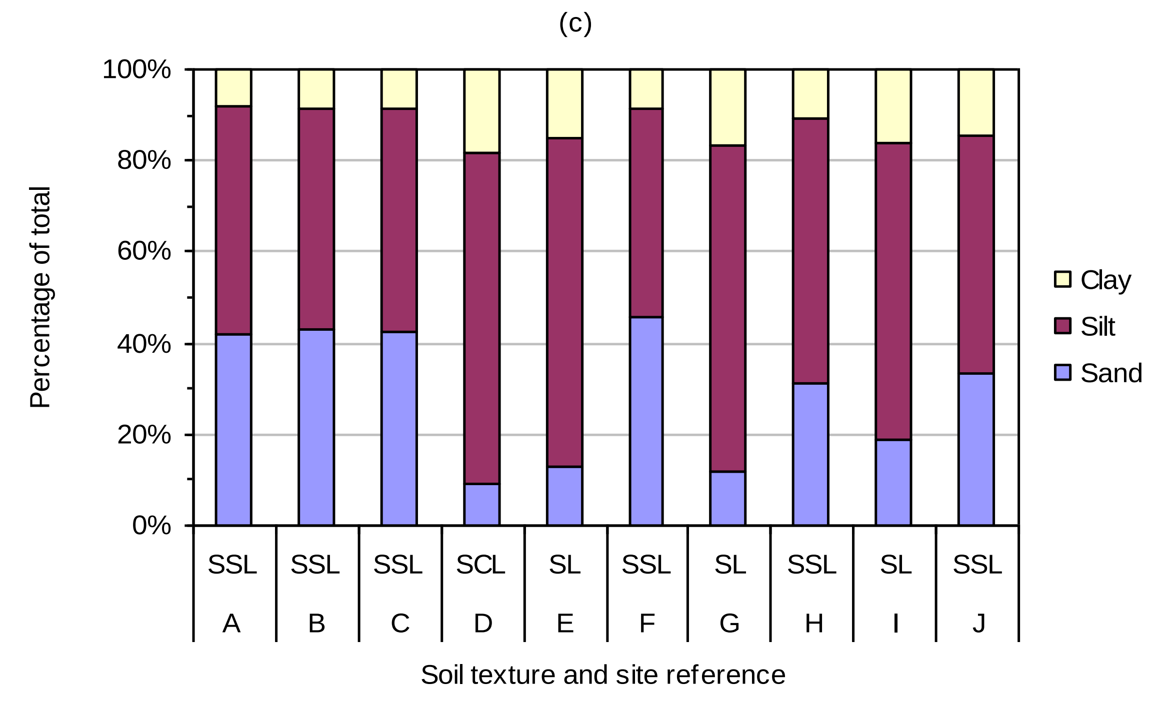


Figure 6. Soil particle size analysis to 30cm for the ten sites: (a) percentage water content (w/w) prior to drying; (b) stones >2mm as percentage of dry soil. Data continued in Figure 7.



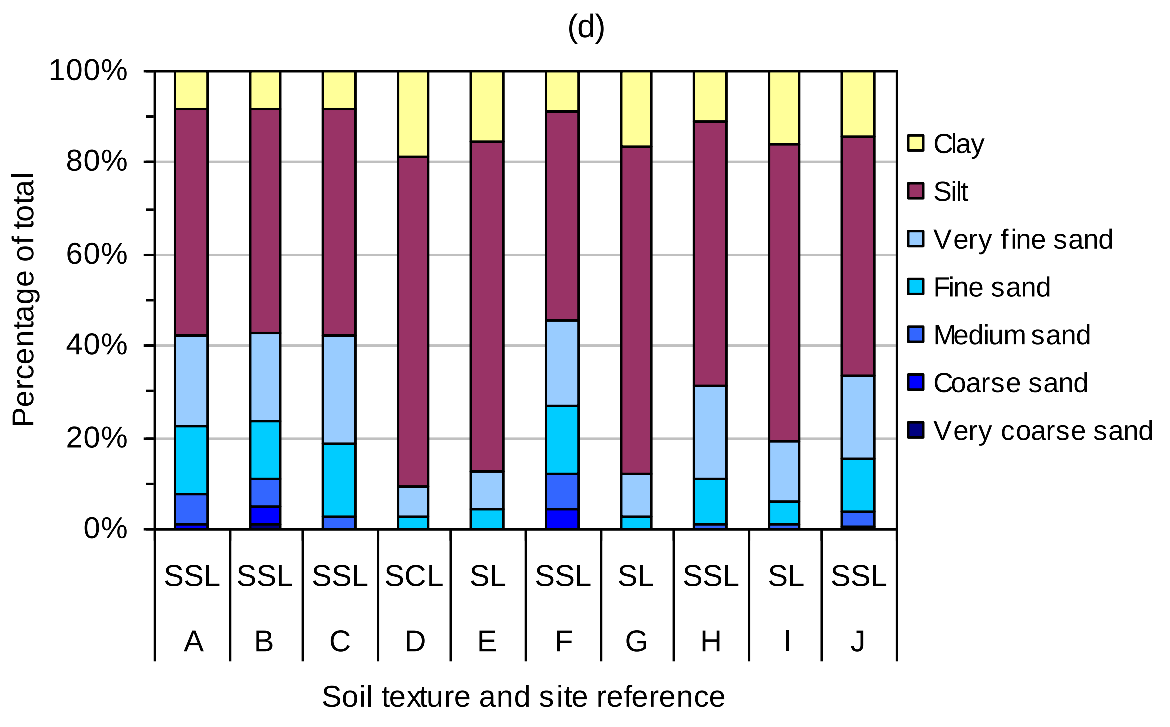


Figure 7. Soil particle size analysis to 30cm for the ten sites (data continued from Figure 6): (c) clay, silt and sand as percentage of dry soil after stones >2mm removed; (d) as (c) but including the breakdown to grades of sand. The soil textures indicated by the particle size analysis are also shown.

At the same time soil structure was assessed using a '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).

Soil horizons

Examination of the soil horizons in autumn 2012 showed that most sites had a relatively shallow, well worked upper layer 20 to 30cm-deep (Figure 8 and Table 4). Bodilly and Rosevidney had a shallow 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 clayey, sandy, gritty or stony with depth. Below 40 to 50cm the soil was difficult to penetrate, except at Roseworthy where the hard layer did not start until about 60cm. The surface contained gravel, stones or rock chips at all sites, and liberally so at Tregiffian, Rosevidney, Roseworthy, Mawla and Fourburrow. The soil appeared particularly prone to capping at St Buryan. All sites appeared to drain well down slopes, though at St Buryan and Rosevidney water tended to stand in the furrows of the plots, these being sited in lower parts of the field.

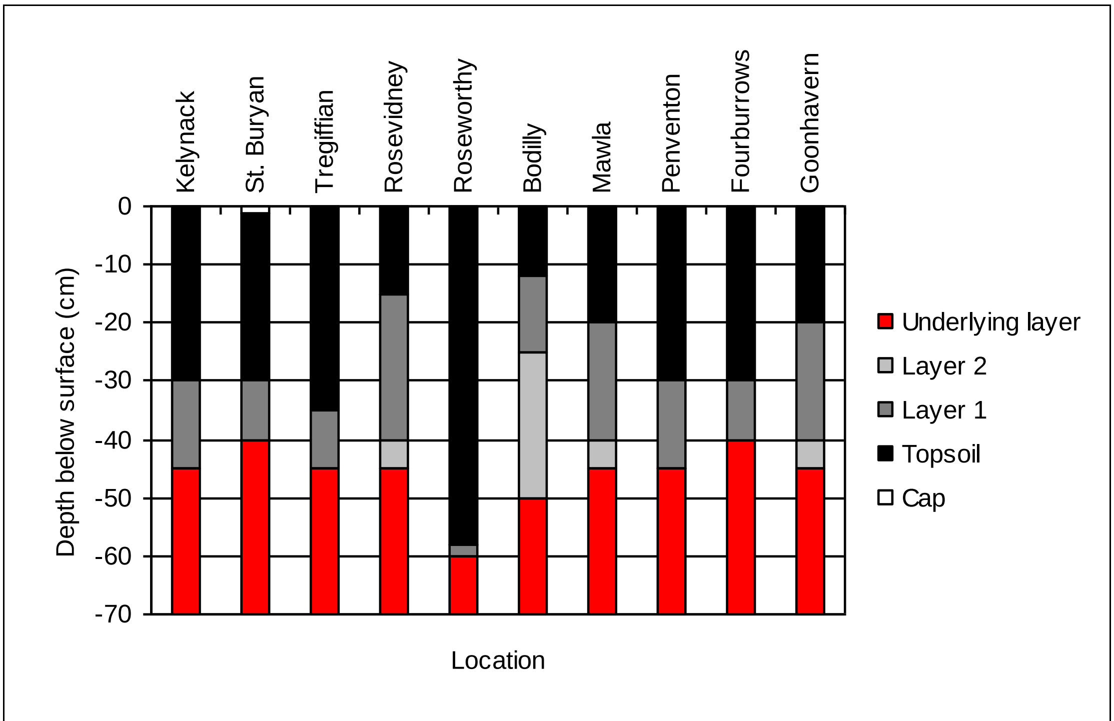


Figure 8. Soil horizons at the ten test sites.

Soil associations

The soil associations at most sites were typical brown earths as used for horticultural crops in west Cornwall (Table 4). However, at St Buryan, Tregiffian and Bodilly there were typically dark brown podzolic soils resulting from acidic weathering, also used for horticultural crops in west Cornwall. At Kelynack the soil was similar, but with a humose or peaty topsoil, a soil not usually used for horticultural crops.

Soil analysis

Table 7 shows the results of standard agricultural soil analysis at the start of the project in autumn 2012. More detailed soil analysis was carried out in spring 2013 (Table 8) and spring 2014 (Table 9 and Table 10).

An unavoidable change of analytical laboratories in 2014 meant changes in some analytical methods (see 'Materials and methods'), and where this applies it is noted in the text and means that comparisons can only be made between sites and not between years.

Table 7. Soil chemical analysis in October 2012 (0-20cm samples).

Site reference and name		pH	NO_3-N (ppm)	P (ppm)	K (ppm)	Mg (ppm)
A	Kelynack	7.4	42.5	164.8	212.8	133.1
B	St Buryan	6.7	41.7	163.6	460.8	233.0
C	Tregiffian	7.0	50.7	128.0	171.2	143.6
D	Rosevidney	6.8	14.8	102.4	442.5	98.5
E	Roseworthy	7.7	17.7	115.2	317.9	102.1
F	Bodilly	7.4	55.5	148.5	171.2	155.6
G	Mawla	7.3	23.7	82.4	314.9	100.9
H	Penventon	6.3	20.0	166.8	117.8	112.8
I	Fourburrow	6.3	13.5	56.5	274.4	57.5
J	Goonhavern	6.8	5.9	57.3	164.4	117.9

Table 8. Soil chemical analysis in March 2013 (0-20cm samples except for total mineral N which used 0-45 or 0-50cm samples depending on site, see text).

Site reference and name		pH	Total mineral N (ppm)		Nutrient concentration (ppm)							
			NO_3-N	NH_4-N	P	K	Mg	Al	Ca	Fe	Mn	Na
A	Kelynack	7.0	10.6	6.0	58.3	199.9	150.9	21.3	4116	1.4	0.5	920
B	St Buryan	6.6	11.0	5.5	50.9	384.5	245.4	16.4	4090	1.9	1.1	866
C	Tregiffian	6.8	16.7	6.0	38.3	185.8	148.9	16.7	3101	2.9	1.4	922
D	Rosevidney	6.7	12.5	4.6	49.1	400.7	104.3	14.3	3011	1.1	3.5	976
E	Roseworthy	7.4	10.8	6.4	52.5	302.5	106.9	15.1	4134	2.9	0.5	985
F	Bodilly	7.0	17.0	5.9	48.6	170.4	140.6	35.2	4097	3.8	1.6	1107
G	Mawla	7.3	11.3	6.7	41.5	329.7	78.3	17.8	3911	1.4	0.9	772
H	Penventon	6.1	9.0	6.2	62.3	104.7	112.7	20.1	2803	1.6	5.1	780
I	Fourburrow	5.9	7.5	4.7	23.2	208.5	58.4	24.5	1709	1.0	5.8	887
J	Goonhavern	6.9	11.7	6.2	22.2	176.1	119.9	19.6	2898	1.2	2.1	799

Table 9. Soil chemical analysis in April 2014 (0-20cm samples except for total mineral N which used 0-45 or 0-50cm samples depending on site, see text).

Site reference and name	pH	Top-soil organic matter (% w/w)	Total mineral N (ppm)		Nutrient concentration (ppm)		
			NO_3-N	NH_4-N	P	K	Mg
A Kelynack	7.4	7.0	23.7	3.1	66.6	105.0	87.6
B St Buryan	6.2	10.3	34.6	3.2	45.0	225.0	163.0
C Tregiffian	6.4	8.4	52.3	3.2	29.6	94.3	114.0
D Rosevidney	6.8	5.8	21.5	2.9	52.6	324.0	73.9
E Roseworthy	7.6	6.2	22.4	3.0	60.2	194.0	71.6
F Bodilly	7.2	7.8	43.1	3.3	43.0	94.3	81.4
G Mawla	7.6	7.0	22.5	3.0	43.8	251.0	55.2
H Penventon	6.0	6.0	30.4	3.1	61.0	58.8	86.9
I Fourburrow	5.6	5.6	38.6	3.0	19.4	152.0	38.8
J Goonhavern	7.4	5.7	38.2	3.1	16.0	77.0	66.3

Table 10. Soil micronutrient analysis in April 2014 (0-20cm samples).

Site reference and name	Nutrient concentration (ppm (mg/L) except where stated)									
	Al (g/kg)	Ca	Fe	Mn	Na	Cu	Zn	Mo	SO_4	B
A Kelynack	12.0	1849	97	0.7	89	3.0	2.2	0.3	29.3	1.0
B St Buryan	13.8	1707	181	0.7	67	1.3	2.2	0.3	37.1	0.9
C Tregiffian	30.4	1561	42	2.2	278	4.0	1.1	0.5	59.5	0.9
D Rosevidney	26.0	1497	66	4.2	41	23.7	4.9	0.4	18.4	1.3
E Roseworthy	42.4	2189	23	4.1	47	18.6	4.2	0.4	20.7	1.3
F Bodilly	25.4	2085	86	1.6	48	5.3	2.2	0.4	52.0	0.6
G Mawla	26.2	1886	50	4.3	40	39.3	7.0	0.4	23.6	1.3
H Penventon	18.1	1576	134	4.0	46	49.3	7.9	0.4	21.9	0.6

I	Fourburrow	16.5	880	46	5.8	37	1.9	1.1	0.2	22.0	0.9
J	Goonhavern	14.7	1425	41	4.7	28	7.4	18.1	0.3	30.8	0.7

Soil pH

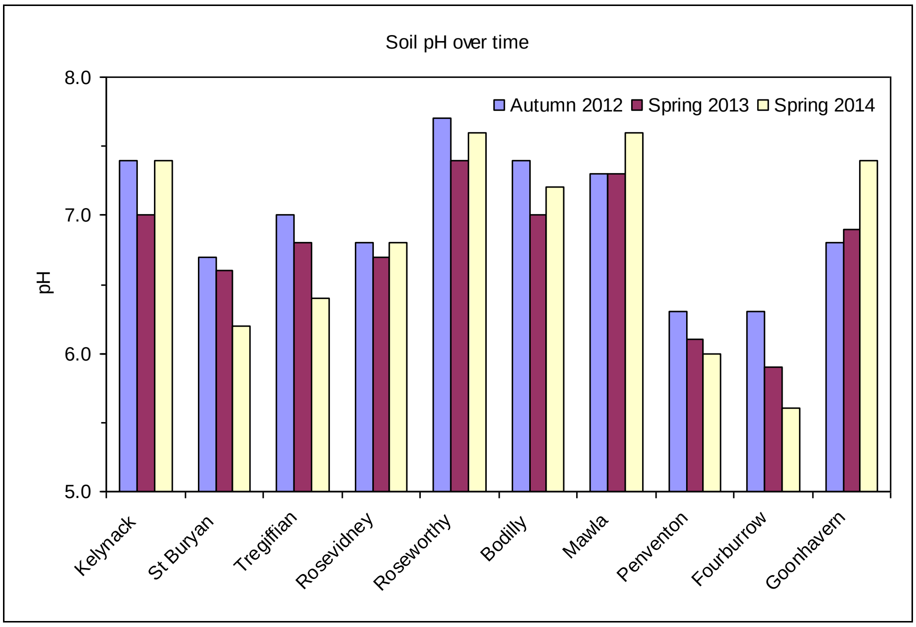


Figure 9. Soil ph at the ten sites, autumn 2012 to spring 2014.

Soil pH initially varied from slightly acid, pH 6.3, at Fourburrow and Penventon, to slightly alkali, pH 7.7 at Roseworthy (indicating a naturally lime-rich soil or a tendency to over-lime) (Figure 9). The remaining sites were neutral (7.0±0.4). Over the next 2 years pH levels fell further at Fourburrow and Penventon (finally to 6.0 and 5.6), while the high pH at Roseworthy was joined by some other sites - Kelynack, Bodilly, Mawla and Goonhavern. These levels would all be regarded as suitable for daffodil growing.

Nitrogen (N)

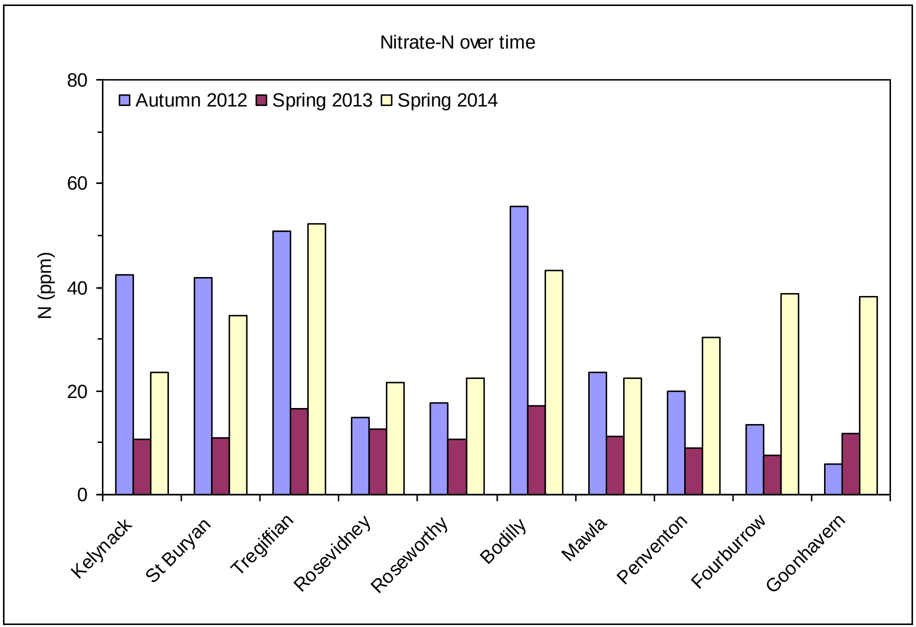


Figure 10. Soil nitrate-N concentrations at the ten sites autumn 2012 to spring 2014 (note: autumn 2012 measurements from routine 0-20cm soil samples, subsequent spring samples from 0-45 to 0-50cm soil samples for total mineral N).

In the autumn 2012 analysis nitrate levels varied from a low of 6ppm at Goonhavern to a high of 56ppm at Bodilly (Figure 10). The larger amounts of N fertiliser that had been applied at Roseworthy and Fourburrow (Table 3) were not evident now. By the next spring mineral N levels were relatively low at all sites, having fallen to 17ppm or less. By spring 2014 the levels were again higher – similar to the initial levels, perhaps due to the milder but much wetter winter leading to increased mineralisation.

Ammonium-N made a small and usually consistent contribution to total mineral N (Table 9).

N levels at Rosevidney, Roseworthy, Mawla and Penventon were consistently low, and overall the results indicate a general requirement for some further N fertiliser at all sites. It would have been expected that the higher levels of mineral N would follow more intensive previous cropping or manuring and would occur on the more organic soils. However, consideration of previous cropping and manuring (Table 3) and of top-soil organic matter (Figure 14) provides no obvious relationship: for example, the only site to which N was applied in 2012 (Roseworthy) and the only site with a history of organic fertiliser (Penventon) each subsequently showed a low level of nitrate. Further, low or high levels of mineral N occurred irrespective of whether or not there had been previous cropping with brassicas. Finally, despite the variations in mineral N levels found in spring 2014, all sites showed relatively similar top-soil organic matter content, only St Buryan standing out as having a somewhat higher organic content – a site with *low* mineral N levels in 2014.

Phosphorus (P)

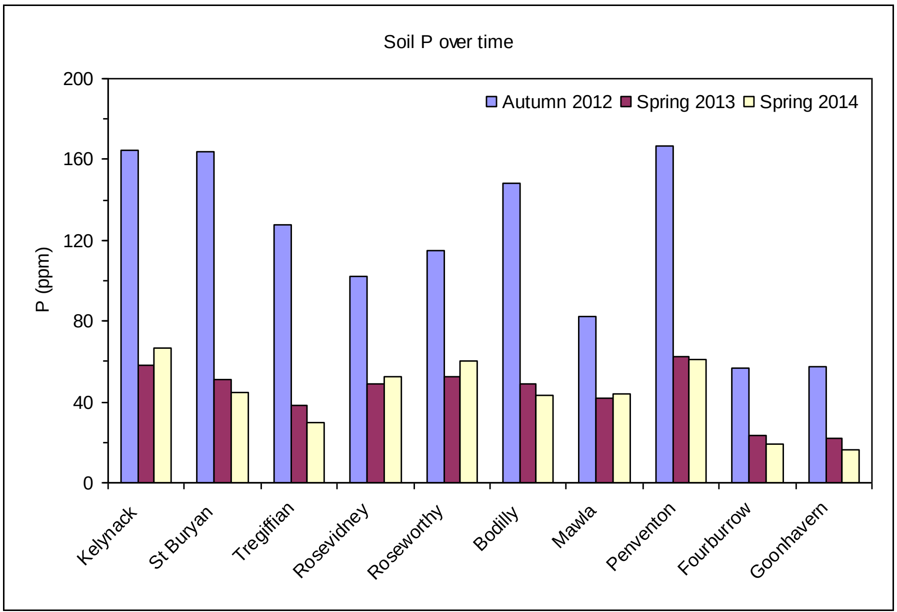


Figure 11. Soil P concentrations at the ten sites autumn 2012 to spring 2014.

Soil P levels were initially adequate at all sites, despite varying greatly between about 60ppm (index 4) at Fourburrow and Goonhavern to about 160ppm (index 7) at Penventon, St Buryan and Kelynack (Figure 11). Over winter P levels fell markedly to give a range of 22–62ppm (index 2 to index 4) by spring 2013. After this P levels, as expected, changed little to the analysis in 2014. The levels of P in autumn 2012 – notably lower at Fourburrow and Goonhavern – did not correspond to the amount of P fertiliser applied earlier that year, when Kelynack and Penventon were the only sites that did not receive substantial applications of P.

The levels of P found in spring 2013 and 2014, between 16 and 67ppm (index 2 to 4), would mean that none of the sites was deficient in P.

Potassium (K)

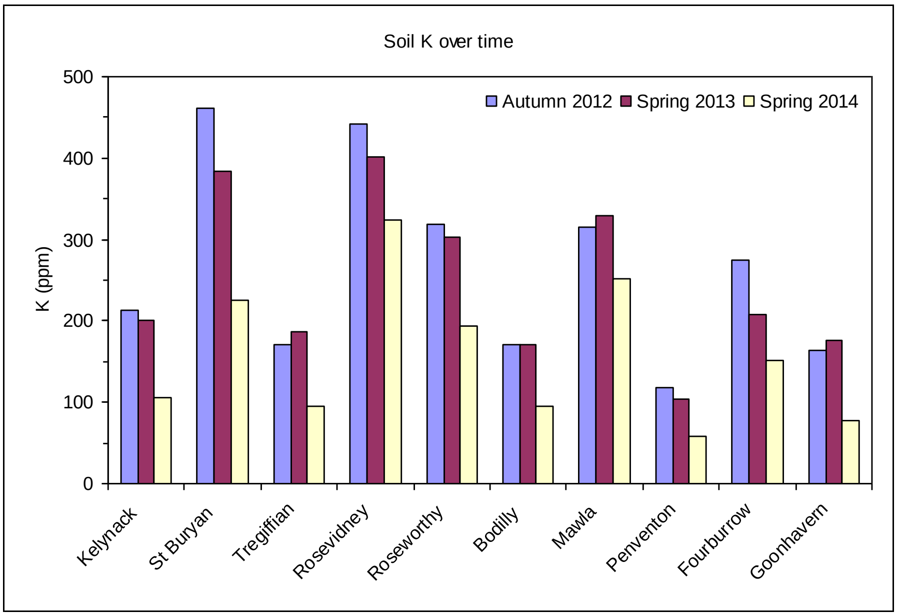


Figure 12. Soil K concentrations at the ten sites autumn 2012 to spring 2014.

In autumn 2012 soil K levels ranged from 118ppm (index 1) at Penventon to around 450ppm (index 4) at St Buryan and Rosevidney (Figure 12). K concentrations fell steadily over the next two years, probably enhanced by the wet winters, to between 59ppm (index 0) at Penventon and 324ppm (index 3) at Rosevidney. Application of potash fertiliser would be indicated at sites with a K index of 0.

Magnesium (Mg)

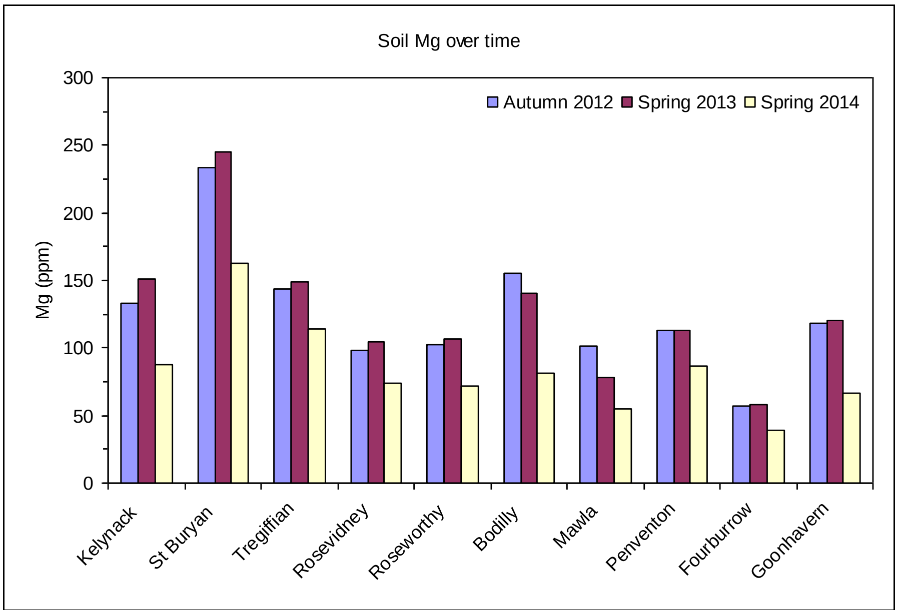


Figure 13. Soil Mg concentrations at the ten sites autumn 2012 to spring 2014.

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 13). Mg concentrations changed little over the next year but fell between spring 2013 and 2014 to between 39ppm (index 1) at Fourburrow and 163ppm (index 3) at St Buryan. At that point only Fourburrow would need Mg fertiliser.

Top-soil organic matter

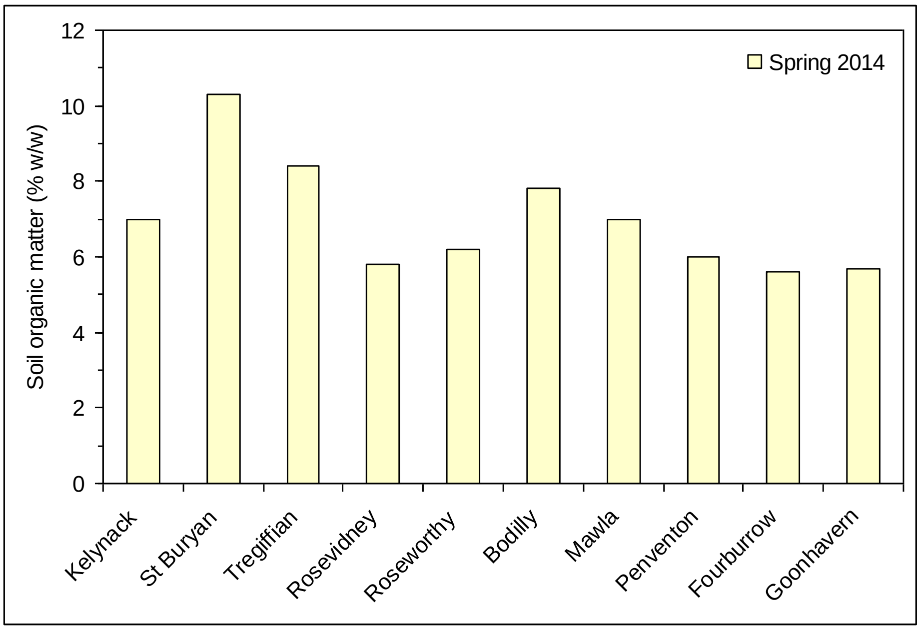


Figure 14. Percentage top-soil organic matter at the ten sites, spring 2014

Top-soil organic matter was also recorded in 2014 (Figure 14). The percentage of top-soil organic matter varied relatively little from site to site, from 6% at Rosevidney, Roseworthy, Penventon, Fourburrow and Goonhavern, to 10% at St Buryan.

Aluminium (Al)

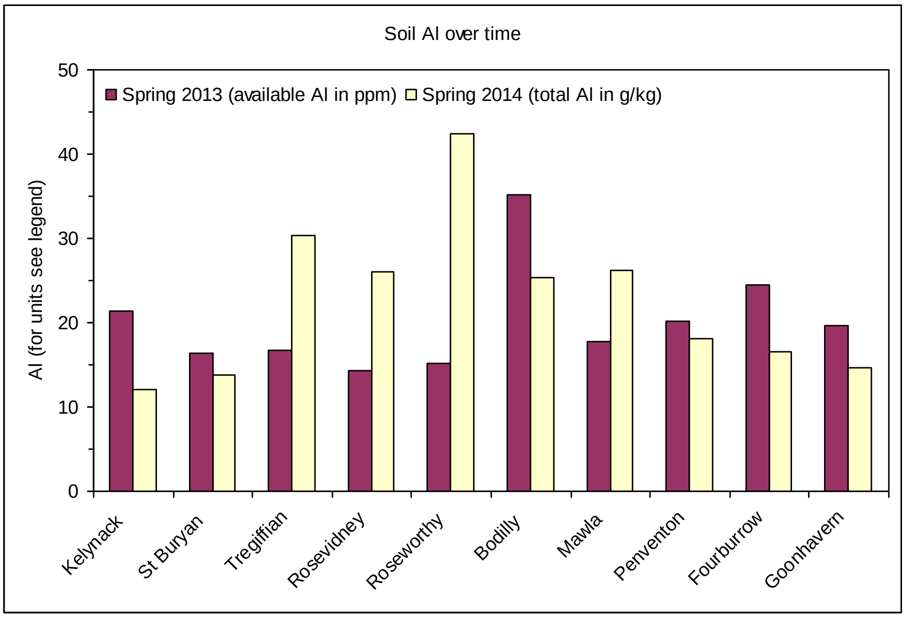


Figure 15. Soil Al concentrations at the ten sites spring 2013 and 2014. Note the difference in units between the years (see legend and text).

A change in analytical methods for soil Al means that the results were given as *available Al* in 2013 but *total Al* in 2014. In 2013 Bodilly had the highest concentration of available Al, 35ppm, with 12-25ppm at the remaining sites (Figure 15). In 2014 Tregiffian, Rosevidney, Roseworthy, Bodilly and Mawla showed higher concentrations of total Al, 25-42g/kg (25,000-42,000ppm) and the remaining sites lower concentrations of 12-18g/kg (12,000-18,000ppm).

Boron (B)

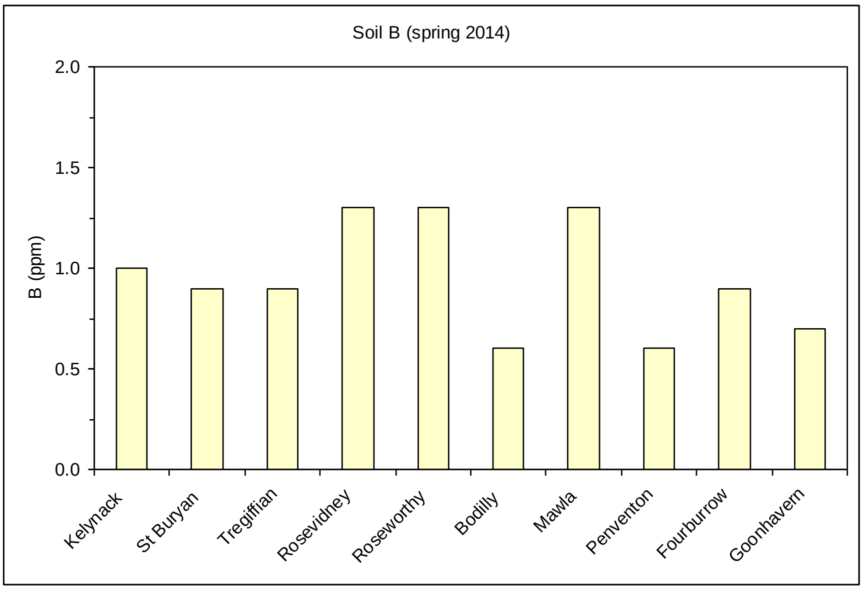


Figure 16. Soil B concentrations at the ten sites spring 2014.

Soil B concentrations (measured only in 2014) were relatively consistent, 1.3ppm at Rosevidney, Roseworthy and Mawla and 0.6 to 0.9ppm at the remaining sites (Figure 16). A boron deficiency would not be expected unless the level in soil was <0.1ppm.

Calcium (Ca)

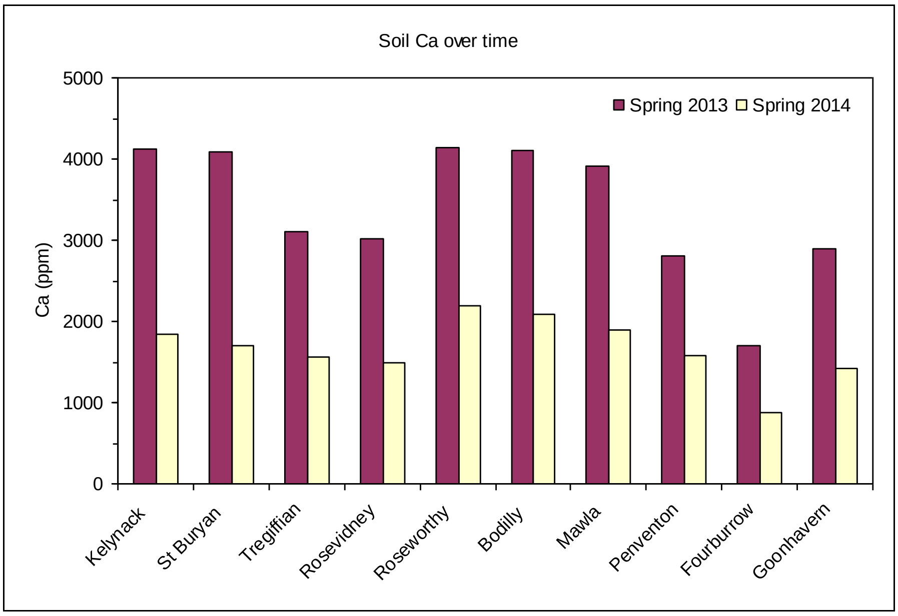


Figure 17. Soil Ca concentrations at the ten sites spring 2013 and 2014.

Levels of Ca in soil fell by about a half between spring 2013 and 2014, perhaps due to the wet weather (Figure 17). 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). By spring 2014 Ca levels varied from 880ppm at Fourburrow to 2189ppm at Roseworthy.

Copper (Cu)

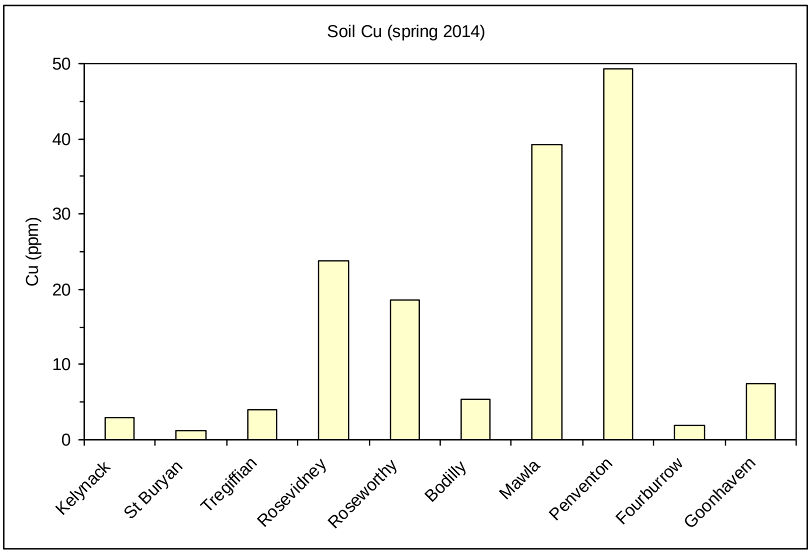


Figure 18. Soil Cu concentrations at the ten sites in spring 2014.

Soil Cu concentrations (measured only in 2014) showed marked differences between the sites (Figure 18). Mawla and Penventon exhibited high levels of Cu (39 or 49ppm) and Rosevidney and Roseworthy also relatively high levels (19 to 24ppm). At the other sites levels varied from 7.4 at Goonhavern to 1.3ppm at St Buryan. On lighter soils deficiencies are possible with Cu levels below 1.6ppm.

Iron (Fe)

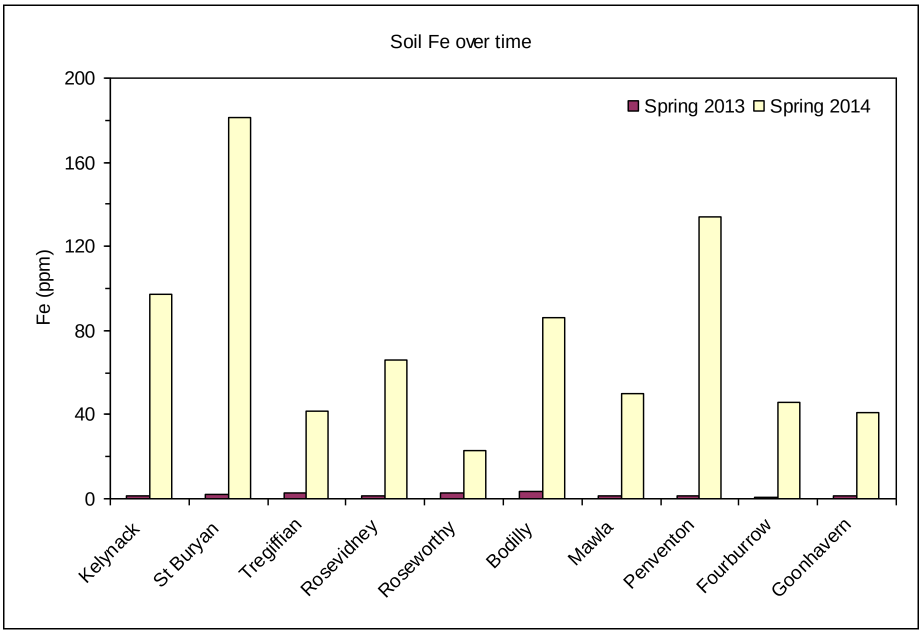


Figure 19. Soil Fe concentrations at the ten sites spring 2013 and 2014 (note that different test methods were used in 2013 and 2014).

As with Al, the analysis of soil Fe was also affected by changes in analytical methods, and where this occurs comparisons can only be made between sites and not between years. In 2014 Fe concentrations varied from 23ppm at Roseworthy to 181ppm at St Buryan, much higher than the 2013 measurements which varied from 1.0ppm at Fourburrow to 3.8ppm at Bodilly (Figure 19). Testing soils for plant-available Fe can be problematic.

Manganese (Mn)

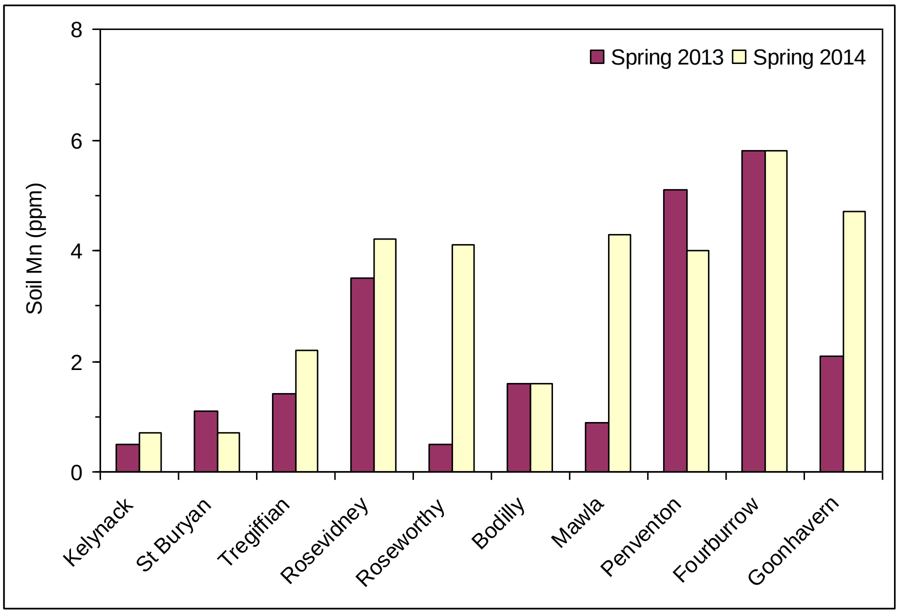


Figure 20. Soil Mn concentrations at the ten sites, spring 2013 and 2014.

Mn concentrations in soils varied considerably between sites, and in a minority of cases – Roseworthy, Mawla and Goonhavern - increased markedly between spring 2013 and spring 2014 (Figure 20). In the 2013 analyses Mn levels at Kelynack, St Buryan, Tregiffian, Roseworthy, Bodilly, Mawla and Goonhavern were relatively low (below about 2ppm) and those at the remaining sites relatively high (3.5-5.8ppm). From the 2014 analyses only Kelynack, St Buryan, Tregiffian and Bodilly remained in this low group, while the levels in the remaining sites were again between 4 and 6ppm. Soil tests for plant-available Mn can be problematic and are influenced by pH, soil texture and organic matter content.

Molybdenum (Mo)

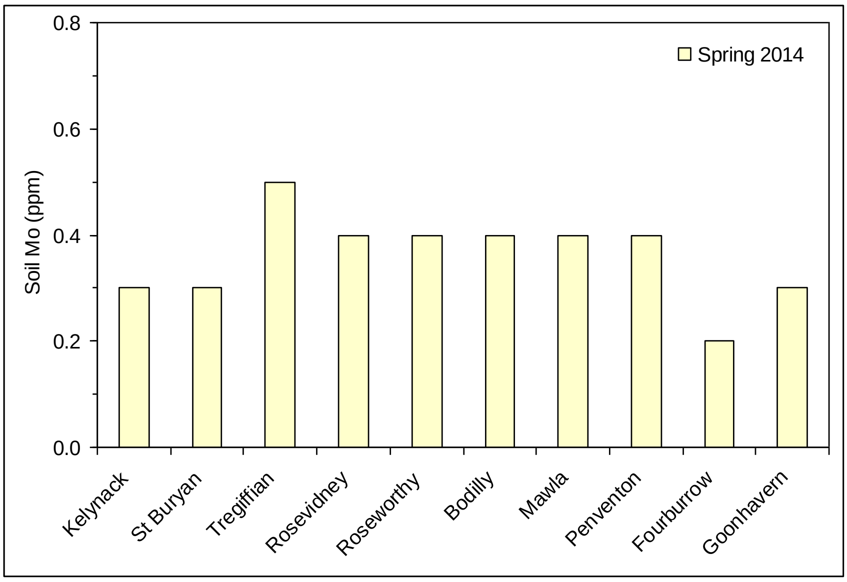


Figure 21. Soil Mo concentrations at the ten sites, spring 2014.

Mo levels in soil (measured in 2014 only) were consistent, between 0.2 and 0.5ppm (Figure 21). Soil Mo concentrations are controlled by pH.

Sodium (Na)

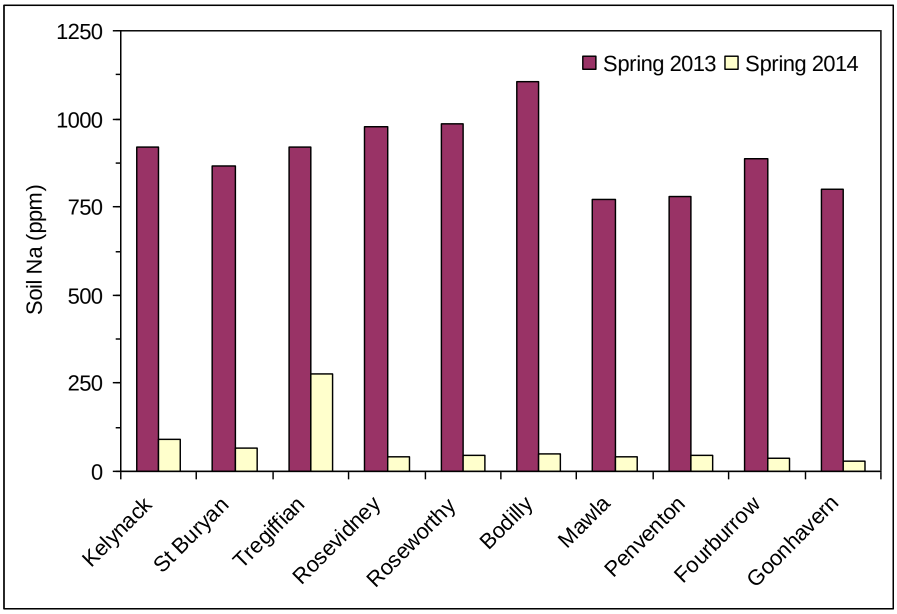


Figure 22. Soil Na concentrations at the ten sites, spring 2013 and 2014.

Soil Na levels, like those for Al and Fe, were also affected by changes in analytical methods between 2013 and 2014, so comparisons can only be made between sites and not between years. The two years' concentrations differed by an order of magnitude (Figure 22). In 2013 concentrations ranged between about 800 and 1,100ppm, with values for Mawla, Penventon, Fourburrow and Goonhavern somewhat lower than the rest. In the 2014 analysis concentrations varied between about 30 and 90ppm, except at Tregiffian where the level was 278ppm.

Sulphate (SO_4)

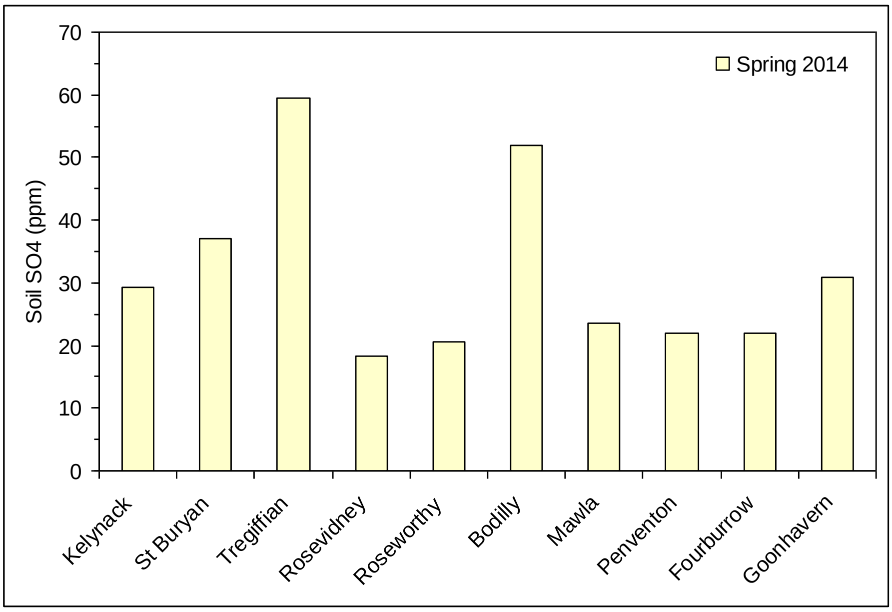


Figure 23. Soil SO_4 concentrations at the ten sites, spring 2014.

Soil sulphate concentrations (measured only in 2014) were relatively high at Rosevidney, Roseworthy, Mawla, Penventon and Fourburrow (18-24ppm), higher at Tregiffian and Bodilly (52 and 60ppm) and intermediate at the remaining sites (Figure 23). Low sulphate levels are likely only on light soils following very wet winters.

Zinc (Zn)

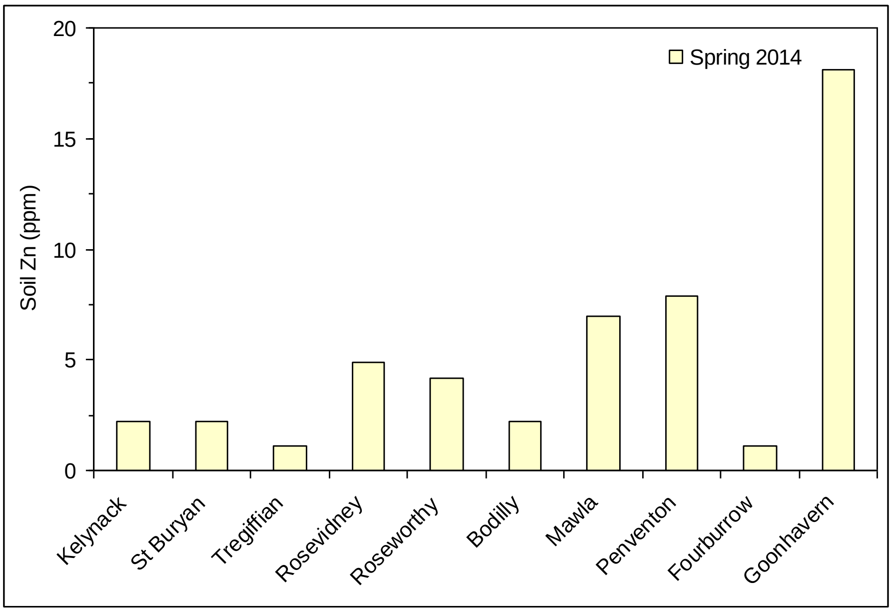


Figure 24. Soil Zn concentrations at the ten sites, spring 2014.

Soil zinc concentrations (measured only in 2014) were high at Goonhavern (18ppm), intermediate at Rosevidney, Roseworthy, Mawla and Penventon (4-8ppm), and low (<2ppm) at the remaining sites (Figure 24). As in the case of sulphate, low Zn levels are likely only on light soils following very wet winters.

Leaf analysis

The leaf analysis results from samples taken in April 2014 are given in Table 11 (major nutrients) and Table 12 (trace elements) and are also shown graphically below. For several of the elements the sites appeared to fall into three distinct groups, with high, intermediate and low levels of nutrients.

Table 11. Leaf major nutrient analysis in April 2014 for the ten sites.

Site reference and name	Nutrient concentration			
	N (% w/w)	P (ppm)	K (ppm)	Mg (ppm)
A Kelynack	3.02	3,962	24,400	1,954
B St Buryan	3.15	3,613	29,800	1,811
C Tregiffian	3.81	3,497	19,300	2,235
D Rosevidney	2.85	4,100	32,000	1,390
E Roseworthy	3.14	4,045	25,100	1,302
F Bodilly	3.18	4,189	19,700	1,517
G Mawla	3.23	3,250	26,900	1,368
H Penventon	2.67	3,714	19,400	1,583
I Fourburrow	3.16	3,226	27,300	1,424
J Goonhavern	3.37	3,094	24,200	1,364

Table 12. Leaf micronutrient analysis in April 2014 for the ten sites.

Site reference and name	Nutrient concentration (ppm)									
	Al	Ca	Fe	Mn	Na	Cu	Zn	Mo	S	B
A Kelynack	47	12,400	92	14.7	2,668	6.8	35.6	3.51	1,967	19.3
B St Buryan	47	9,000	122	14.7	1,252	7.6	47.0	1.42	2,209	20.1
C Tregiffian	99	14,600	204	56.4	2,281	8.1	29.2	0.43	2,352	23.4
D Rosevidney	101	9,300	206	38.1	605	8.4	44.3	2.15	2,041	20.8
E Roseworthy	92	10,300	198	50.5	856	7.9	29.0	3.99	2,031	19.3
F Bodilly	52	10,700	99	22.7	343	7.7	37.9	3.62	1,962	17.8
G Mawla	112	9,800	216	45.7	723	8.3	38.4	2.23	2,078	22.5
H Penventon	24	10,600	64	45.8	215	6.7	85.1	1.40	1,732	27.0
I Fourburrow	77	9,400	173	54.0	653	6.9	29.3	0.13	1,999	20.0
J Goonhavern	39	8,800	97	27.0	167	7.8	73.0	0.70	2,094	16.4

Nitrogen (leaf)

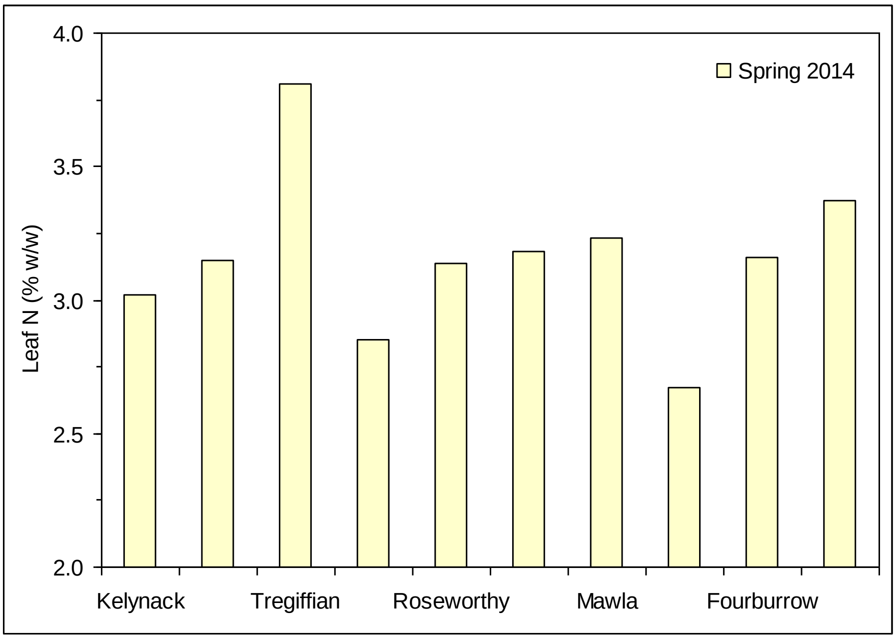


Figure 25. Leaf N concentrations at the ten sites, spring 2014.

Leaf N content was highest at Tregiffian (3.8% w/w), relatively low at Penventon and Rosevidney (2.7 and 2.8%) and intermediate at the remaining sites (3.0-3.4%) (Figure 25). N content can be affected by the size of the plant, but in these cases crop vigour was similar at all sites.

Phosphorus (leaf)

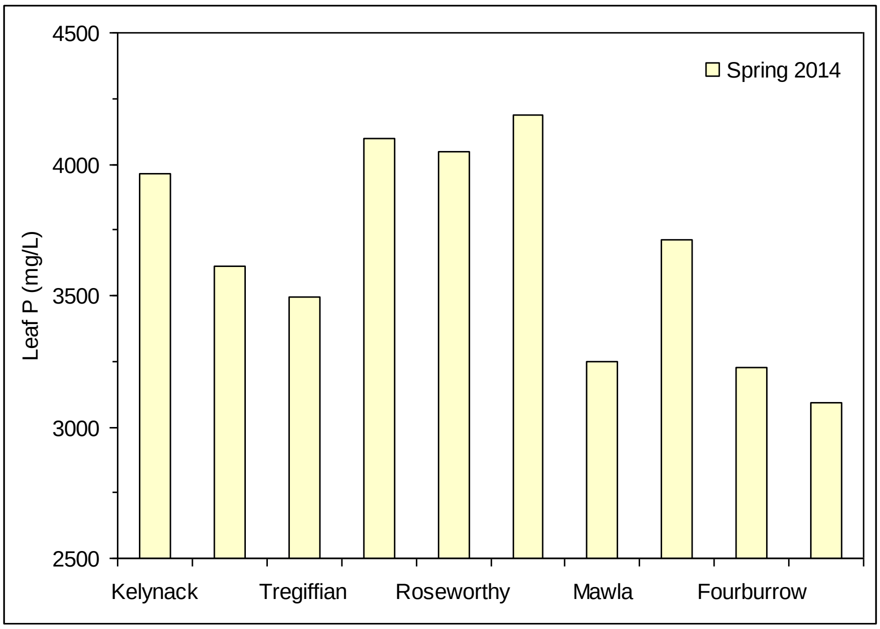


Figure 26. Leaf P concentrations at the ten sites, spring 2014.

Leaf P was high at Kelynack, Rosevidney, Roseworthy and Bodilly (around 4.0-4.2ppm) and relatively low at Mawla, Fourburrow and Goonhavern (3.1-3.2ppm) (Figure 26).

Concentrations were intermediate at the remaining sites.

Potassium (leaf)

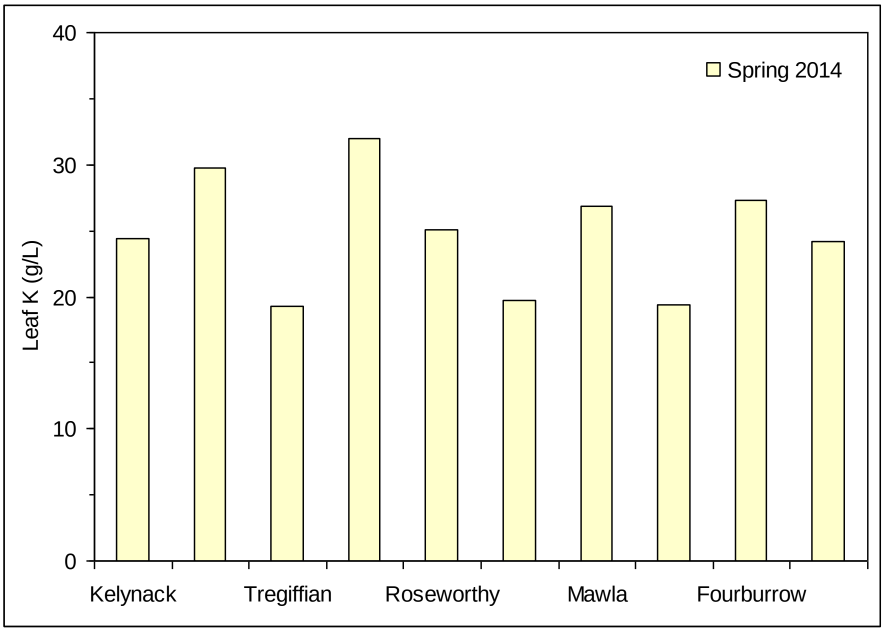


Figure 27. Leaf K concentrations at the ten sites, spring 2014.

Leaf K was present in relatively high levels at St Buryan and Rosevidney (29,800-32,000ppm), at low levels at Tregiffian, Bodilly and Penventon (19,300-19,700ppm), and intermediate at the other sites (Figure 27).

Magnesium (leaf)

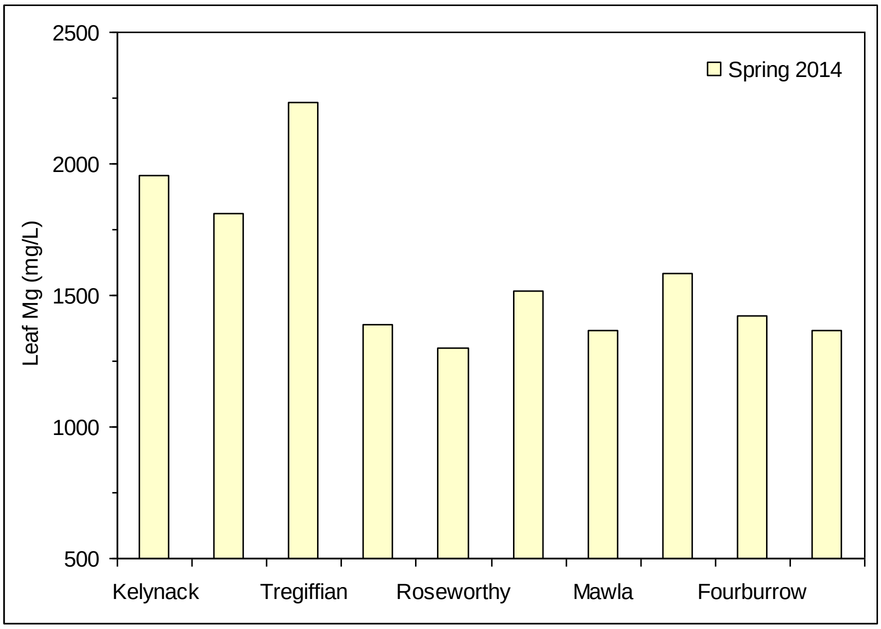


Figure 28. Leaf Mg concentrations at the ten sites, spring 2014.

Leaf Mg concentrations were higher at the three western-most sites, Tregiffian, Kelynack and St Buryan (1,811-2,235ppm) and lower elsewhere (1,302-1,583ppm; Figure 28).

Aluminium (leaf)

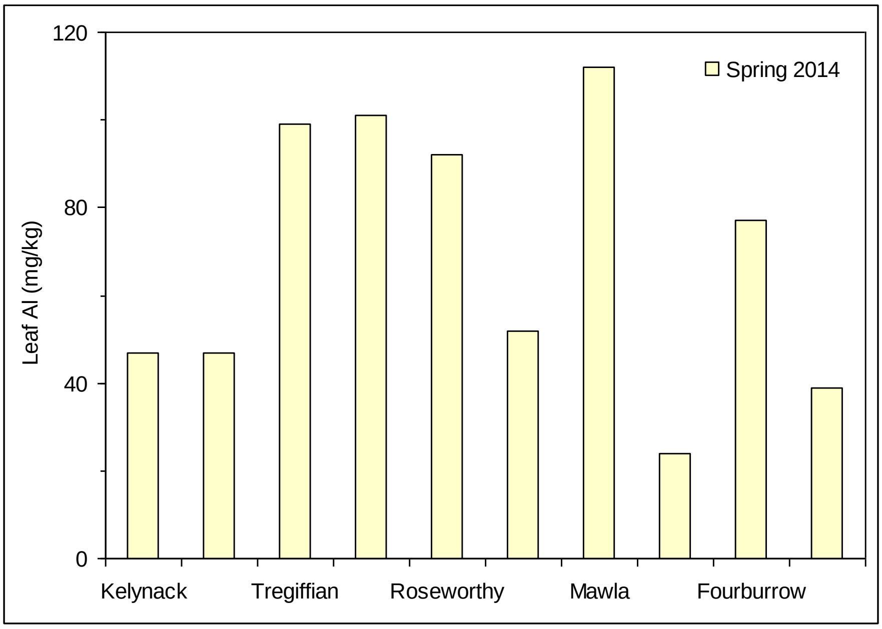


Figure 29. Leaf Al concentrations at the ten sites, spring 2014.

Leaf Al levels ranged quite widely, from 24ppm at Penventon to 112ppm at Mawla (Figure 29). Kelynack, St Buryan, Bodilly, Penventon and Goonhavern formed a low-level group (24-52ppm) and Tregiffian, Rosevidney, Roseworthy and Mawla a high-level group (92-112ppm). Fourburrow samples showed an intermediate concentration. More acidic soils would normally be expected to show higher Al concentrations, though here the range of pH values varied only between 5.6 and 7.6, and the most acidic sites (Fourburrow, Penventon and St Buryan (with pH of 5.6, 6.0 and 6.2, respectively) had relatively *low* Al levels.

Calcium (leaf)

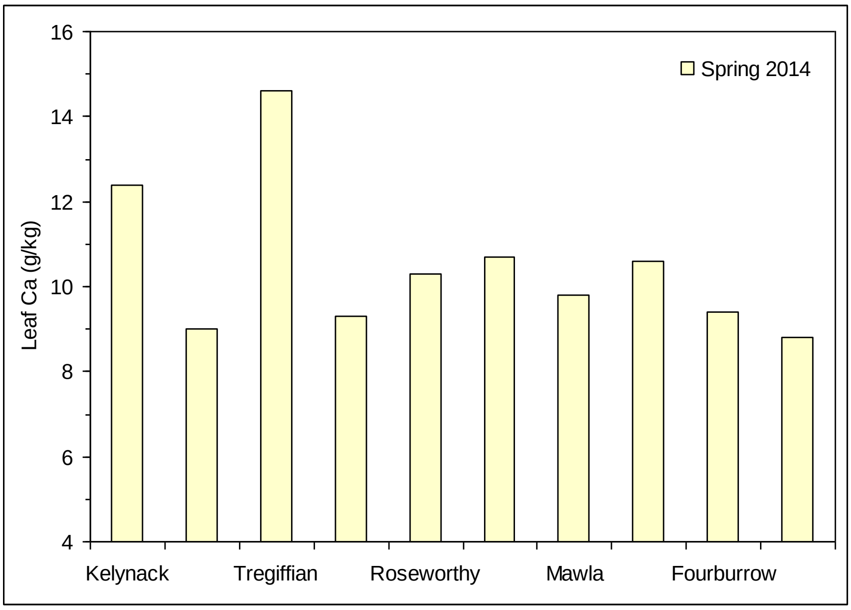


Figure 30. Leaf Ca concentrations at the ten sites, spring 2014.

Ca in leaves was found at higher concentrations at two sites, Tregiffian and Kelynack, both <1km to the sea (14,600 and 12,400ppm, respectively; Figure 30). At all other sites leaf Ca concentrations were lower, varying between 8,800 and 10,700ppm.

Iron (leaf)

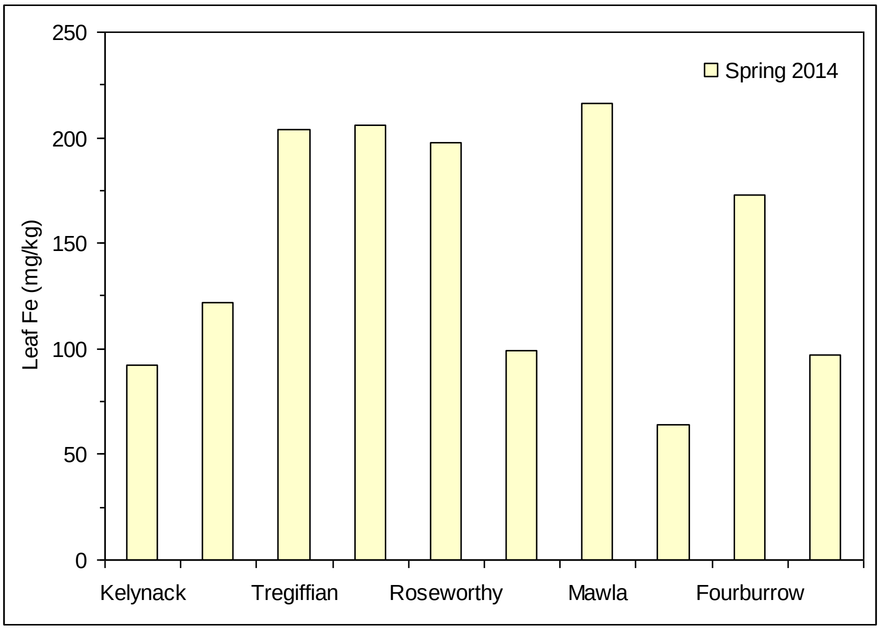


Figure 31. Leaf Fe concentrations at the ten sites, spring 2014.

Leaf Fe levels fell into two groups (Figure 31). Levels were high (173-216mg/kg) at Tregiffian, Rosevidney, Roseworthy, Mawla and Fourburrow, and low (64-122mg/kg) at the remaining sites.

Manganese (leaf)

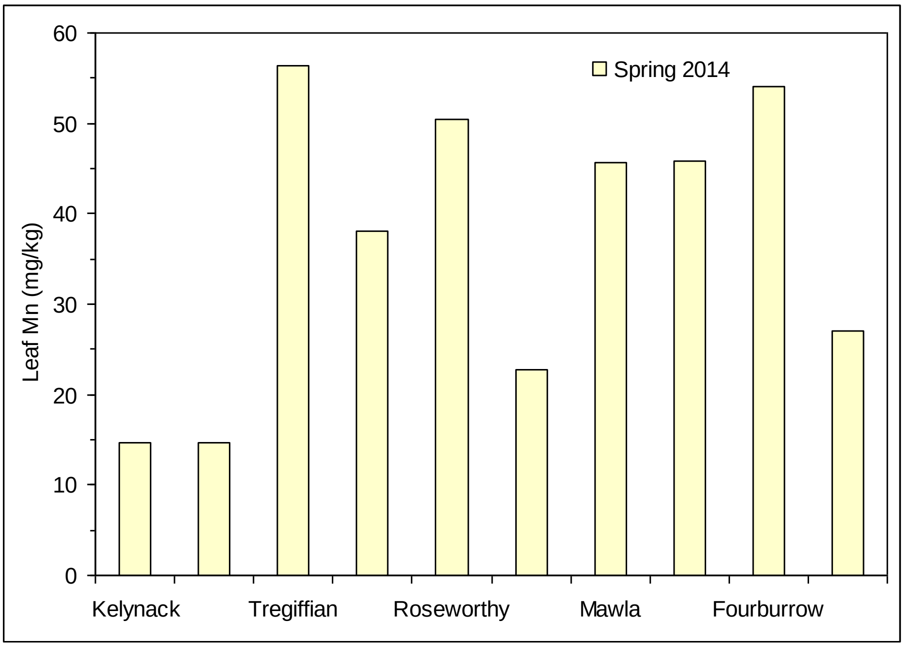


Figure 32. Leaf Mn concentrations at the ten sites, spring 2014.

Leaf Mn concentrations were variable (Figure 32). At Tregiffian, Roseworthy, Mawla, Penventon and Fourburrow levels were relatively high at 46-56ppm, while low values occurred at Kelynack and St Buryan (both 15ppm); concentrations were intermediate at the remaining sites. Leaf Mn concentrations below 20ppm could be considered deficient, so the crops at Kelynack and St Buryan may be at risk.

Sodium (leaf)

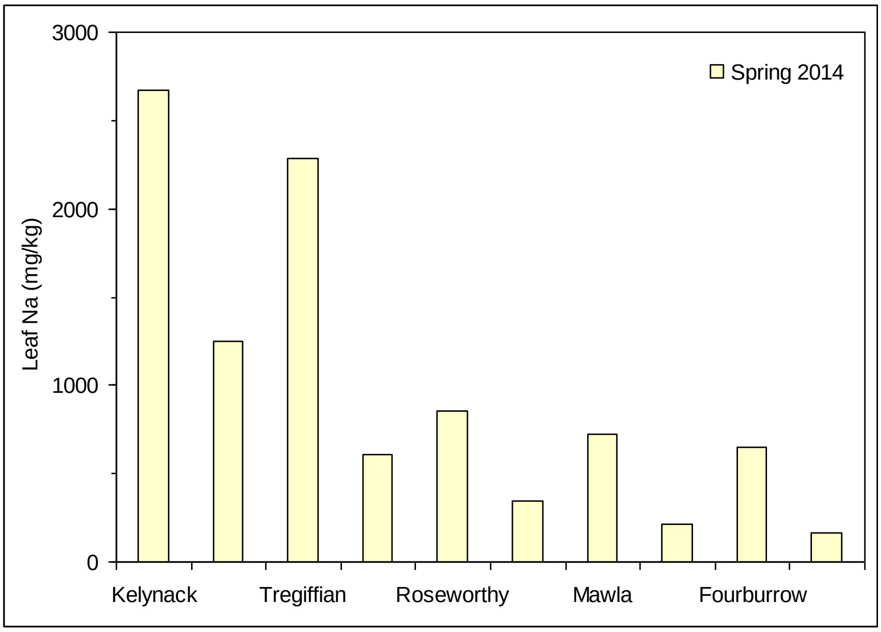


Figure 33. Leaf Na concentrations at the ten sites, spring 2014.

Leaf Na was found at high concentrations at Kelynack and Tregiffian, both close to the sea

(2,668 and 2,281ppm respectively; Figure 33). At the nearby St Buryan, Na concentrations were intermediate, and at the remaining sites they were lower (167-856ppm).

Copper (leaf)

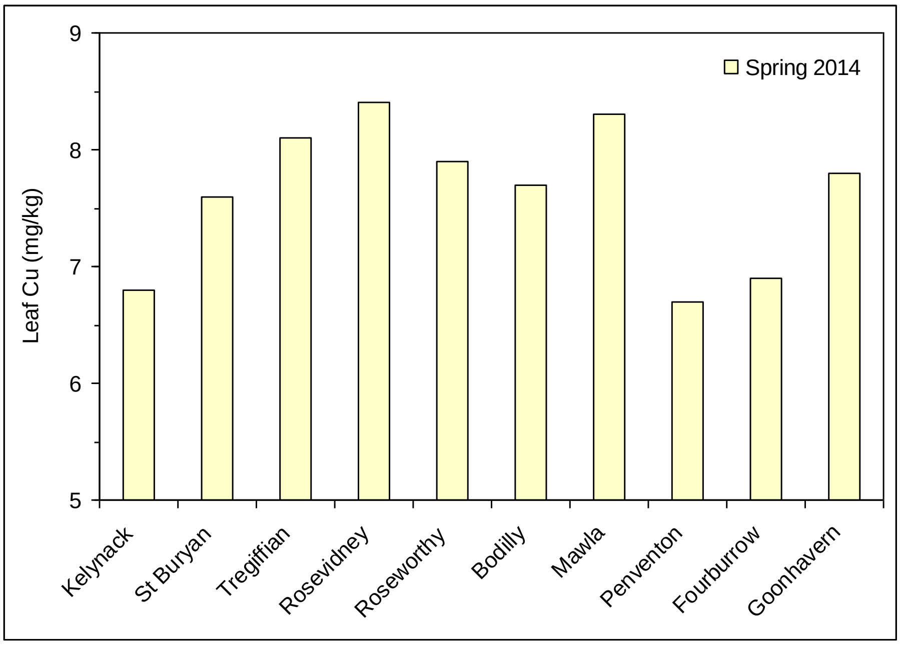


Figure 34. Leaf Cu concentrations at the ten sites, spring 2014.

Cu concentrations in leaves varied from a low of 6.7ppm at Penventon to highs of 8.3-8.4ppm at Rosevidney and Mawla (Figure 34). Leaf Cu concentrations below 3ppm could be considered deficient, so no deficiencies would be expected at the trial sites.

Zinc (leaf)

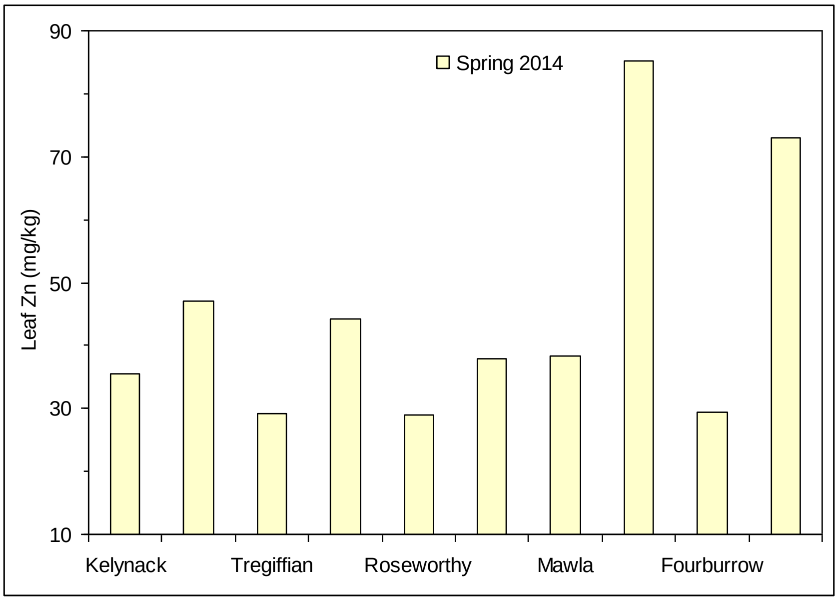


Figure 35. Leaf Zn concentrations at the ten sites, spring 2014.

Leaf Zn levels were much higher at Penventon (85ppm) and Goonhavern (73ppm) than at the other sites, where concentrations ranged from 29 to 47ppm (Figure 35).

Molybdenum (leaf)

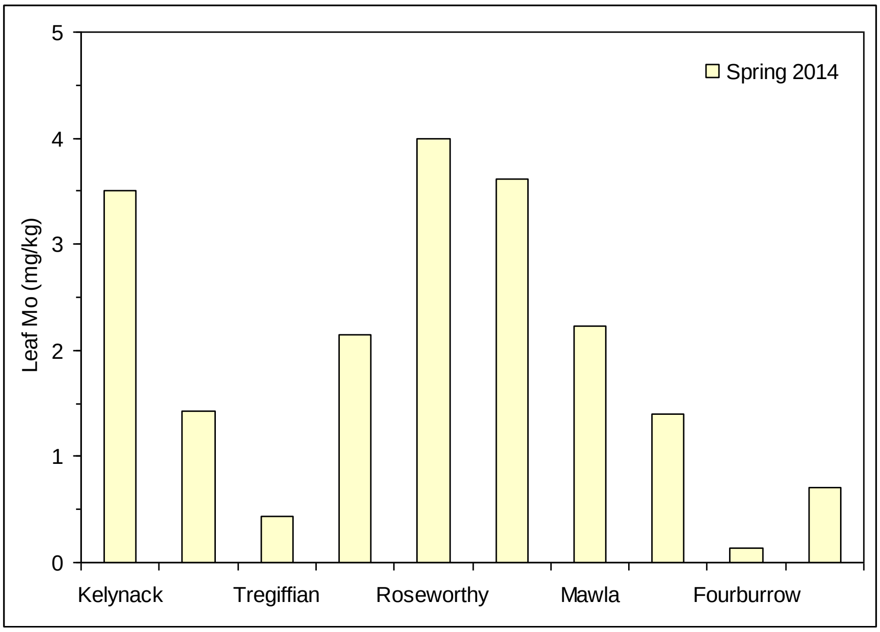


Figure 36. Leaf Mo concentrations at the ten sites, spring 2014.

Leaf Mo concentrations varied from 0.1ppm at Fourburrow to 4.0ppm at Roseworthy (Figure 36). Leaf Mo concentrations below 0.1ppm could result in deficiency, especially where the soil pH is <5.5, so the crop at Fourburrow – with a pH of 5.6 – could be at risk.

Sulphur (leaf)

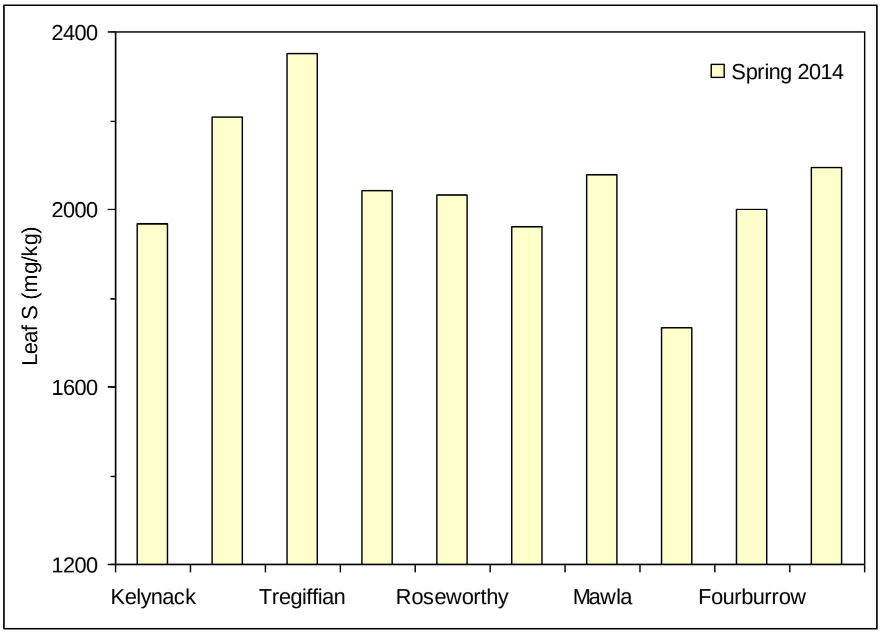


Figure 37. Leaf S concentrations at the ten sites, spring 2014.

Leaf S concentrations varied continuously across the sites, from a low of 1,732ppm at Penventon to a high of 2,352ppm at Tregiffian (Figure 37).

Boron (leaf)

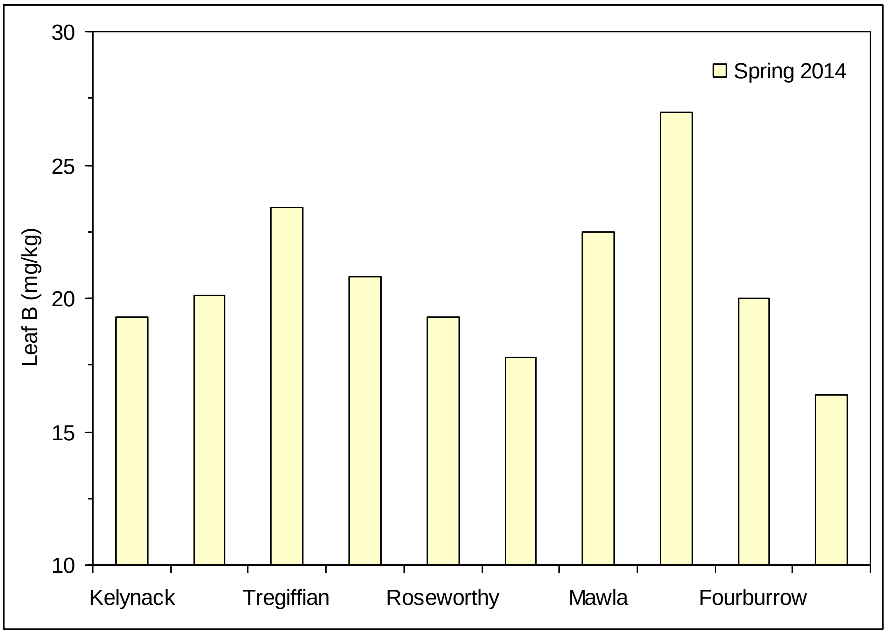


Figure 38. Leaf B concentrations at the ten sites, spring 2014.

Leaf B also varied continuously across the sites, from a low of 16ppm at Goonhavern to a high of 27ppm at Penventon (Figure 38). Leaf Bo concentrations <20ppm may cause deficiency if on a light soil with a high pH. With a soil pH of 7.4 the crop at Goonhavern may be marginally at risk.

Fungal and bacterial disease diagnostics

No evidence of bacterial infection was seen in any of the samples during microscopic observation. In some samples the presence of *Ramularia* sporophores and conidia was very obvious (despite the efforts to avoid plants with white mould symptoms), but no other fungi were noted. Isolations for bacteria from lesions with water-soaked margins resulted in either sterile plates or an inconsistent mix of different bacteria.

Microscopic observation of cleared and stained tissues did not provide any insights in the cause of the rust symptoms.

A *Stemphylium* species – with dark brown/black solitary muriform or multi-septate, slightly verruscose conidia born terminally on pale brown condiophores - was the most consistently isolated fungus from both surface-sterilised and non-surface-sterilised rust lesions, being isolated from samples of eight out of the ten sites. The two samples where *Stemphylium* was not isolated had either predominantly (Rosevidney) or exclusively 'streak' symptoms (Penventon) rather than rust spots. It appeared that the streak symptoms may have a different origin from the typical, more discrete rust spots. It did not appear that that the discrete rust spots develop into streaks. *Stemphylium* was also obvious during humid box incubation in five out of the ten samples. *Ramularia* and *Botrytis* spp. were also isolated from some samples. These results are summarised in

Table **13**.

Table 13. Summary of lesion appearance, fungi seen in rust lesions under damp incubation, and fungi isolated from rust lesions across the ten sites in April 2014. For comparison, rust incidence and severity scores (from Figure 47) are also shown.

Site	**Description of lesions found on stems**	**Fungi observed in damp incubation**	**Fungi isolated (number of cultures out of total)**	**Rust incidence and severity scores**
Kelynack	Brown lesions, some with water-soaked margin, also yellow streaks	Stemphylium	Stemphylium (8/10)	5, 2
St Buryan	Pale *Ramularia* lesions, dark rust spots	Fluffy white mycelium (probably *Ramularia*)	Stemphylium (2/12) Also Ramularia	5, 2
Tregiffian	Pale spots with obvious sporulation (*Ramularia*), brown spots and streaks, some water-soaked	Fluffy white mycelium (probably *Ramularia*)	Stemphylium (5/8)	5, 3
Rosevidney	Mostly streaks rather than discrete spots	Mixed fungi including *Stemphylium*	None	4, 2
Roseworthy	Pale areas and *Ramularia* lesions, some dark specks	Stemphylium	Stemphylium (3/8)	5, 2
Bodilly	Obvious *Ramularia* lesions, some water-soaking around rust spots	Botrytis	Stemphylium (2/12) Also Botrytis	5, 2
Mawla	Mostly brown spots, a few streaks	Botrytis, Stemphylium and others	Stemphylium (2/8)	5, 2
Penventon	All streaks rather than rust spots	None	None	4, 2
Fourburrow	Mostly pale *Ramularia* spots	Stemphylium	Stemphylium (3/8)	4, 2
Goonhavern	Very few small discrete spots	None	Stemphylium (1/8) Also Botrytis	5, 2

Crop and rust assessments

<u>2013: Pre-picking stage</u>

The first assessment was carried out on 7–8 February 2013, when plants at most sites were at a stem extension stage with many buds visible (GS 2.4) (Figure 39). Although plants at most sites had reached this stage, those at Fourburrow (one of the most easterly sites) and at Goonhavern, Mawla and Bodilly (the three late-planted sites) showed slightly later crop development, with more plants at GS 2.2 (shoots elongating, no buds visible) or 2.3 (shoots elongating, tips of buds visible). Earlier GS were matched by shorter shoot/leaf and stem lengths (also shown in Figure 39).

With one exception, no characteristic rust lesions were seen on either stems or leaves at this stage. The exception was Tregiffian, where, over the whole plot, just two small rust spots were seen on each of two stems (Figure 42). At Penventon inconspicuous depressions or pitting of the stems was noted, subsequent observations suggesting these may have been a widely occurring formative stage of rust lesions.

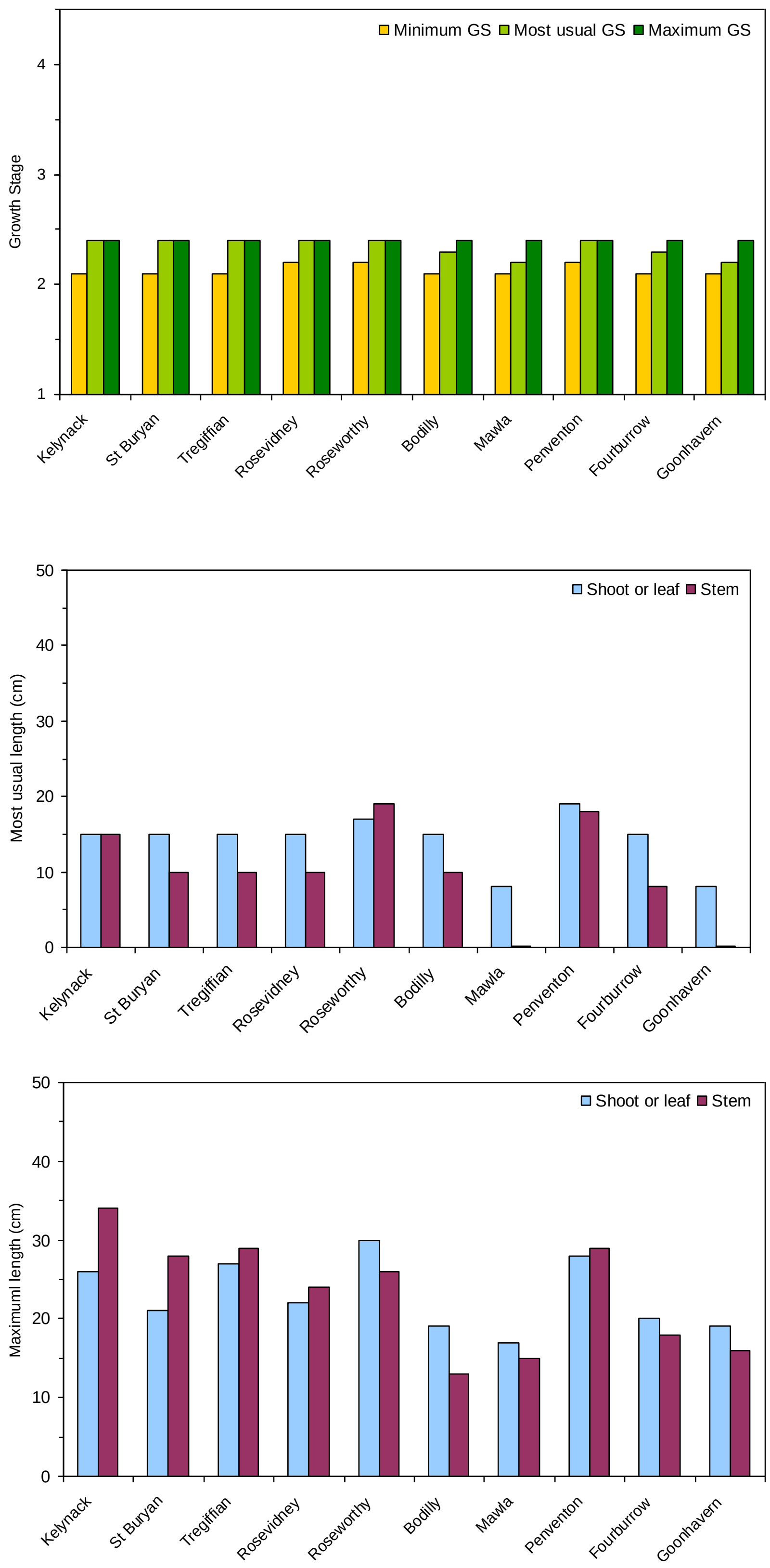


Figure 39. Crop development at ten sites assessed pre-picking in 2013: (top) minimum, most usual and maximum GS, (middle) most usual shoot/leaf and stem lengths and (bottom) maximum shoot/leaf and stem lengths.

2013: Picking stage

The second assessment was carried out over 27 February to 1 March 2013, when most plants were at a late picking stage (GS 3.3). At the four late sites (see above) development was delayed, usually with most plants at an ideal picking or slightly earlier stage (GS 3.1 or 3.2), though at Goonhavern the delay was greater with most buds still in the foliage (GS 2.4) and some still emerging (GS 2.1) (Figure 40). Measurements of shoot/leaf and stem lengths confirmed these findings (Figure 40).

Only infrequent, small and isolated rust lesions were found, and at only five of the sites. At Penventon and St Buryan a single stem with one rust lesion was seen at each site, and at Fourburrow one stem bore an elongated lesion and a second two small spots. At Roseworthy there were five stems with one to three lesions each, and at Tregiffian, where trace symptoms had been noted earlier, there were ten stems with either a single lesion or several spots near ground level (Figure 42). All five sites therefore scored 1 for both severity and incidence of rust, well below the minimum scores that would warn of a potentially commercially damaging level of rust.

In addition to the typical rust lesions, the plots were seen to contain numerous stems with the faint marks referenced under the pre-picking assessment at Penventon. Resembling mild lengthways pitting or blistering and pale or yellowing spots or longitudinal tracks, none had the characteristic appearance of rust lesions. Assessments at each site showed the lesions were always mild in severity, but between sites their incidence score varied between 2 and 5.

Leaves were also examined for rust. The foliage at Tregithian, Penventon, Bodilly, Roseworthy and St Buryan bore occasional brown, rusty streaking, usually near the leaf-tip.

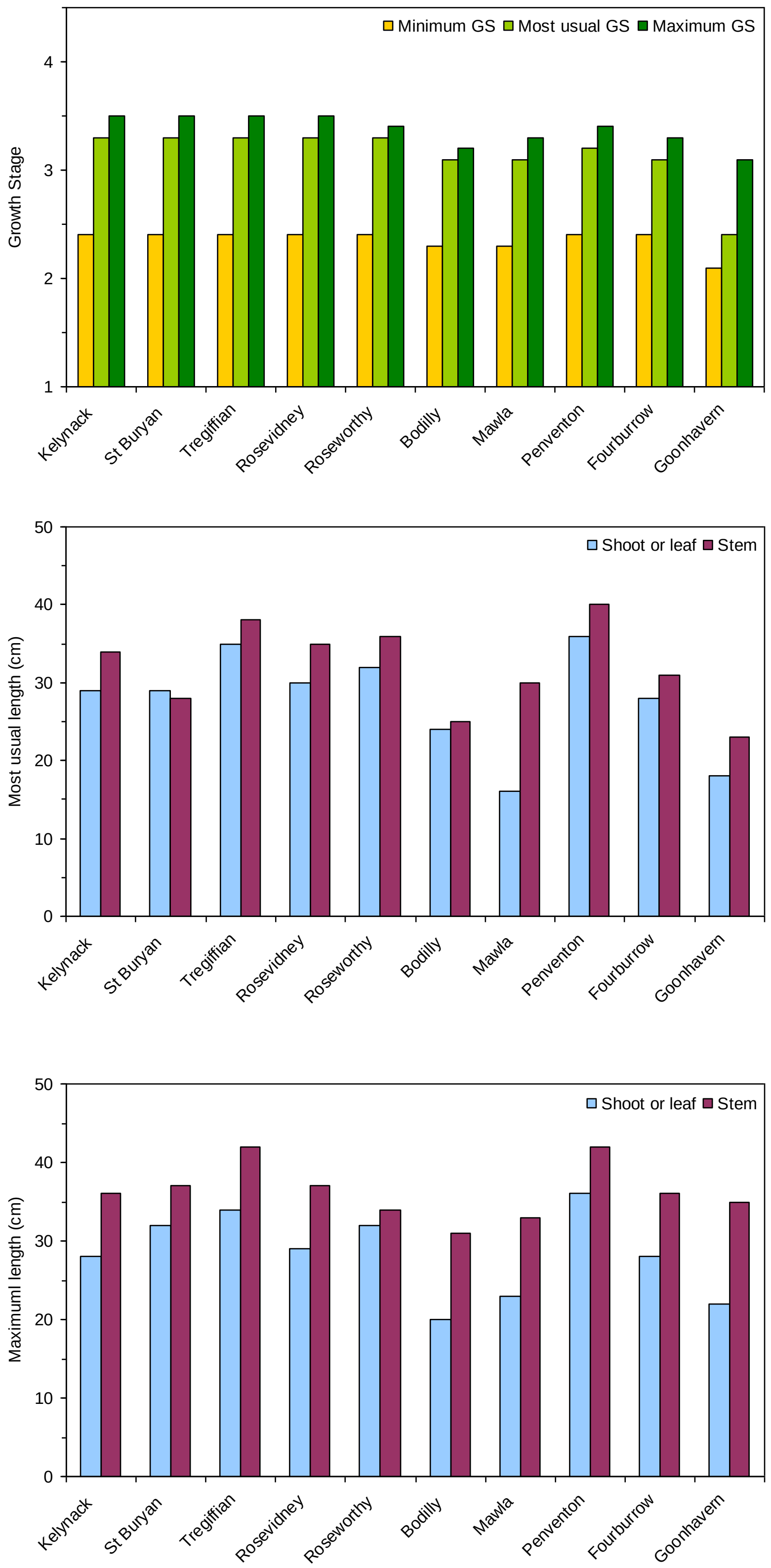


Figure 40. Crop development at ten sites assessed at picking in 2013: (top) minimum, most usual and maximum GS, (middle) most usual shoot/leaf and stem lengths, (bottom) maximum shoot/leaf and stem lengths.

2013: Post-picking stage

The third assessment was carried out over 26–28 March 2013. At the earliest site – Kelynack – most flowers were now fully senescent (GS 3.8), while at the latest – Goonhavern – there was wide variation between GS 3.1 (early picking stage) and GS 3.6 (flowers fully open) (Figure 41). The five more westerly crops were consistently at post-cropping, while the five more easterly ones were later, though this may have been partly due late planting at Goonhavern, Mawla and Bodilly.

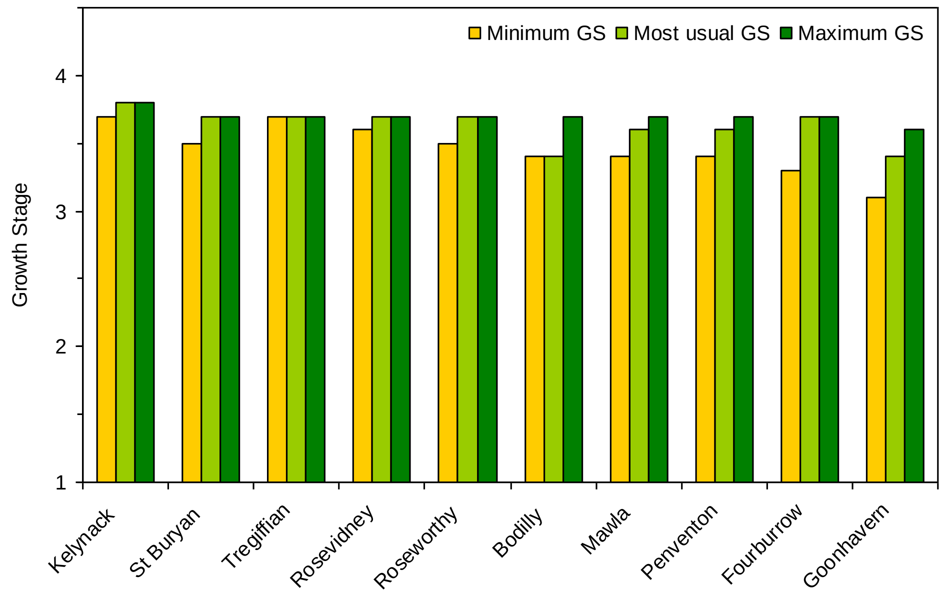


Figure 41. Crop development at ten sites assessed at post-picking stage, 2013. Minimum, most usual and maximum GS are shown.

Small but characteristic rust lesions were seen at all sites except Fourburrow, a site where its prior incidence had been very low. Its severity varied from one or two small spots (or occasionally streaks) per stem at Goonhavern, to individual small spots and groups of up to *ca* 15 small spots at Tregiffian, the earliest site to have evidenced rust and the only site to reach a severity score of 2. Rust incidence varied from <10stems/plot affected at Goonhavern (incidence score of 1) to >100 at St Buryan, Tregiffian, Rosevidney and Penventon (incidence scores of 4) (Figure 42). 'Early-stage lesions' were seen at all sites though no attempt was made to record their severity or incidence.

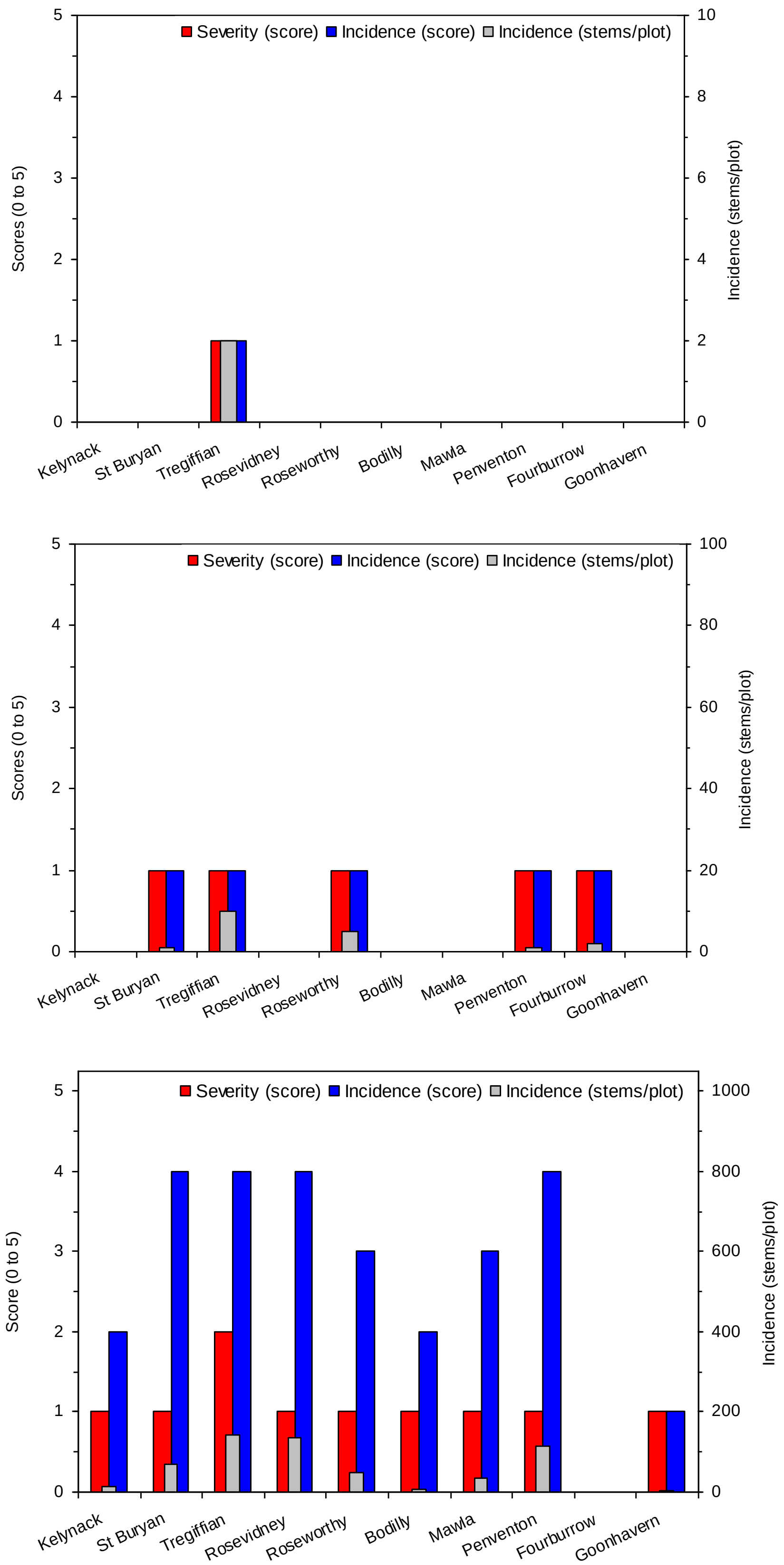


Figure 42. Severity and incidence scores for rust at ten sites assessed in 2013 at (top) pre-picking, (middle) picking and (bottom) post-picking stages. Incidence is shown as both a 0–5 score and as the number of stems per plot with rust.

Rust lesions were also seen on leaves at all sites. At most sites their severity was low (a single or a few small spots or streaks per leaf), as was incidence (a few up to about 10% of leaves affected) (Figure 43). However at Kelynack, St Buryan and Tregiffian severity was much greater, with more extensive markings (often streaks running down from the leaf tips) and greater incidence, with more than 50% of leaves affected. Rust-like lesions were occasionally seen on leaf sheaths and flower spathes. The relationship of foliar lesions to stem lesions is not known.

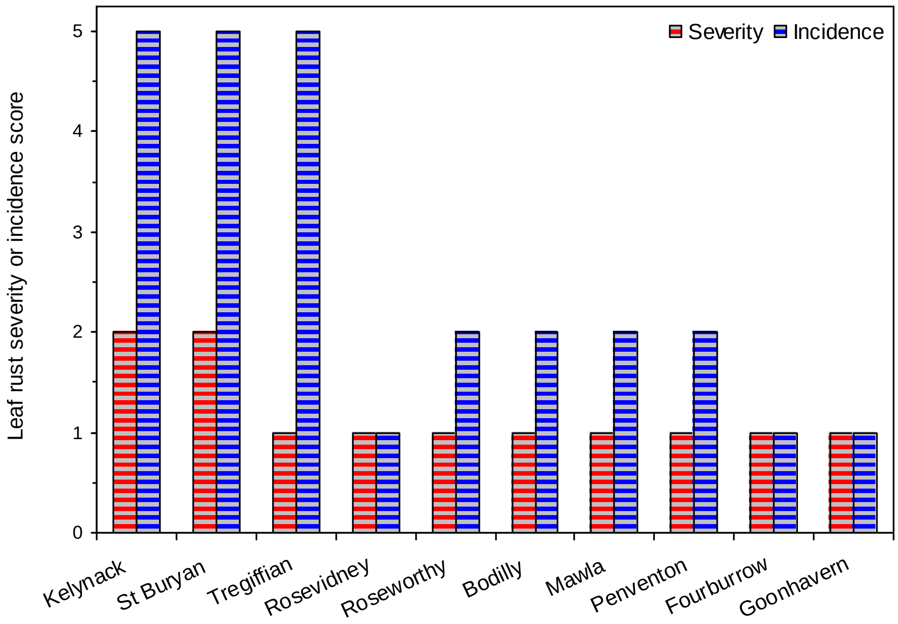


Figure 43. Severity and incidence scores for leaf lesions at ten sites in 2013, assessed at post-picking stage.

<u>2014: Pre-picking stage</u>

The first crop and rust assessment of 2014 was carried out on 12–14 February. As in the previous year, the plants at most sites were at an early stem extension stage with many buds visible (GS 2.4) (Figure 44). However, the relatively later development in 2013 at Fourburrow and the three late-planted sites was no longer apparent, and the same can be said of shoot/leaf and stem extension (Figure 44); this is typical of the regression to the population mean seen in successive years of daffodil crops as the effects of initial planting conditions lessen.

A low level of rust was found at the three most westerly sites (Kelynack, St Buryan and Tregiffian) and at Bodilly, with both severity and incidence scores of 1 (Figure 47) At Tregiffian, the only site where rust had been recorded pre-picking in 2013, the number of leaves affected was higher (6/plot) than at the other three sites (1–2/plot). Rust was not seen at the other six sites.

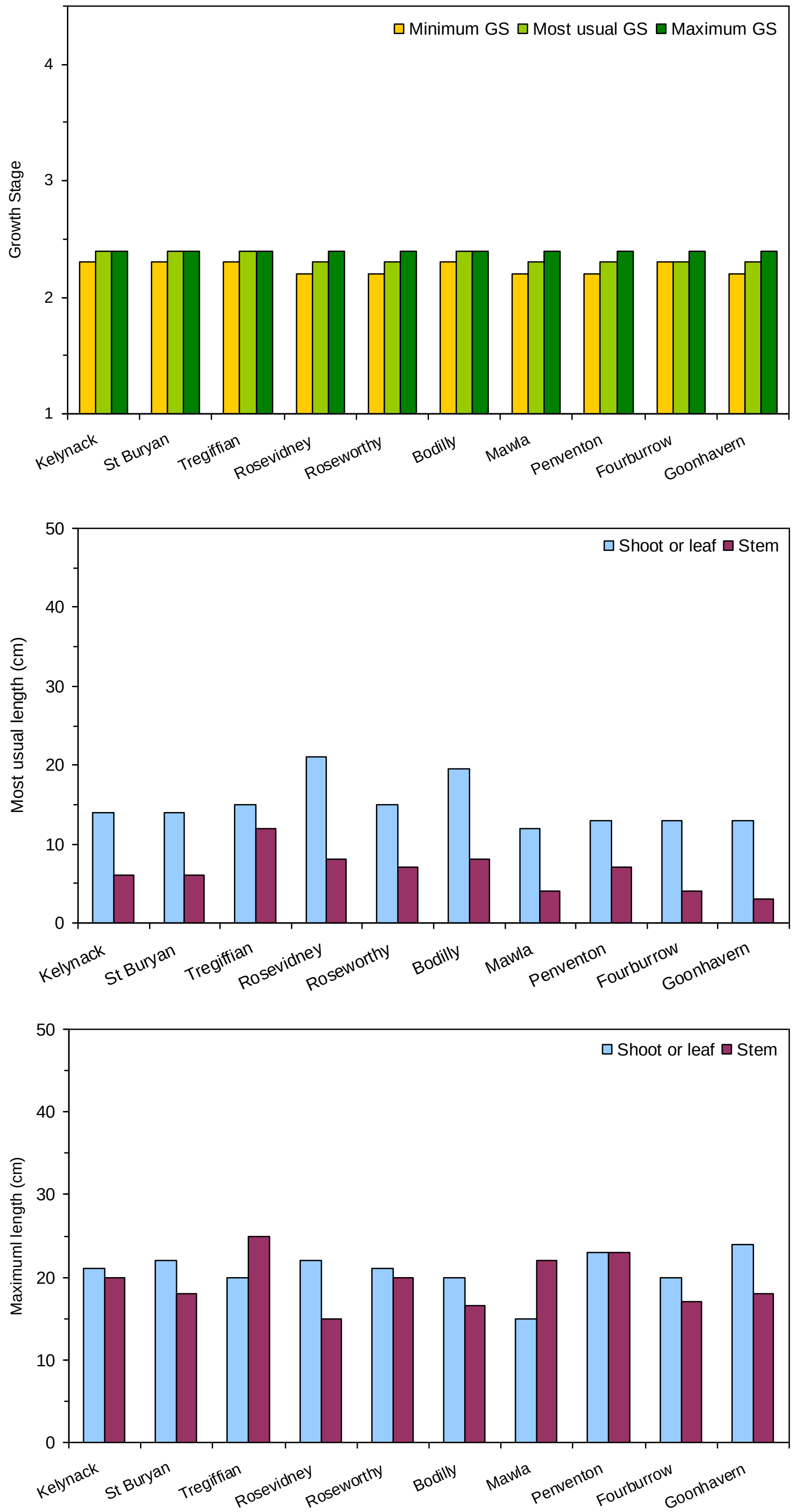


Figure 44. Crop development at ten sites assessed at pre-picking stage in 2014: (top) minimum, most usual and maximum GS, (middle) most usual shoot/leaf and stem lengths and (bottom) maximum shoot/leaf and stem lengths.

2014: Picking stage

The second assessment of 2014 was carried out over 4–6 March, close to picking stage. Most plants were again at a relatively late picking stage (GS 3.3), though a proportion of the crop was late, with GS 2.4, at Kelynack, St Buryan, Fourburrow and Goonhavern, the two most westerly and the two most easterly sites (Figure 45). Measurements of shoot/leaf and stem lengths confirmed these trends (Figure 45).

Rust levels had increased markedly since the pre-picking assessment, and were also substantially greater than in 2013 (Figure 47). Most sites had severity and incidence scores of 2, though severity was higher at Tregiffian and incidence higher at Fourburrow (both 3), and both scores were just 1 at Rosevidney, Mawla and Goonhavern. Measured as the number of stems affected by rust, incidence was highest at Fourburrow (>60/plot), relatively high at Kelynack, Tregiffian and Penventon (*ca* 40/plot), about 20/plot at St Buryan, Roseworthy and Bodilly and <10/plot at the remaining sites Rosevidney, Mawla and Goonhavern).

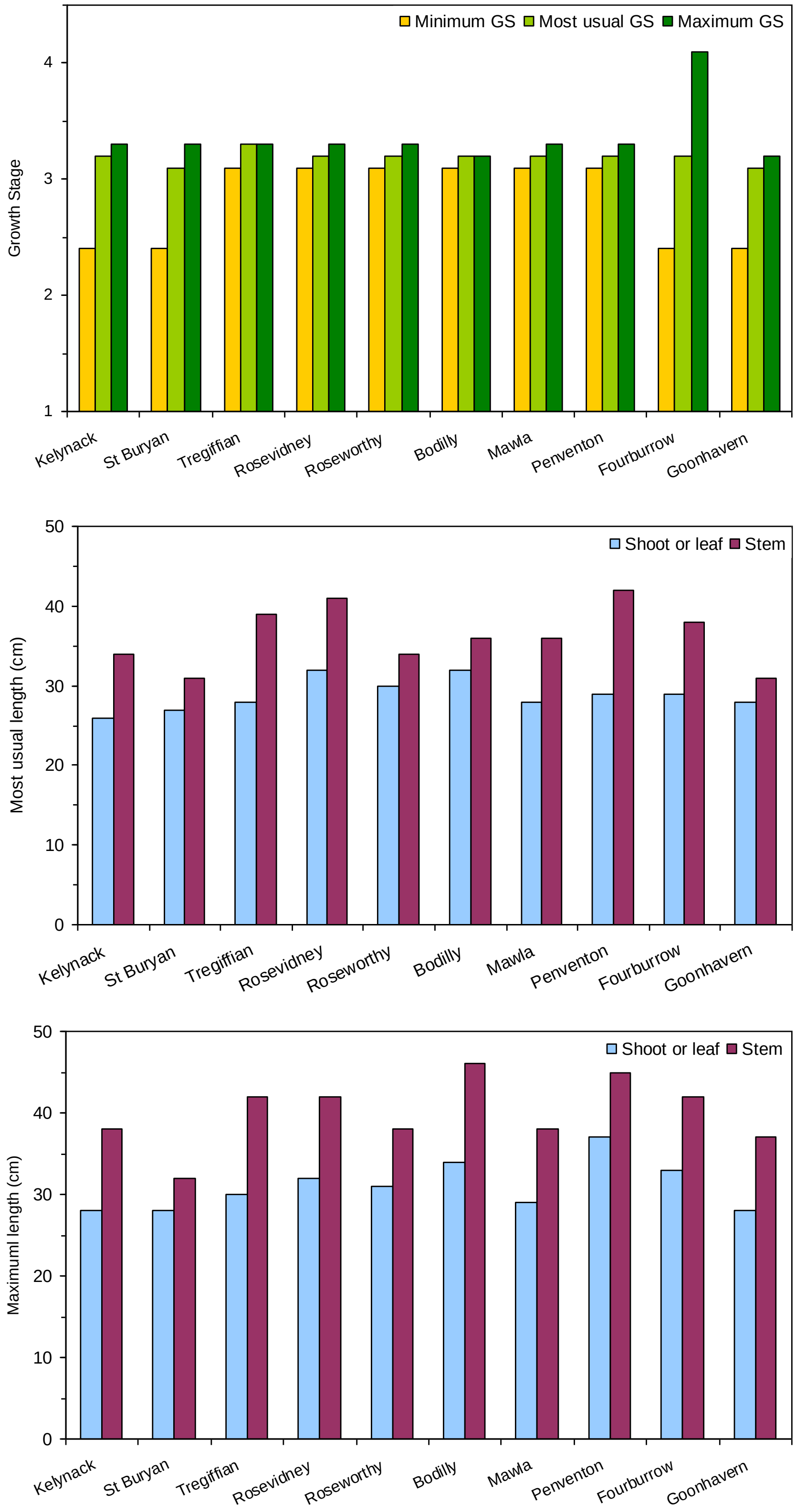


Figure 45. Crop development at ten sites assessed at picking stage in 2014: (top) minimum, most usual and maximum GS, (middle) most usual shoot/leaf and stem lengths and (bottom) maximum shoot/leaf and stem lengths.

2014: Post-picking stage

The third assessment of 2014 was carried out on 5–7 April. The developmental stage of the crop was now consistent across all sites, at GS 3.6 (flowers fully open) to 3.8 (flowers fully senescent) (Figure 46).

The incidence of rust had increased substantially at all sites (Figure 47). Severity, however, remained low and below what would constitute a commercial problem, with scores of 2 except at Tregiffian, where the occurrence of some stem cracking raised the severity score to 3, not a commercially sensitive level but one where a close eye would have to be kept on the crop for any further increase. At seven sites the majority and usually all of the stems were affected by rust (an incidence score of 5), with scores of 4 at Rosevidney, Penventon and Fourburrow where rust incidence affected around 200–400 stems/plot.

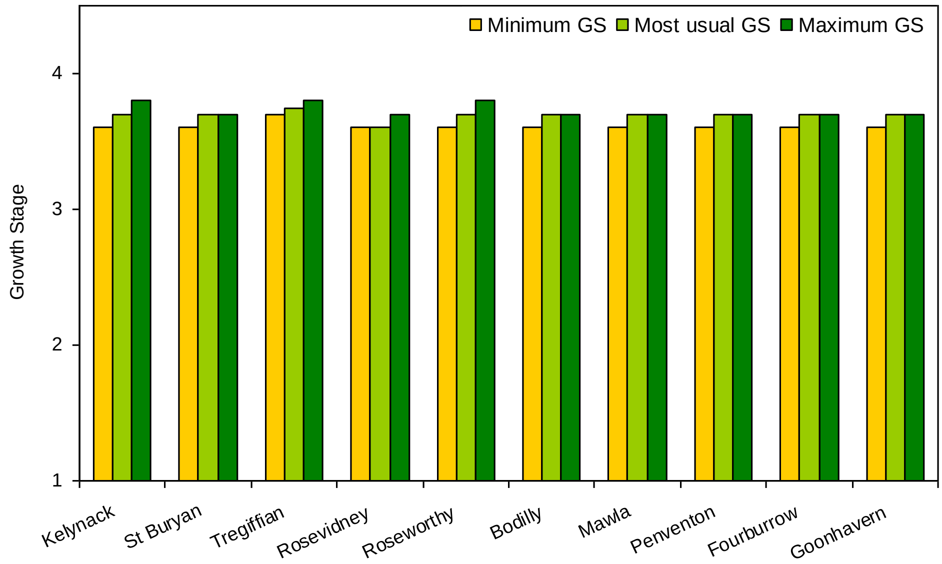


Figure 46. Crop development at ten sites assessed at post-picking stage in 2014, showing minimum, most usual and maximum GS.

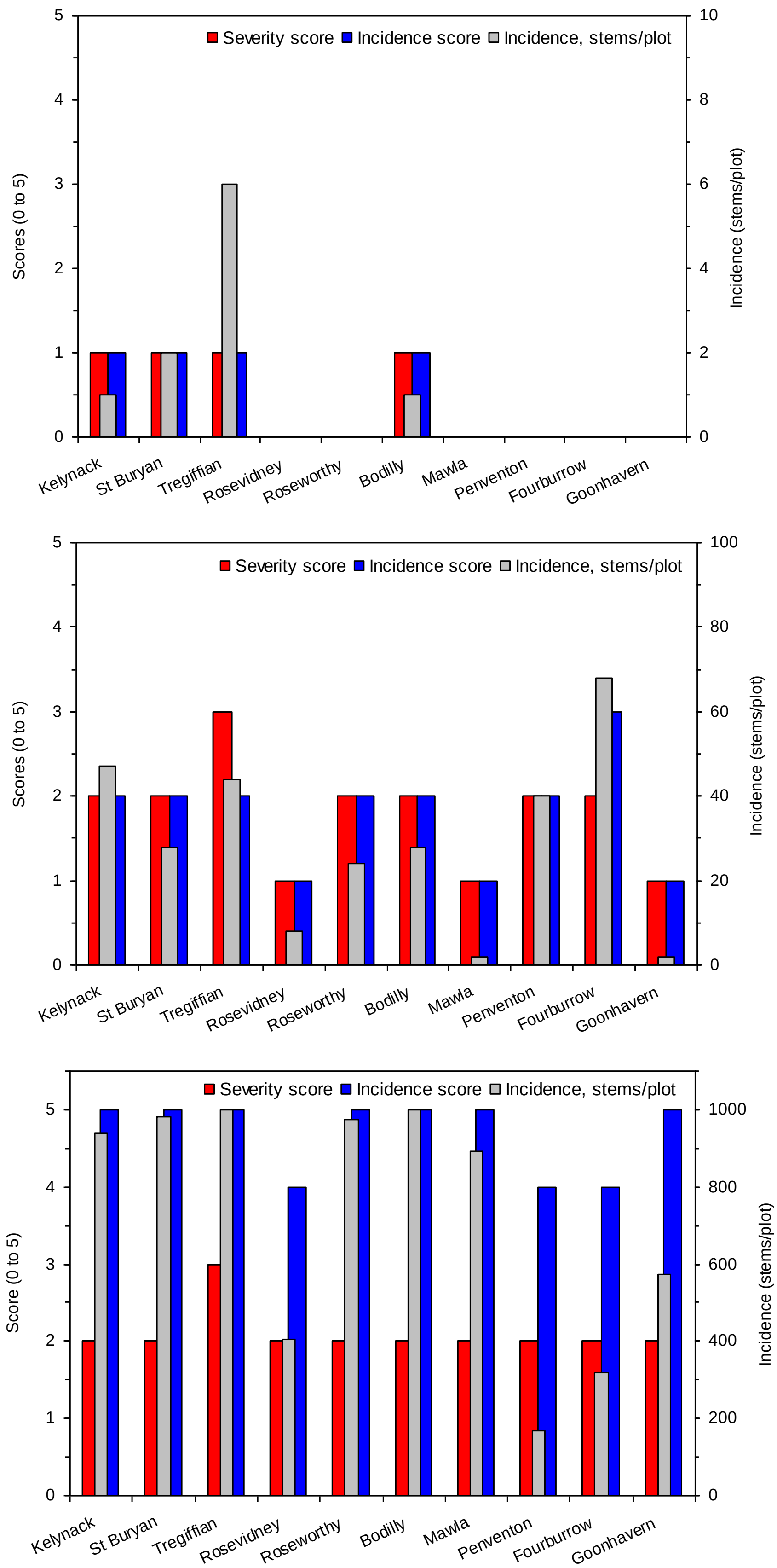


Figure 47. Severity and incidence scores for rust at ten sites assessed in 2014 at (top) pre-picking, (middle) picking and (bottom) post-picking stages. Incidence is shown as both a 0–5 score and as the number of stems per plot with rust.

2014: Leaf rust

Low levels of leaf rust were noted at all sites at the regular assessment dates, but were not quantified then - a further assessment was carried out on 1–2 June 2014 when foliage was at an early senescence stage (GS 4.1 or 4.2) specifically to assess the levels of leaf rust (Table 14). At the different sites between 54 and 88% of leaves sampled had rust lesions, these varying from a few small lesions per leaf to larger groups of lesions or streaks. The much greater levels of leaf rust seen in 2013 at Kelynack, St Buryan and Tregiffian were not evident in 2014. However, in 2014 the assessment of leaf rust was hampered by the appearance of substantial levels of white mould at many sites; at Fourburrow white mould had been severe and no daffodil foliage remained by June.

Table 14. Crop GS and incidence of leaf rust on 1–2 June 2014. In assessing GS the exaggerated leaf senescence as a result of white mould was ignored.

Site and name		GS	% leaves with rust lesions
A	Kelynack	4.2	82
B	St Buryan	4.2	73
C	Tregiffian	4.1	88
D	Rosevidney	4.1	75
E	Roseworthy	4.1	72
F	Bodilly	4.2	81
G	Mawla	4.1	66
H	Penventon	4.1	67
I	Fourburrow	5.2	(crop desiccated)
J	Goonhavern	4.1	54

2014: Early-stage rust lesions

Although not specifically assessed, presumptive ‘early-stage lesions’ (as mentioned above in the 2013 results) were often noted at the regular assessments. They consisted of small patches or larger tracts of ‘pitting’ and depressed, paler areas on the stems as well as the ‘blistering’ previously described.

Observations on lesion development

To discover whether the small, sparse rust lesions being encountered in this project might develop to more damaging levels after picking, on 1 March 2013 a sample of ten such stems 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. The lesions showed no further development. The impression that rust lesions do not develop further following picking has been confirmed by comments from growers and merchants, though it should be confirmed through more formal testing under a wider range of conditions.

Normally rust assessments include only the green, above-ground part of the stem. To determine whether rust lesions could be found on the underground part of the stem – either 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 ten sites for further examination. The plants were placed in loosely closed polythene bags, transported under ambient conditions and kept in an un-heated room until 19 February, when the entire stems were dissected out. Stems were carefully wiped clean with paper tissue and examined for the presence of lesions. Of the 89 stems examined, only five had a typical, small rust lesion, one per stem (four in the green section and one in the yellow section). However, 20 of the stems had numerous groups of paler yellow marks or pitting – presumptive early-stage lesions - in the yellow sections of the stems. To record any further development of lesions, 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 section of the stem. Neither type of lesion was found on the white part of the stem, though some patches of roughened surface were observed here.

Summary of rust severity and incidence 2013 and 2014

The severity and incidence of rust were much greater in 2014 than in 2013, although in both years rust levels varied across the ten sites. Rust severity scores are summarised in Figure 48. In 2013 only one site, Tregiffian, scored positive for rust at the pre-picking stage, but rust had occurred at all ten by the third assessment around a month later. Apart from the final assessment at Tregiffian, severity scored just 1, meaning that only slight rust markings had been seen – almost unnoticeable in combination with a low incidence. In 2014 rust lesions occurred sooner and at more sites, reaching a severity score of 2 at all sites except Kelynack where it was 3. A severity score of 2 indicates the occurrence of sparse but typical rust lesions that would certainly cause no commercial concerns over product quality, though it would indicate a crop “to keep an eye on”; the severity score of 3 at Kelynack indicates a moderate level of rust lesions that are beginning to become disfiguring – at the top-end of this score there would be the possibility of product down-grading.

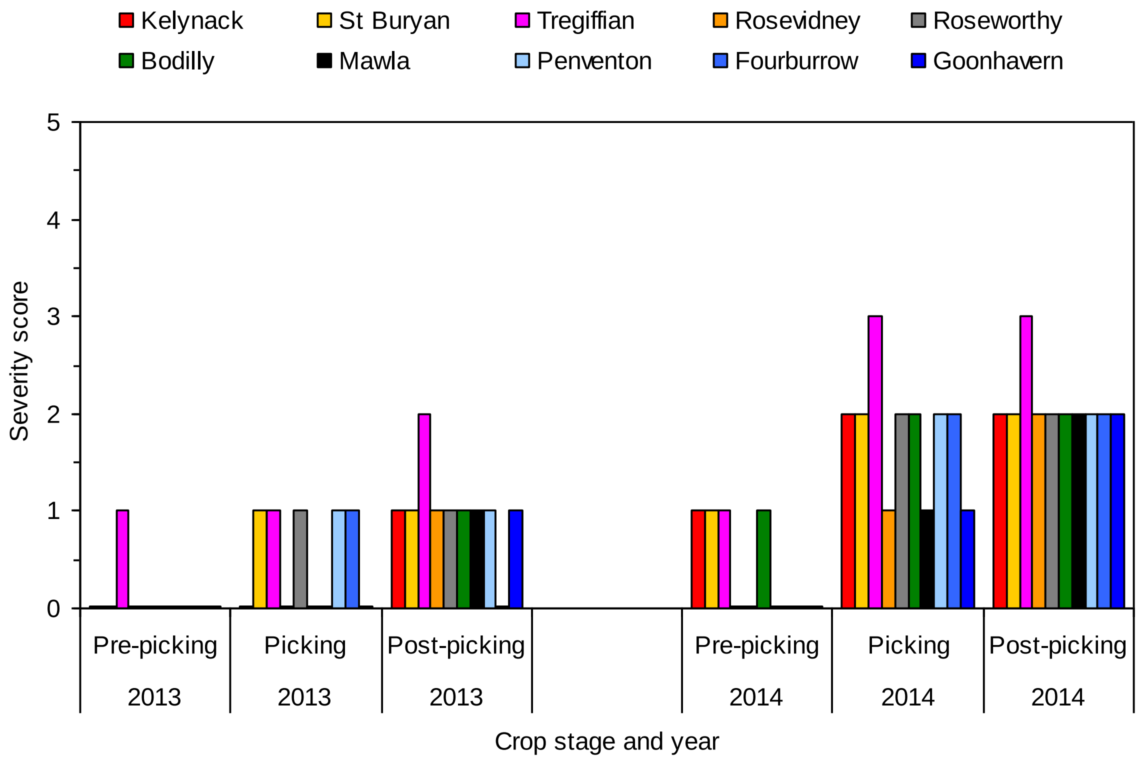


Figure 48. Severity scores for rust at the ten sites assessed in 2013 and 2014 at pre-picking, picking and post-picking stages.

Severity scores have to be considered in conjunction with scores for incidence, shown in Figure 49. High severity scores can be tolerated if incidence scores are low, and vice versa. In 2013 incidence scores did not exceed 1 (meaning that up to 1% of stems were affected) at the first two assessments, and occurred in only half of the sites – as for the severity scores – but by the post-picking stage incidence scores were between 2 and 4 at eight of the sites, meaning that up to 50% of stems were affected at some sites. 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, meaning that at seven sites up to 100% of stems were affected. Fortunately for the crops these scores were not accompanied by high severity scores, meaning that - commercially speaking - there would be no concerns about loss of product quality; further, rust symptoms occurring after flower picking is of little or no concern to the industry.

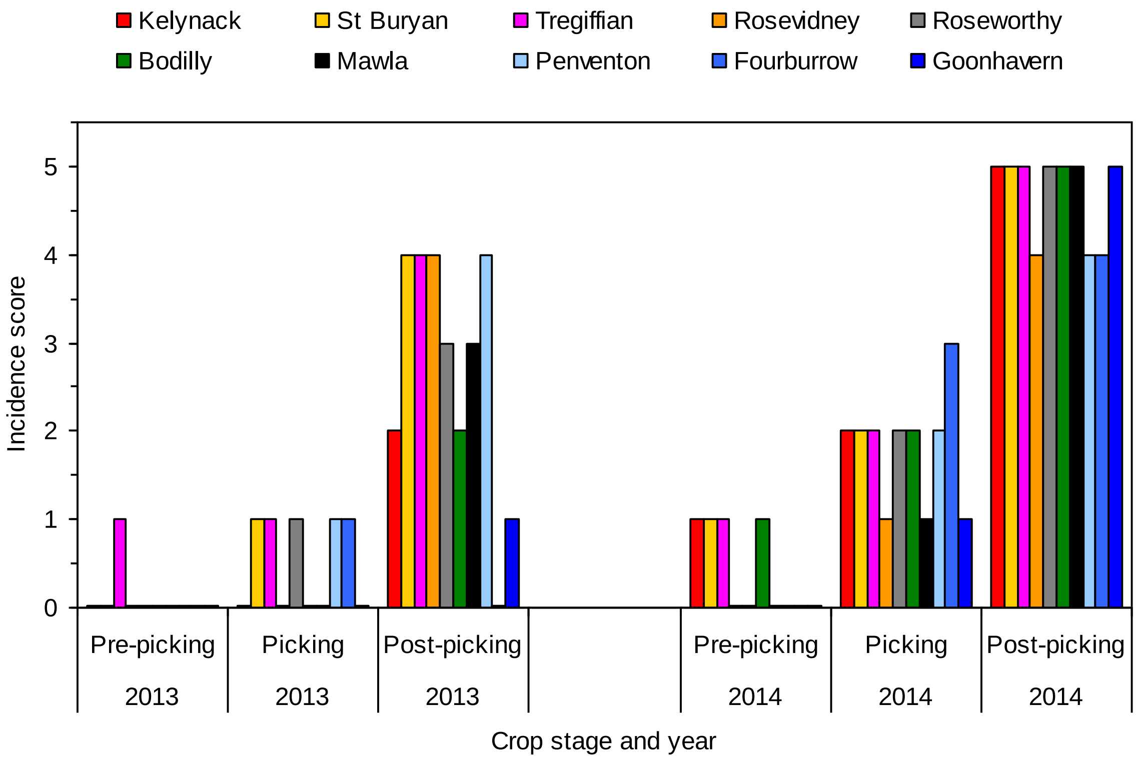


Figure 49. Incidence scores for rust at the ten sites assessed in 2013 and 2014 at pre-picking, picking and post-picking stages.

The severity score is a relatively crude way of expressing the numbers of stems with rust symptoms – in a five-point scale it needs to cover from 0 to 100% of stems affected. Figure 50 shows rust incidence as the number of stems per plot (of 1,000 stems). This shows that six sites – Kelynack, St Buryan, Tregiffian, Roseworthy, Bodilly and Mawla – have 80–100% of stems showing rust to some extent; at the remaining sites between 17 and 57% of stems are affected, the lowest level at Penventon. All these results are presented with sites in a west-to-east order, and it is evident that there is no west-east effect on rust levels.

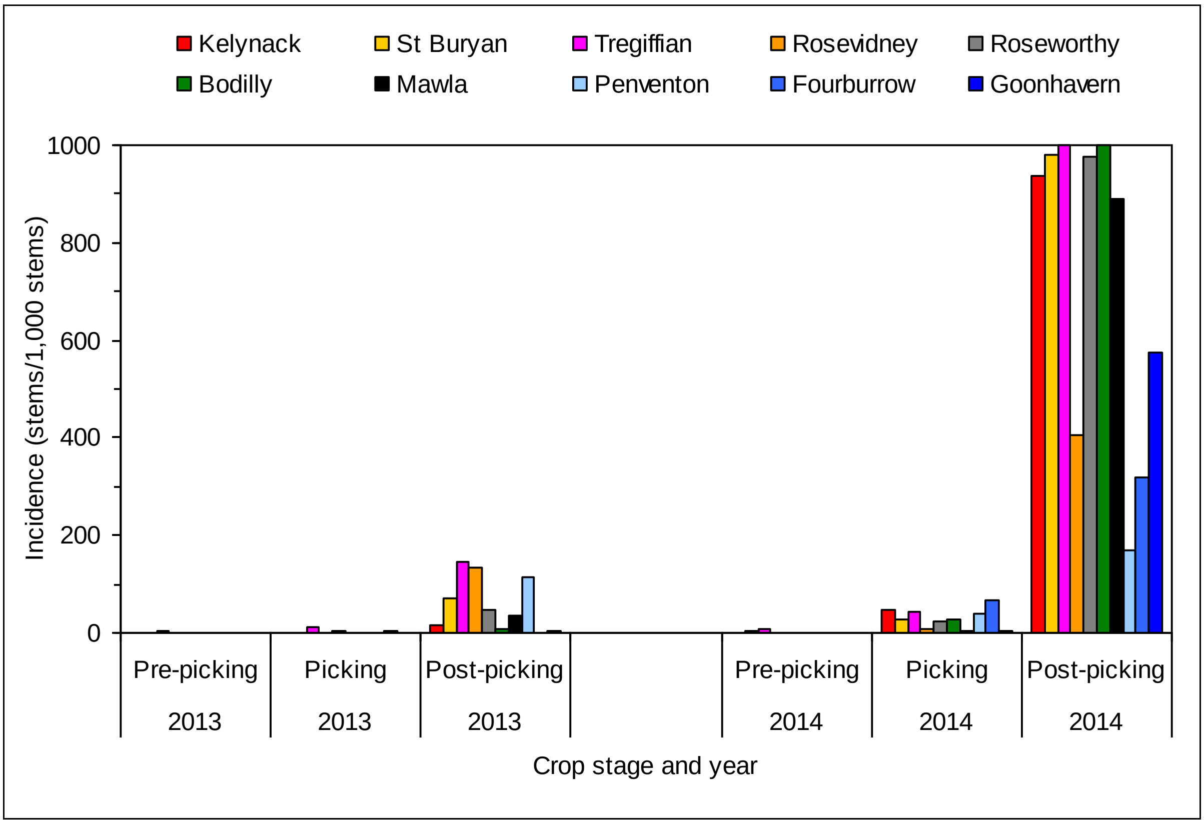


Figure 50. The incidence of stems with rust lesions at the ten sites assessed in 2013 and 2014, expressed as the number of stems per 1,000 stems affected.

<u>Other pests, diseases, disorders and problems</u>

The more notable occurrences of pests, diseases, etc. are summarised in Table 15. Weather-related damage played a large part, especially hail in the pre-picking and picking periods in spring 2014. Hail seriously reduced normal productivity at some sites (Kelynack, St Buryan and Tregiffian). Damage from frost was widespread, with dead leaf-tips and yellow leaf-tipping and banding (Kelynack, St Buryan, Tregiffian and Mawla), as was wind damage, resulting in many broken stems especially in 2014 (Kelynack, Roseworthy, Bodilly and Goonhavern). Human interactions played their part, despite efforts to avoid them, with damage from tractors to parts of three plots (Kelynack, Mawla and Fourburrow) and, at two plots, flowers being picked (Kelynack and Goonhavern). Two plots exhibited poor, sparse growth with yellowish leaves in 2013, probably as a result of unavoidably late planting and unsuitable conditions at planting (Mawla and Goonhavern), but they recovered remarkably by 2014. Pale foliage was noted at one site in 2013 only (Kelynack). The only major pest or disease impact was the serious incidence of white mould relatively late in spring 2014 (St Buryan, Tregiffian, Bodilly, Penventon and Fourburrow) (Table 15). In carrying out the regular assessments the seriously affected areas of plots were avoided.

Other pests, diseases and disorders occurred at low levels and are not specified in Table

15. 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. Predation of stems, leaves and flowers by snails and slugs was widespread. There were occasional flower-opening disorders (buds failing to develop or failing to open normally), mainly in 2013 and probably largely a result of prior hot-water treatment. These issues were not considered site-specific, appearing to be due to a stock problem or conditions generally.

Table 15. Notes on pests, diseases, disorders and problems at the ten sites.

Site and name		Pests, diseases, disorders and problems
A	Kelynack	2013: ends of outer row substantially damaged by tractors. Numerous yellow leaf-tips and bands (frost). 2014: substantial damage by frost (5cm of leaf-tips killed), wind (many broken stems) and by the picking stage some hail damage at one end of plot (trial plot less severely affected than adjacent, earlier cultivars); some flowers picked. White mould June 2014: Controlled levels of white mould on adjacent crops but relatively little spread to plot.
B	St Buryan	2013: numerous yellow leaf-tips and bands (frost). 2014: by picking stage some hail damage (5% of plants with up to 5cm of leaf-tips killed, exacerbated by smoulder lesions); white mould clearly evident at post-picking stage. Plants at top end of rows larger and more advanced, possibly planted in error. White mould June 2014: a 4m-long stretch of the plot was desiccated, part of a swathe of white mould across this part of the field, starting to encroach onto the rest of the plot.
C	Tregiffian	2013: some yellow leaf-tips and bands (frost). 2014: substantial hail damage pre-picking (up to 10cm of leaf-tip killed, damage to flower-buds and top of stem, about 10% of buds killed) especially on outer row (avoided for assessments), adjacent earlier cultivars more severely affected: white mould clearly evident at post-picking stage. White mould June 2014: surrounding crops largely desiccated but plot only slightly affected.
D	Rosevidney	2014: No hail damage evident. White mould June 2014: Plot and rest of field healthy.
E	Roseworthy	2014: substantial damaged by wind (broken stems) but no hail damage evident. White mould June 2014: plot and rest of field generally healthy, a little white mould.
F	Bodilly	2014: substantial damage by wind (broken stems); no hail damage evident; severe damage from white mould (actively sporulating) especially on outer row (not assessed) (50% of plants with up to 10cm dead leaf-tips). White mould June 2014: generally controlled, but some desiccated patches in field and plot.
G	Mawla	2013: some yellow leaf-tips and bands; wind damage. Weak, late and uneven growth, pale foliage (some foliage yellowish-green).

		2014: crop quality considerably improved (despite over-close wheeling by outer row). No hail damage evident. White mould June 2014: low, controlled levels on adjacent crops, but only a few plants affected in plot.
H	Penventon	Well-grown crop, best in trial. 2014: white mould coming in at post-picking stage. White mould June 2014: some in plot, levels vary in rest of field (a variety collection) but largely controlled.
I	Fourburrow	2013: two patches near row ends damaged by tractor. 2014: severe damage from white mould (actively sporulating) before picking stage, some lesions dried-out (controlled) but many rotting or dead flower-buds and up to 15cm dead leaf-tips; no hail damage evident. Growth better at lower end of plot. White mould June 2014: plot and whole field desiccated.
J	Goonhavern	2013: weak, late and uneven growth, pale foliage (some foliage yellowish-green); wind damage. 2014: crop quality considerably improved by 2014. No hail damage evident. Some flowers had been picked. White mould June 2014: traces on plot, otherwise healthy.

Relationships between levels of rust, SWC and other factors

Preliminary data analysis based on box-and-whisker plots and scatter plots was used to assess which, if any, of the SWC and other factors recorded might have a relationship with the level of rust. In making these assessments the level of rust was initially expressed in terms of incidence scores, severity scores and the numbers of stems per plot with rust recorded before, around and after the flower-picking stage. Of these indicators, incidence and severity scores exhibited low variability at any one recording time, which limited their usefulness, while the generally greater development of rust symptoms between the flower-picking stage and the post-picking assessment meant that the number of stems per plot with rust at the post-picking stage was the most useful indicator and was therefore used extensively. Flower-picking was over at all sites by late-March or early-April, so the maximum expression of rust relevant to the flower crop would have been reached by that time. Once data from the third-year of the crop has been obtained in 2015 as part of the AHDB Horticulture-funded project extension (BOF 76a), multiple regression analysis will be used to explore the relationships of the whole data-set.

Rust and SWC: Crop-year 1, 2012-2013

SWC and other weather data had been logged from 1 November 2012, once all plots had been planted, so there were five months' data to explore for effects on the level of rust at picking 2013.

Figure 51 is a box-and-whisker plot of average SWC (the average of the 10, 20 and 30 cm-deep readings) over the five-month period November 2012 to March 2013 in relation to rust incidence (expressed as the number of stems per plot with rust at the end of March 2013).

The legend of Figure 51 serves as a general example of how the box-and-whisker plots are set out. To help visualise relationships the sites were colour-coded according to their rust level - red for high, yellow for middle, green for low or white for zero. The most noticeable feature was that three of the four sites with a high incidence of daffodil rust were associated with high values of SWC – St Buryan, Tregiffian and Rosevidney, the exception being Penventon. Figure 52 summarises data from the separate sensors at each soil depth, and the same trend, though somewhat weaker, can be seen.

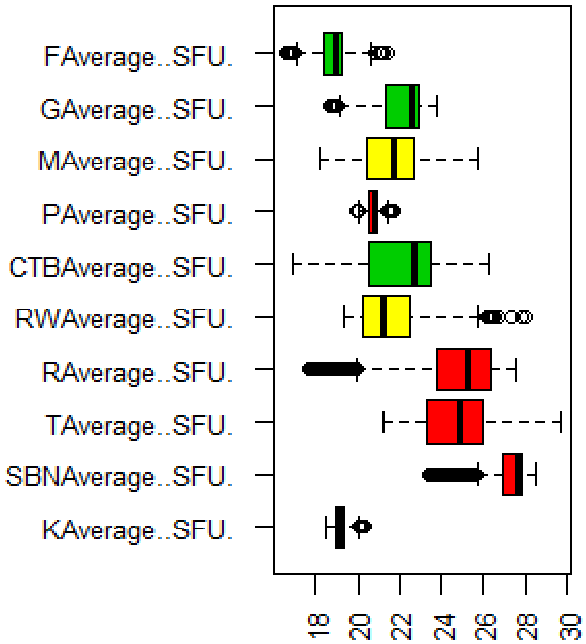


Figure 51. Box-and-whisker plot showing the level of daffodil rust at the ten sites in relation to average SWC recorded in the preceding five-month period (1 November 2012 to 31 March 2013). Daffodil rust levels were expressed as the number of stems per plot with symptoms in late-March 2013, shown by the boxes coloured red, yellow, green or white for high, middle, low and zero levels, respectively. SWC, shown on the horizontal axis, is the average across sensors 10, 20 and 30cm deep. The sites are coded (from top to bottom on the vertical axis) in roughly east-to-west order: **F**ourburrow, **G**oonhavern, **M**awla, **P**enventon, **[CT]B**odilly, **R**ose**W**orthy, **R**osevidney, **T**regiffian, **S**t **B**uryan and **K**elynack. Note the high SWC values associated with the rust-prone sites at St Buryan, Tregiffian and Rosevidney but not Penventon.

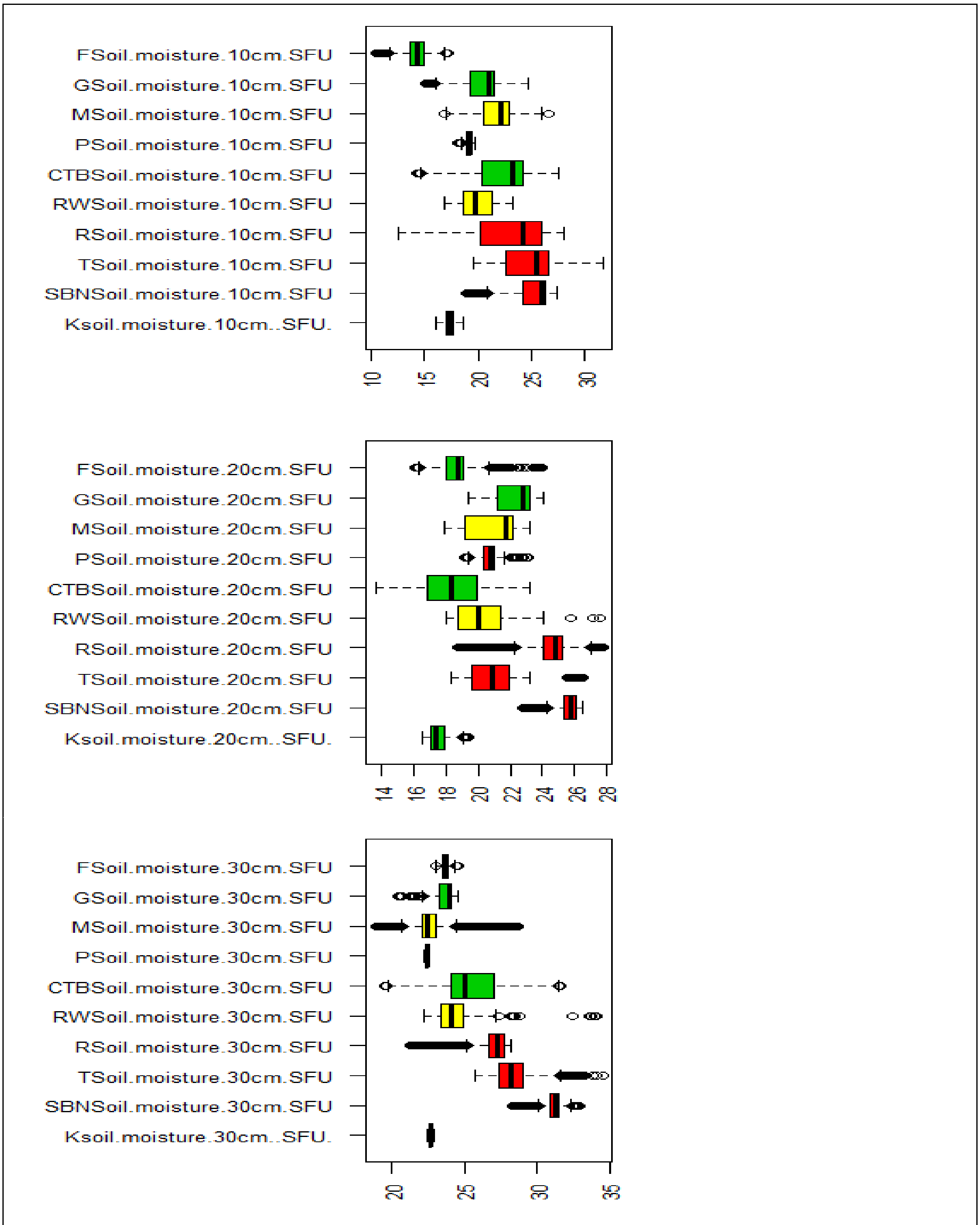


Figure 52. Box-and-whisker plots showing the level of daffodil rust at the ten sites in relation to SWC at 10cm (top), 20cm (middle) and 30cm soil depth (bottom) recorded in the preceding five-month period (1 November 2012 to 31 March 2013). For other details, see legend to Figure 51.

Figure 53 is a plot of average SWC similar to that of Figure 51, but with rust incidence expressed as incidence scores rather than number of leaves affected. It shows that both ways of expressing rust incidence gave similar results, and that the results using SWC separately at 10, 20 and 30cm-soil depth were also the same as before (data not shown).

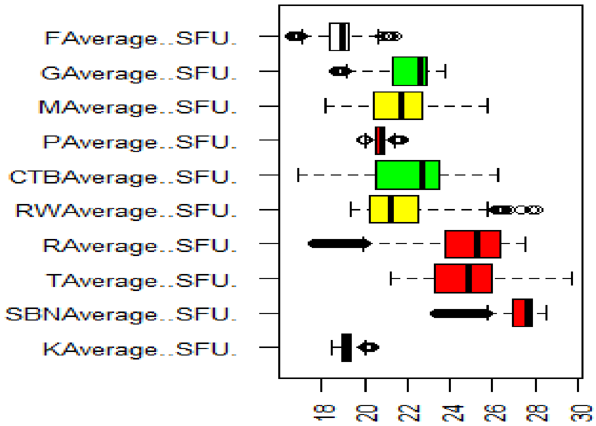


Figure 53. Box-and-whisker plot showing the level of daffodil rust at the ten sites in relation to average SWC recorded in the preceding five-month period (1 November 2012 to 31 March 2013). Daffodil rust levels are expressed as the incidence scores in late-March 2013. SWC, shown on the horizontal axis, is the average across sensors 10, 20 and 30cm deep. Note the high SWC values associated with the rust-prone sites at St Buryan, Tregiffian and Rosevidney but not Penventon. For other details, see legend to Figure 51.

These data suggest the possibility of an association between SWC over the five-month period prior to flower picking (November to March), but this might be a short-term effect (say, SWC affects stem growth at the time of rapid shoot growth that immediately precedes flowering) or a longer-term one (perhaps an accumulating effect of SWC over the previous months, or at some earlier key-stage in development). To investigate these possibilities, the equivalent data for shorter periods were also examined, i.e. December to March (Figure 54), January to March (Figure 55), February to March (Figure 56) and March only (Figure 57). Comparing this series of box-and-whisker plots (Figure 51 and Figure 54 to Figure 57), it can be seen that the apparently rust-enhancing effect of high SWC has lessened by the January to March data-set and was not evident thereafter, implying that the key-period of the putative SWC effect was November to December rather than January to March. One rust-prone site, St Buryan, exhibited very high levels of SWC throughout. As found with the first data-set (November to March), these later sets showed that the effect of SWC was less evident using readings from the individual SWC sensors at different depths (data not shown), suggesting that the overall SWC is the important factor. If the rust level was expressed instead as the incidence score, the same conclusions were drawn (data not shown). All the above assessments of rust levels were based on the number of stems affected by rust or the incidence score in late-March 2013, when the disorder was considered fully expressed. It was not practical to use the severity score as this was relatively low in all cases. Neither was it practical to use the rust assessments made in early- or late-February 2013, since the levels of rust at this time were very low.

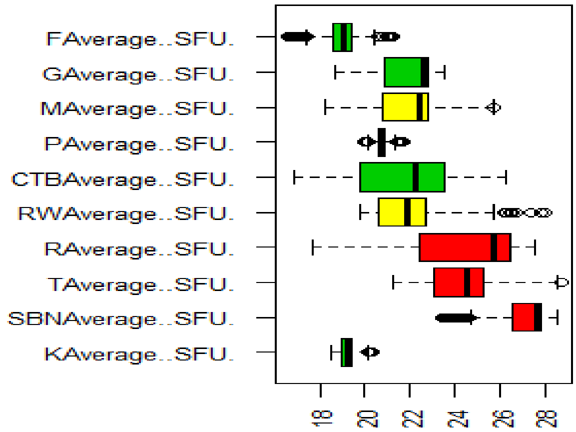


Figure 54. Box-and-whisker plot showing the level of daffodil rust at the ten sites in relation to average SWC recorded in the preceding four-month period (1 December 2012 to 31 March 2013). Rust levels are expressed as the number of stems per plot with symptoms in late-March 2013. SWC, shown on the horizontal axis, is the average across sensors 10, 20 and 30cm deep. Note the high SWC values associated with the rust-prone sites at St Buryan, Tregiffian and Rosevidney (but not Penventon). For other details, see legend to Figure 51.

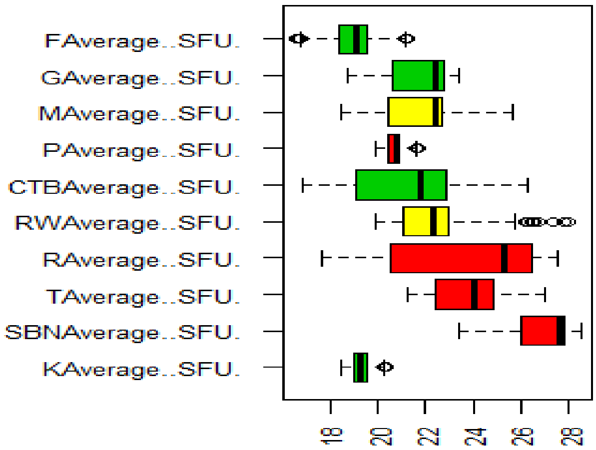


Figure 55. Box-and-whisker plot showing the level of daffodil rust at the ten sites in relation to average SWC recorded in the preceding three-month period (1 January 2012 to 31 March 2013). Rust levels are expressed as the number of stems per plot with symptoms in late-March 2013. SWC, shown on the horizontal axis, is the average across sensors 10, 20 and 30cm deep. For other details, see legend to Figure 51.

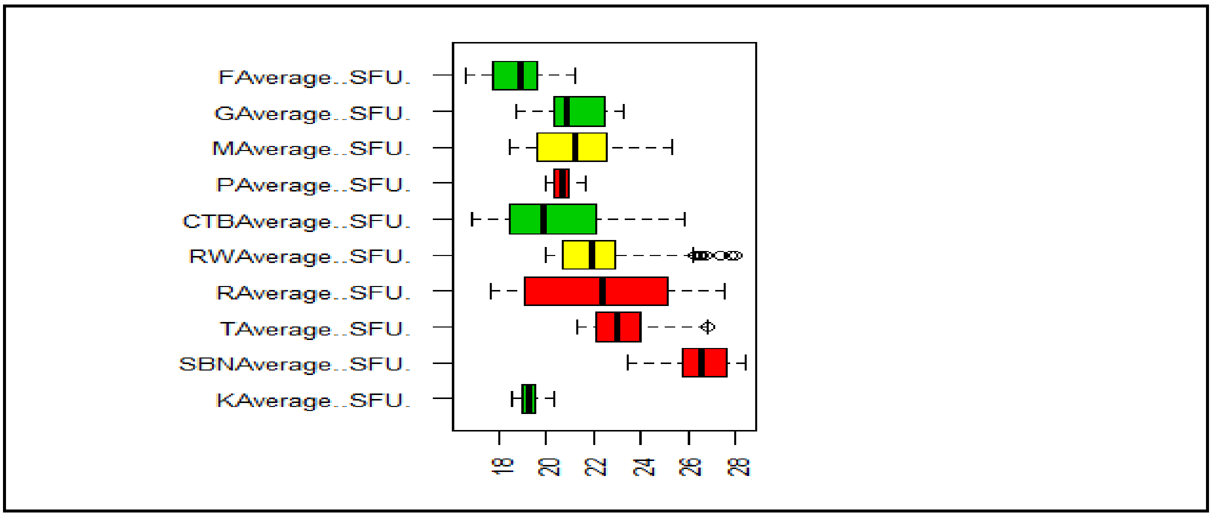


Figure 56. Box-and-whisker plot showing the level of daffodil rust at the ten sites in relation to average SWC recorded in the preceding two-month period (1 February 2012 to 31 March 2013). Rust levels are expressed as the number of stems per plot with symptoms in late-March 2013. SWC, shown on the horizontal axis, is the average across sensors 10, 20 and 30cm deep. For other details, see legend to Figure 51.

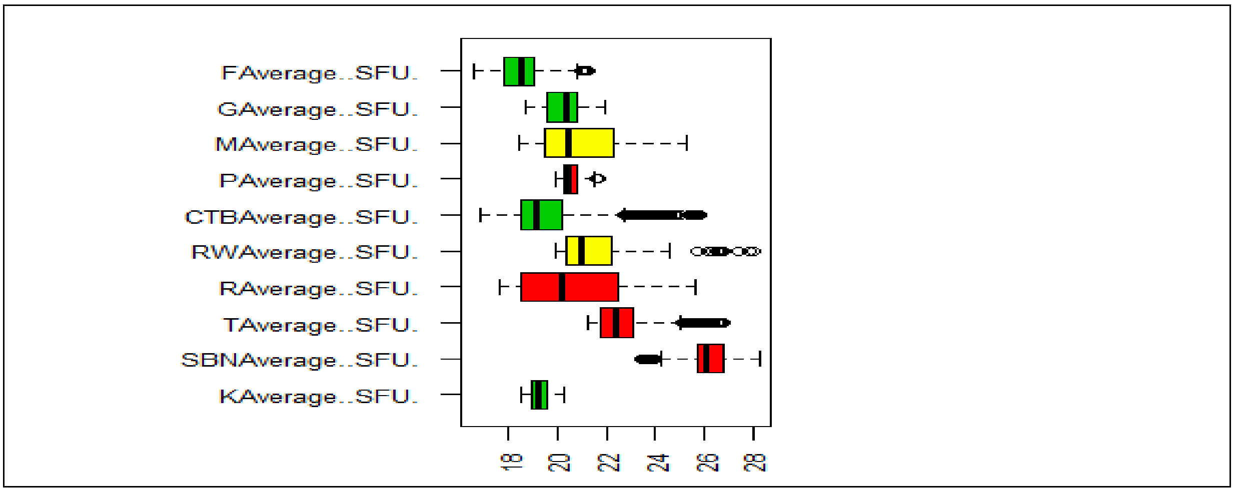


Figure 57. Box-and-whisker plot showing the level of daffodil rust at the ten sites in relation to average SWC recorded in the preceding month (March 2013). Rust levels are expressed as the number of stems per plot with symptoms in late-March 2013. SWC, shown on the horizontal axis, is the average across sensors 10, 20 and 30cm deep. For other details, see legend to Figure 51.

In order to narrow-down further the key period in which SWC appears to affect subsequent rust levels, the SWC data were next considered month-by-month and week-by-week. SWC data for the four months February to November are given in Figure 58 to Figure 61, respectively (the data for March were presented in Figure 57). These results showed that SWC during November and December appeared to be associated with higher levels of rust, while in January, February and March the effect was diminished. As previously noted, the same relationship held true for rust incidence scores as for the number of stems affected, and a weaker effect was again seen when SWC data were expressed as the readings of the sensors at different soil depths (data not shown).

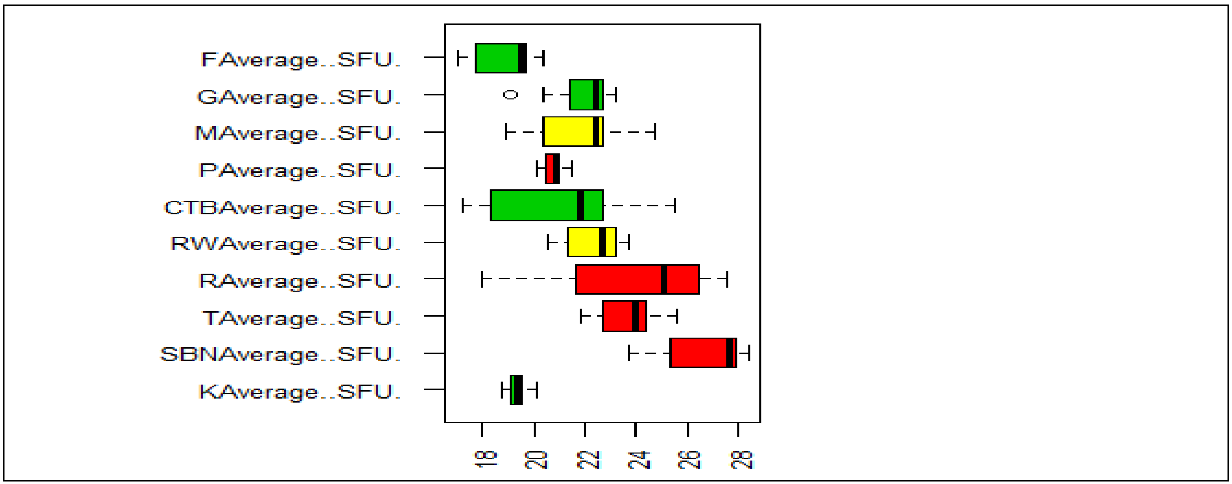


Figure 58. Box-and-whisker plot showing the level of daffodil rust at the ten sites in relation to average SWC recorded in February 2013. Rust levels are expressed as the number of stems per plot with symptoms in late-March 2013. SWC, shown on the horizontal axis, is the average across sensors 10, 20 and 30cm deep. For other details, see legend to Figure 51.

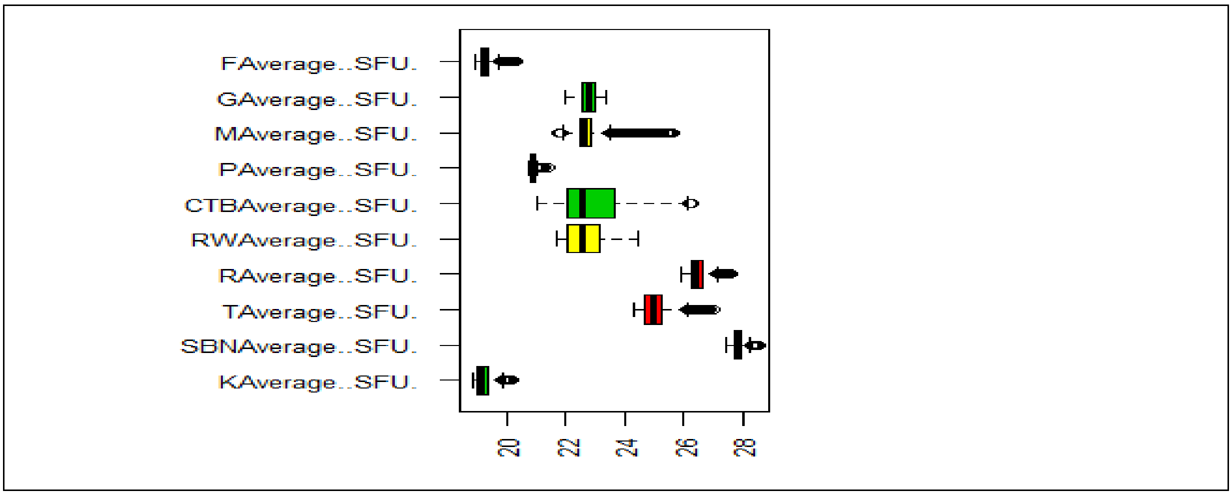


Figure 59. Box-and-whisker plot showing the level of daffodil rust at the ten sites in relation to average SWC recorded in January 2013. Rust levels are expressed as the number of stems per plot with symptoms in late-March 2013. SWC, shown on the horizontal axis, is the average across sensors 10, 20 and 30cm deep. For other details, see legend to Figure 51.

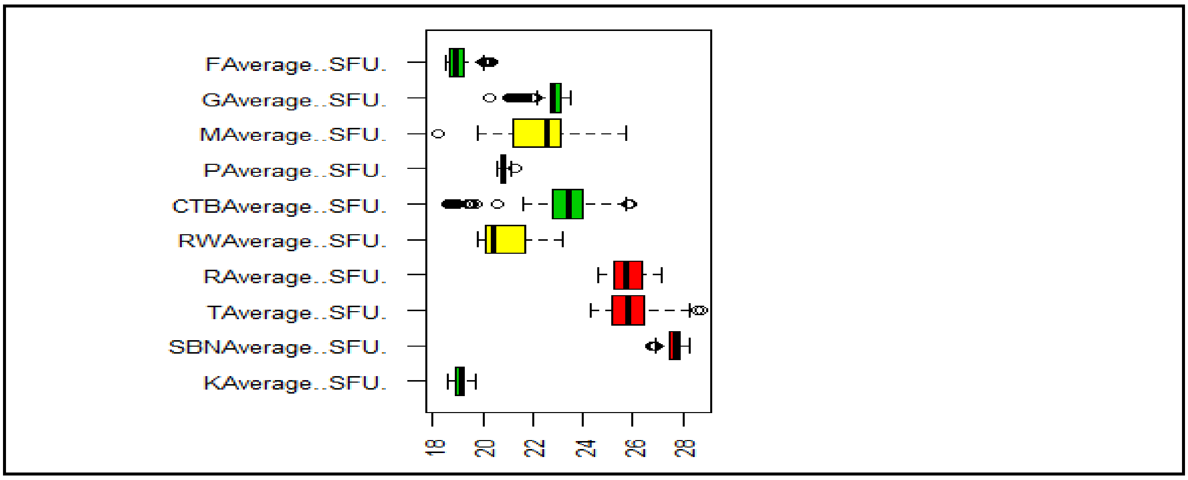


Figure 60. Box-and-whisker plot showing the level of daffodil rust at the ten sites in relation to average SWC recorded in December 2012. Rust levels are expressed as the number of stems per plot with symptoms in late-March 2013. SWC, shown on the horizontal axis, is the average across sensors 10, 20 and 30cm deep. For other details, see legend to Figure 51.

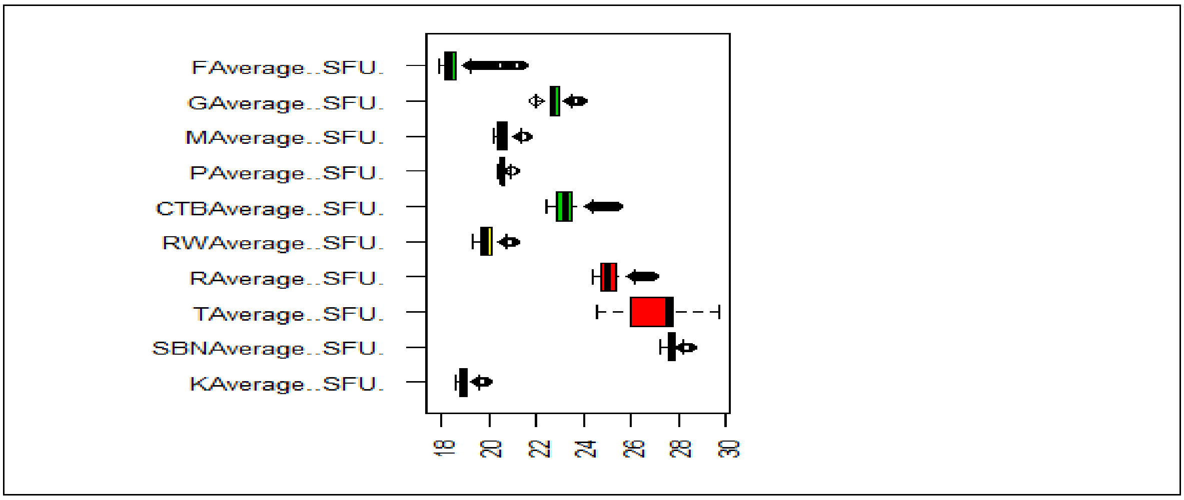


Figure 61. Box-and-whisker plot showing the level of daffodil rust at the ten sites in relation to average SWC recorded in November 2012. Rust levels are expressed as the number of stems per plot with symptoms in late-March 2013. SWC, shown on the horizontal axis, is the average across sensors 10, 20 and 30cm deep. For other details, see legend to Figure 51.

SWC data were also examined for the separate weeks of November 2012 and March 2013. In the November data there was a clear association of high SWC and high rust levels at the three sites previously noted, while in the March data the effect was weak. Examples are shown in Figure 62 and Figure 63.

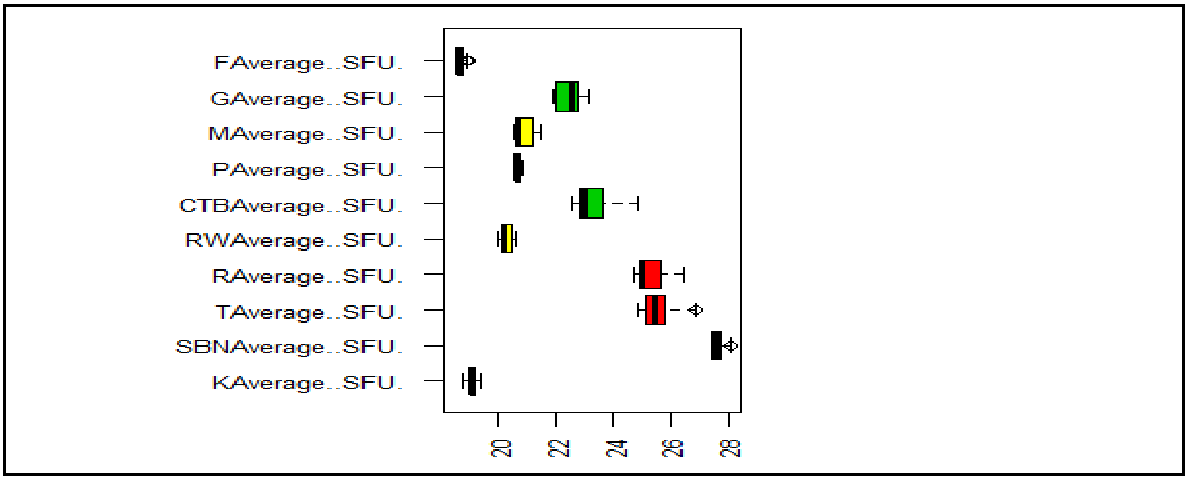


Figure 62. Box-and-whisker plot showing the level of daffodil rust at the ten sites in relation to average SWC recorded during the week 29 November to 5 December 2012. Rust levels are expressed as the number of stems per plot with symptoms in late-March 2013. SWC, shown on the horizontal axis, is the average across sensors 10, 20 and 30cm deep. For other details, see legend to Figure 51.

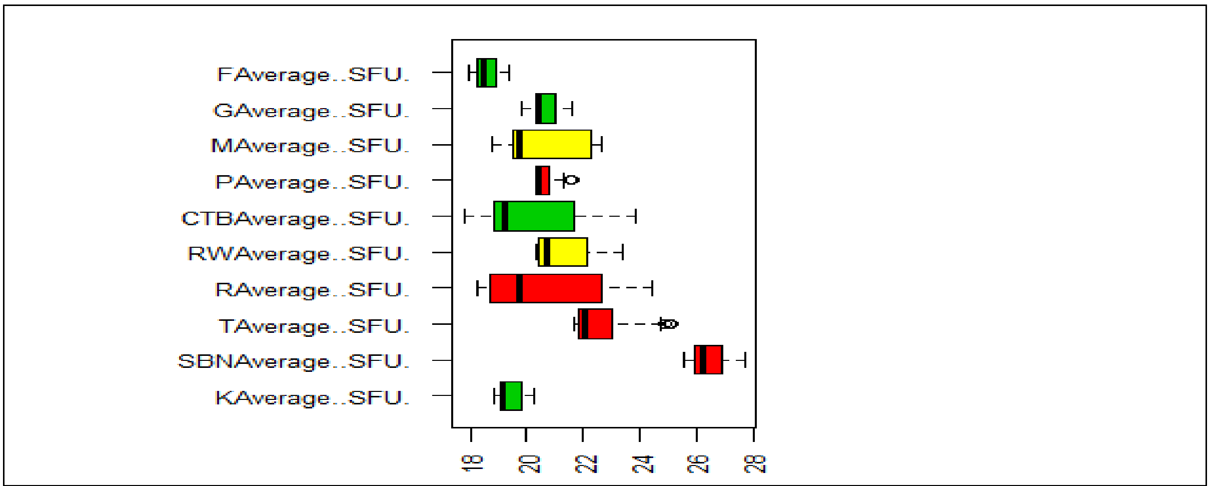


Figure 63. Box-and-whisker plot showing the level of daffodil rust at the ten sites in relation to average SWC recorded during the week 11 to 17 March 2013. Rust levels are expressed as the number of stems per plot with symptoms in late-March 2013. SWC, shown on the horizontal axis, is the average across sensors 10, 20 and 30cm deep. For other details, see legend to Figure 51.

Rust and SWC: Crop-year 2, 2013-2014

The suggestion of an association between higher winter SWC and higher subsequent levels of rust was further investigated for the 2014 flower crop, which had the advantage of a longer run (>1 year) of prior SWC and other meteorological data.

Rust levels were examined in relation to the previous year's SWC data (01 April 2013 to 01 April 2014) as severity and incidence scores around picking and as the number of stems per plot with rust post-picking. Assessed as the severity score around picking, Figure 64 shows there was a tendency for higher rust scores (yellow and orange boxes) to fall at the wetter end of the SWC scale and for lower rust scores (green boxes) to fall at the drier end, though all sites (e.g. Fourburrow, with relatively high rust levels) did not conform exactly. Although this tendency can be seen at all three sensor depths (10, 20 and 30cm deep), it is clearest in the 30cm readings. Four sites – St Buryan, Tregiffian, Roseworthy and Bodilly – were consistent in having high SWC, while in general Rosevidney, Mawla and Goonhavern were amongst the drier sites. SWC is greater at 10cm- and 30cm-depth (associated with surface flooding and water-logging above the deeper impervious layers, respectively?) that at 20cm (indicating reasonable drainage at bulb depth?). Similar results, including finding the clearest distinctions at 30cm-depth, can be seen when rust was expressed as incidence scores (Figure 65), as the number of stems per plot with rust (Figure 66, note highest rust levels indicated by brown boxes), and as SWC averaged across the three depths (Figure 67).

In order to see the effects of SWC over a longer period, the number of stems per plot with rust at post-picking 2014 was plotted against SWC in the periods 01 March 2014 to 01 April 2014 (in the month of picking), 01 February 2014 to 01 April 2014 (over the two months up to picking), 01 January 2014 to 01 April 2014 (over the three months up to picking), and so on up to the period 01 November 2012 to 01 April 2014 (over the 17 months up to picking). Three examples are shown in Figure 68, and the full set of results is included in Appendix 1; all use SWC at 10cm depth as an example. For all these periods the results were consistent (with a slightly weaker effect for the one-month period immediately preceding the post-picking stage) and mirrored those described above, with a strong tendency for higher rust scores (brown, orange and red boxes) to fall at the wetter end of the SWC scale and lower rust scores (white, yellow and green boxes) to fall at the drier end (Figure 68 and Appendix 1). This consistency suggests that the effect of SWC on rust levels relates to an ongoing process – perhaps climate-related – rather than a particular short-term effect.

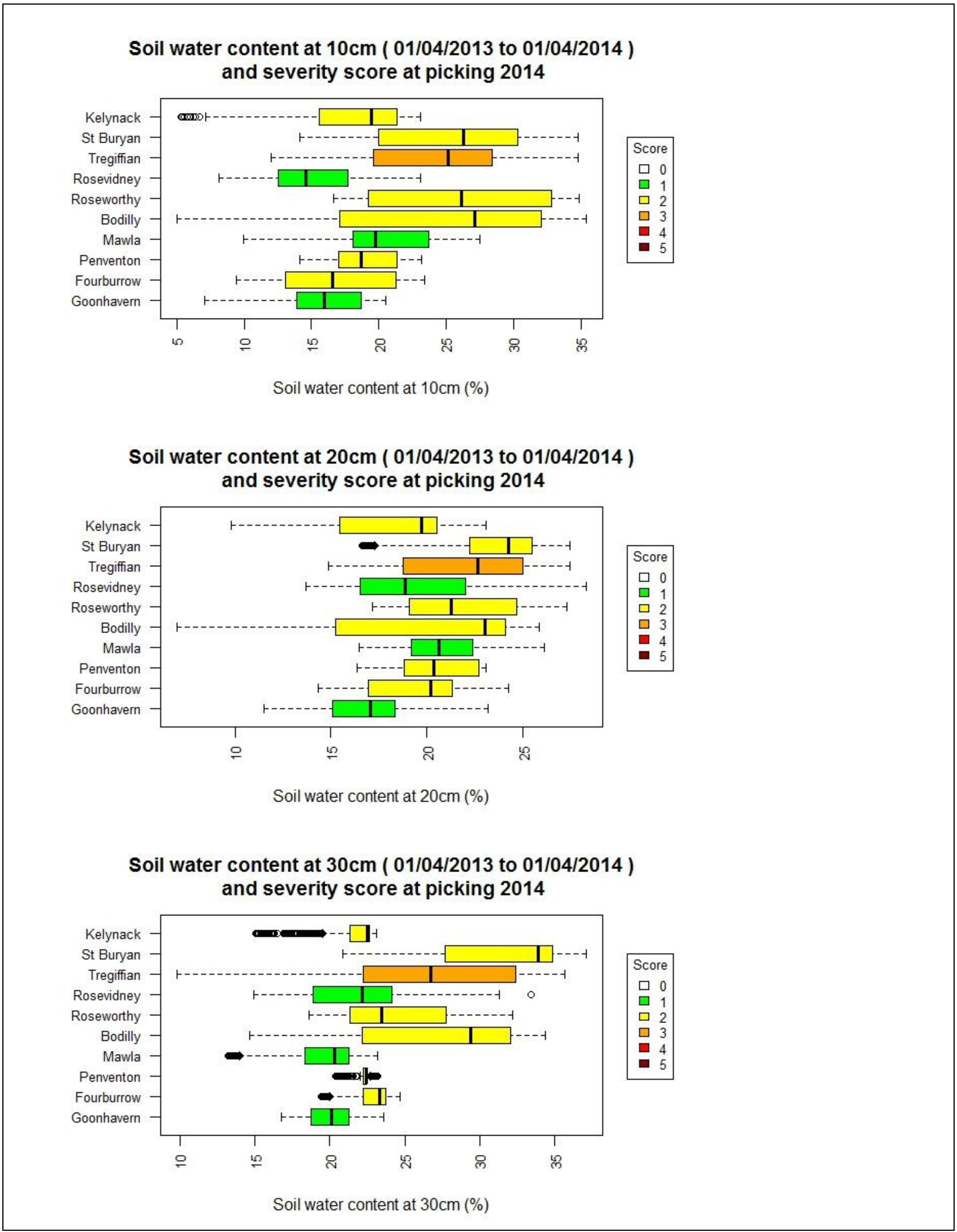


Figure 64. Box-and-whisker plots showing rust severity scores at the ten sites at picking 2014 in relation to SWC 10cm (top), 20cm (middle) and 30cm deep (bottom) between 01 April 2013 and 01 April 2014. Note that the scales of the x-axes vary between depths, the middle zone being drier than both surface and lower layers.

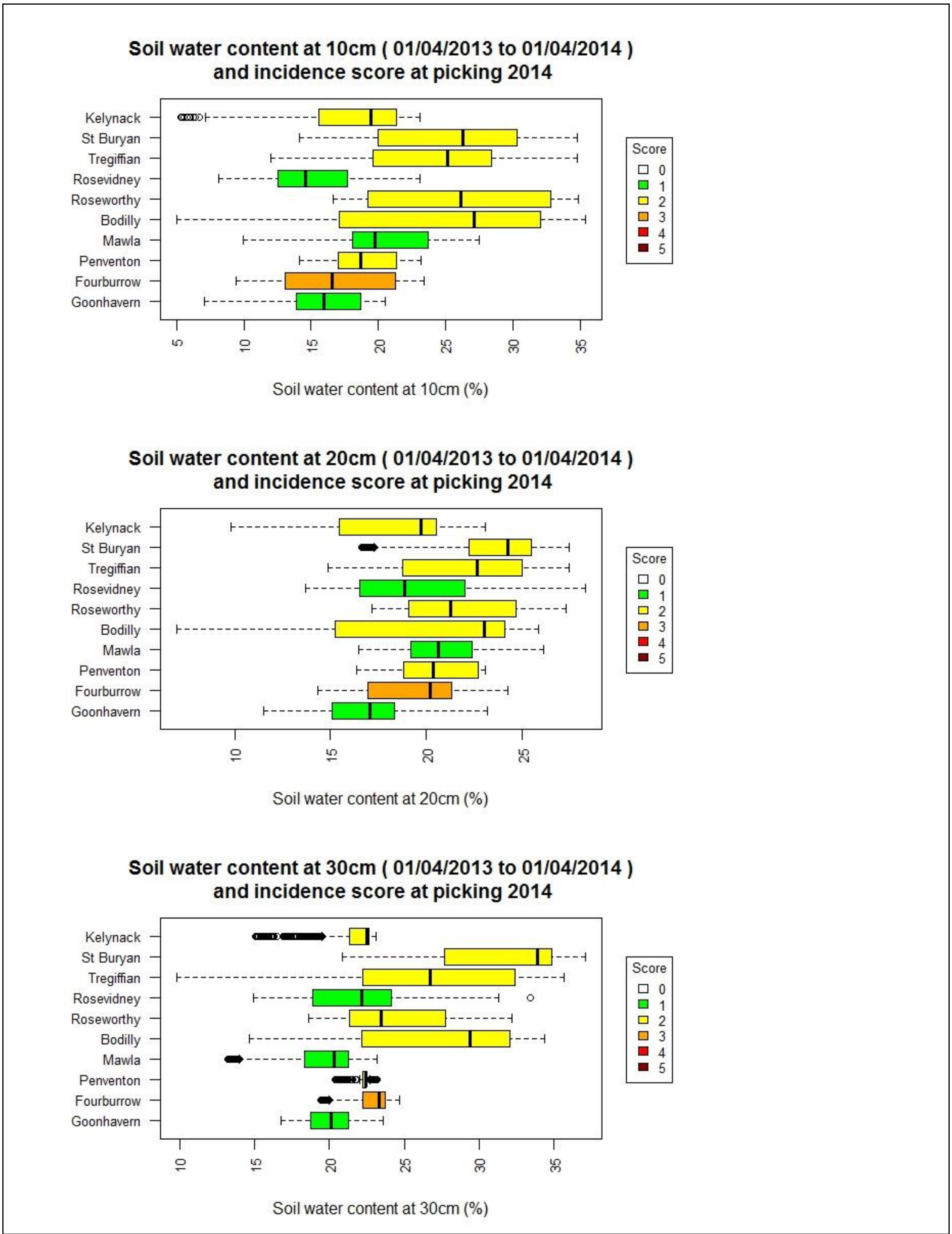


Figure 65. Box-and-whisker plots showing rust incidence scores at the ten sites at picking 2014 in relation to SWC 10cm (top), 20cm (middle) and 30cm deep (bottom) between 01 April 2013 and 01 April 2014. Note that the scales of the x-axes vary between depths, the middle zone being drier than both surface and lower layers.

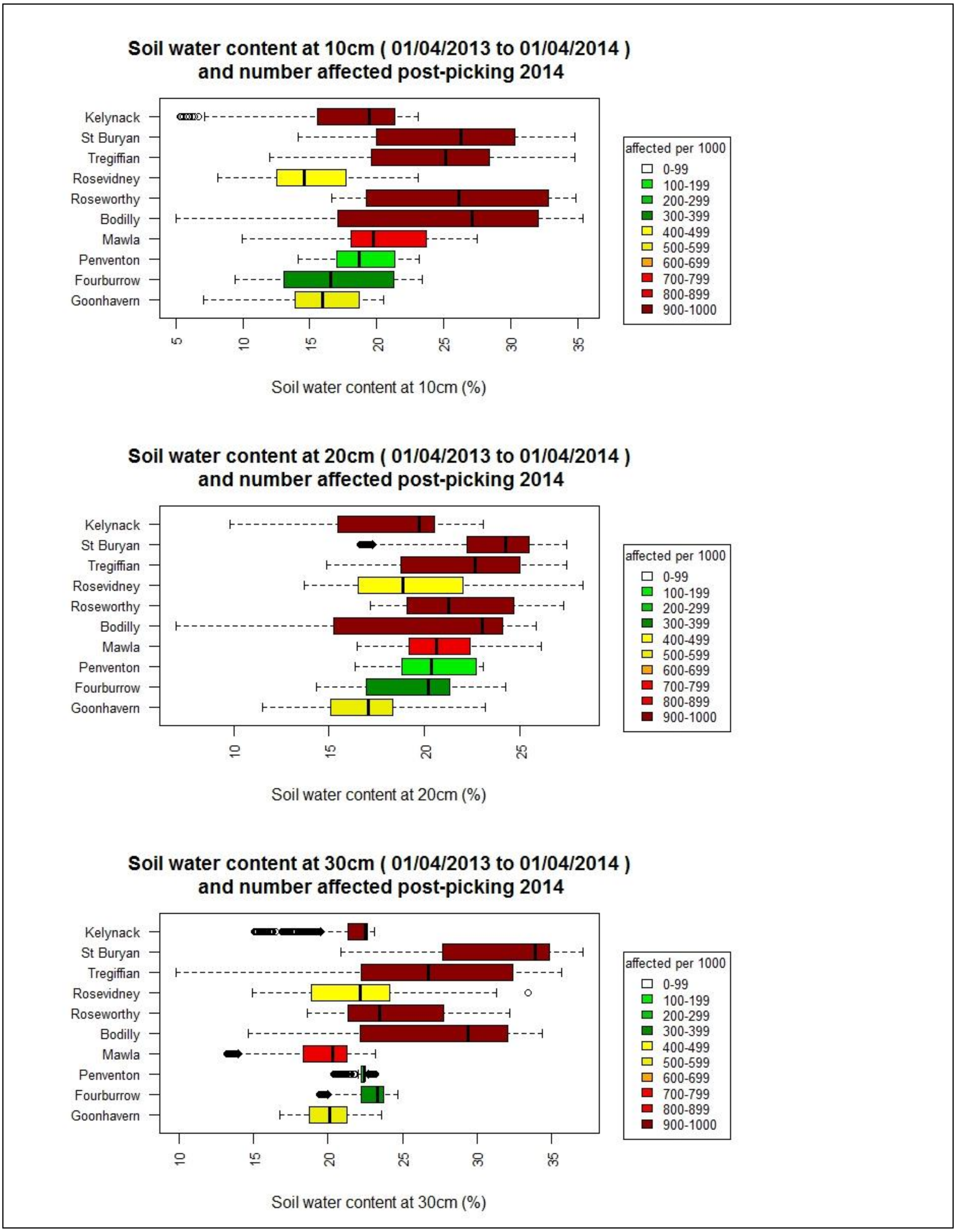


Figure 66. Box-and-whisker plots showing the number of stems per plot with rust at the ten sites post-picking 2014 in relation to SWC 10cm (top), 20cm (middle) and 30cm deep (bottom) between 01 April 2013 and 01 April 2014. Note that the scales of the x-axes vary between depths, the middle zone being drier than both surface and lower layers.

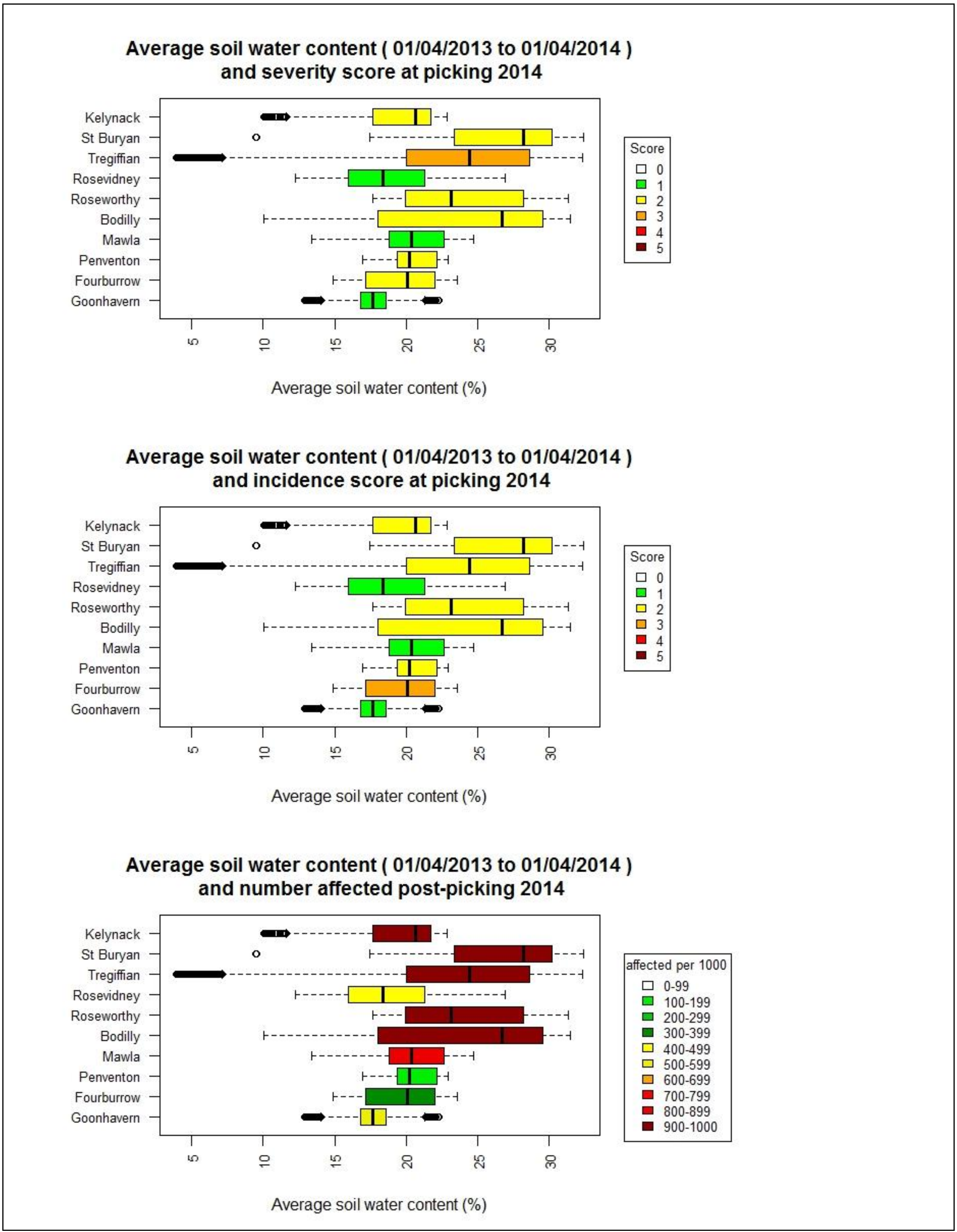


Figure 67. Box-and-whisker plots showing rust severity score at picking 2014 (top), incidence score at picking 2014 (middle) and number of stems per plot with rust post-picking 2014 (bottom) at the ten sites in relation to SWC averaged across measurements 10, 20 and 30cm deep between 01 April 2013 and 01 April 2014.

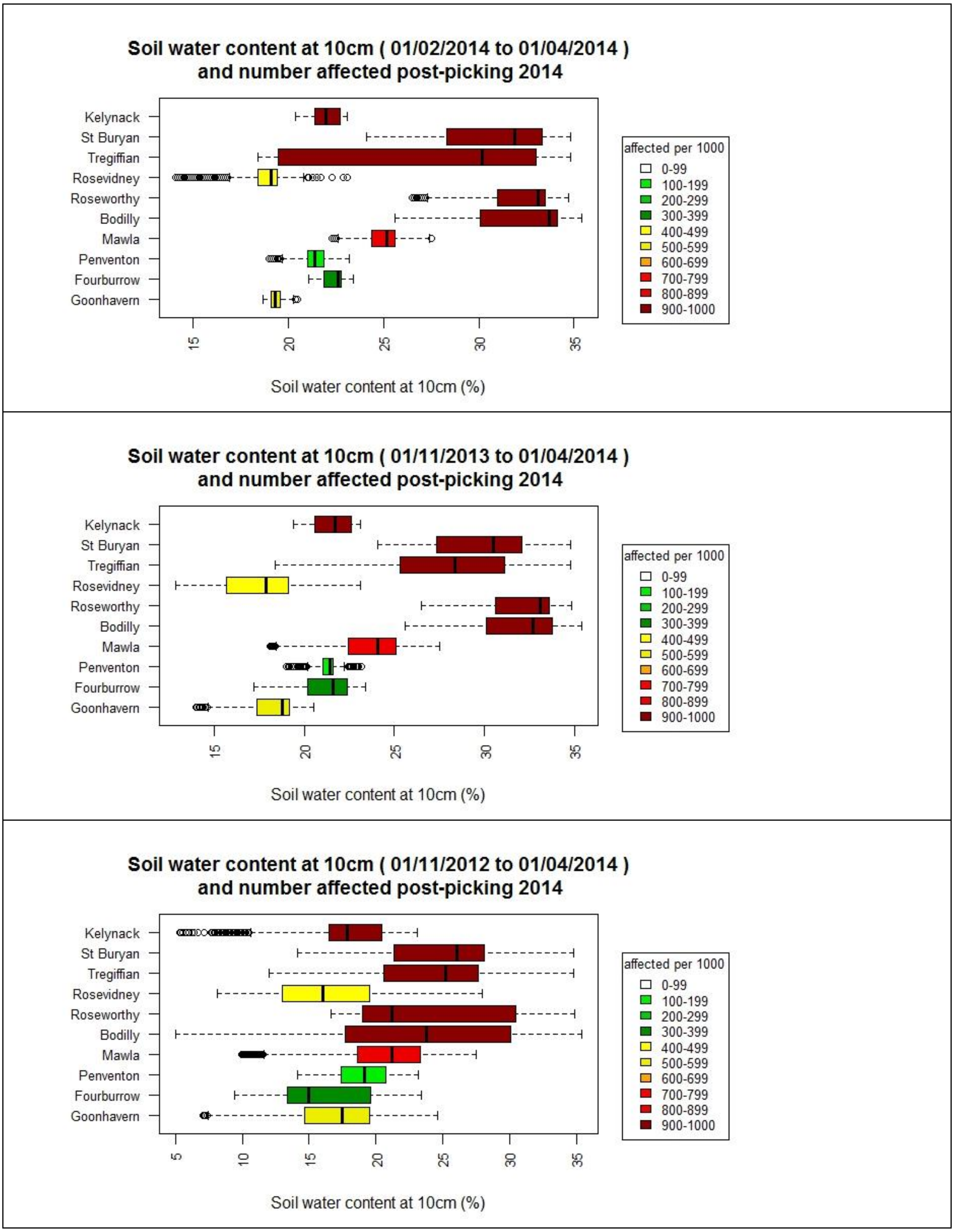


Figure 68. Box-and-whisker plots showing the number of stems per plot with rust at the ten sites post-picking 2014 in relation to SWC 10cm deep (bottom) between 01 February 2014 and 01 April 2014 (top), 01 November 2013 and 01 April 2014 (middle) and 01 November 2012 and 01 April 2014 (bottom).

Rust and temperature and humidity: Crop-year 1, 2012-2013

The levels of rust at the ten sites were also examined in relation to air temperature and RH in various periods from the beginning of monitoring (01 November 2012) until post-picking in 2013. However, using the same approach as described above for SWC, there was no suggestion of much variation in air temperature or RH across the ten sites. In all cases the ranges of air temperature or RH were similar. Two examples are shown for air temperature (Figure 69 and Figure 70) and two for RH (Figure 71 and

Figure **72**).

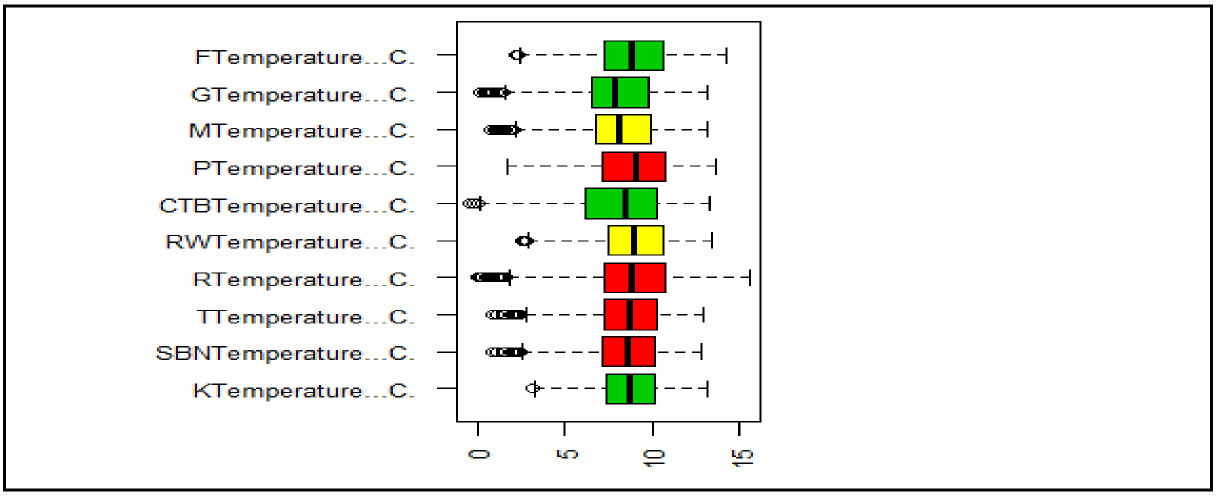


Figure 69. Box-and-whisker plot showing the level of daffodil rust at the ten sites in relation to air temperature recorded in November 2012. Daffodil rust levels are expressed as the number of stems per plot with symptoms in late-March 2013, shown by the boxes coloured red, yellow and green for high, middle and low levels, respectively. Temperature (°C) is shown on the horizontal axis. For other details, see legend to Figure 51.

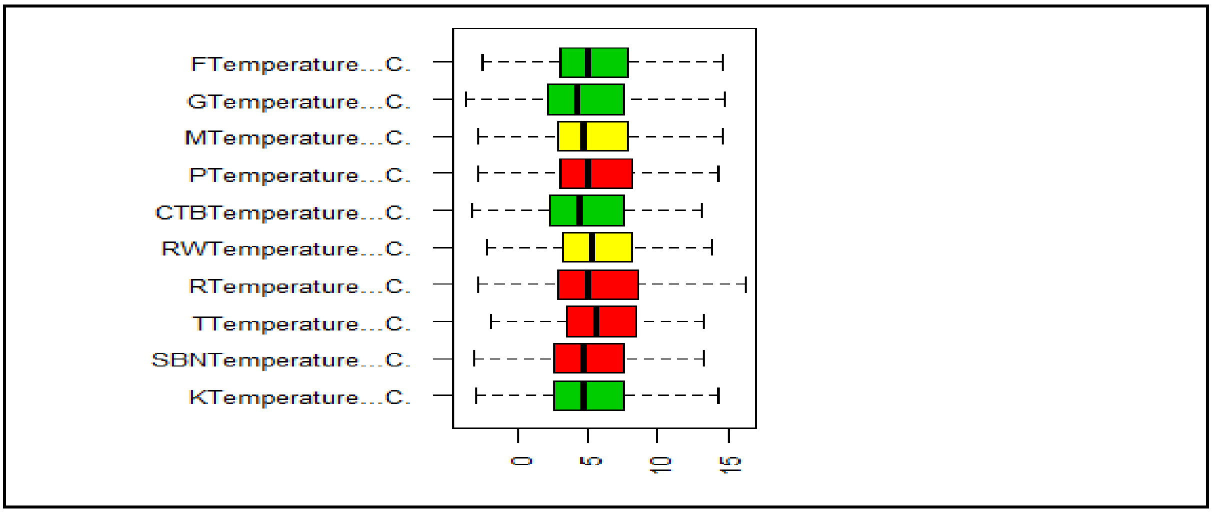


Figure 70. Box-and-whisker plot showing the level of daffodil rust at the ten sites in relation to air temperature recorded in March 2013. Daffodil rust levels are expressed as the number of stems per plot with symptoms in late-March 2013, shown by the boxes coloured red, yellow and green for high, middle and low levels,

respectively. Temperature (°C) is shown on the horizontal axis. For other details, see legend to Figure 51.

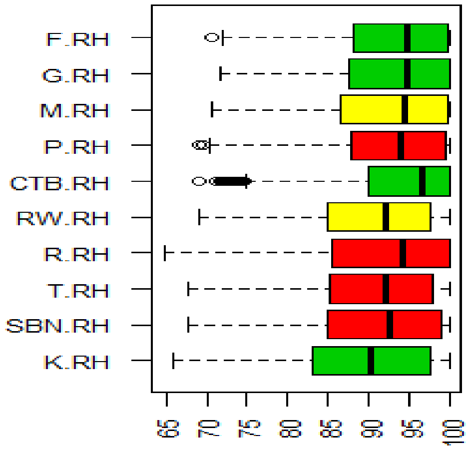


Figure 71. Box-and-whisker plot showing the level of daffodil rust at the ten sites in relation to RH recorded in November 2012. Daffodil rust levels are expressed as the number of stems per plot with symptoms in late-March 2013, shown by the boxes coloured red, yellow and green for high, middle and low levels, respectively. RH (%) is shown on the horizontal axis. For other details, see legend to Figure 51.

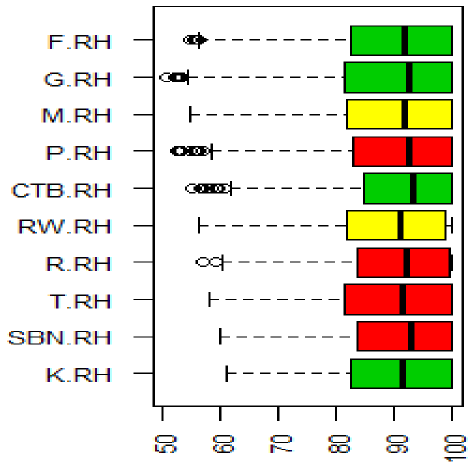


Figure 72. Box-and-whisker plot showing the level of daffodil rust at the ten sites in relation to RH recorded in March 2013. Daffodil rust levels are expressed as the number of stems per plot with symptoms in late-March 2013, shown by the boxes coloured red, yellow and green for high, middle and low levels, respectively. RH (%) is shown on the horizontal axis. For other details, see legend to Figure 51.

<u>Rust and temperature and humidity: Crop-year 2, 2013-2014</u>

Rust levels were also examined in relation to the previous year's soil temperature (measured at 15cm-depth), air temperature and RH data (01 April 2013 to 01 April 2014). Compared with SWC, the range of soil and air temperatures and RH across the ten sites was small and, confirming the findings of the previous year, rust levels (measured as severity or incidence scores or as the number of stems per plot with rust) could not be related to these variables (Figure 73 to Figure 75).

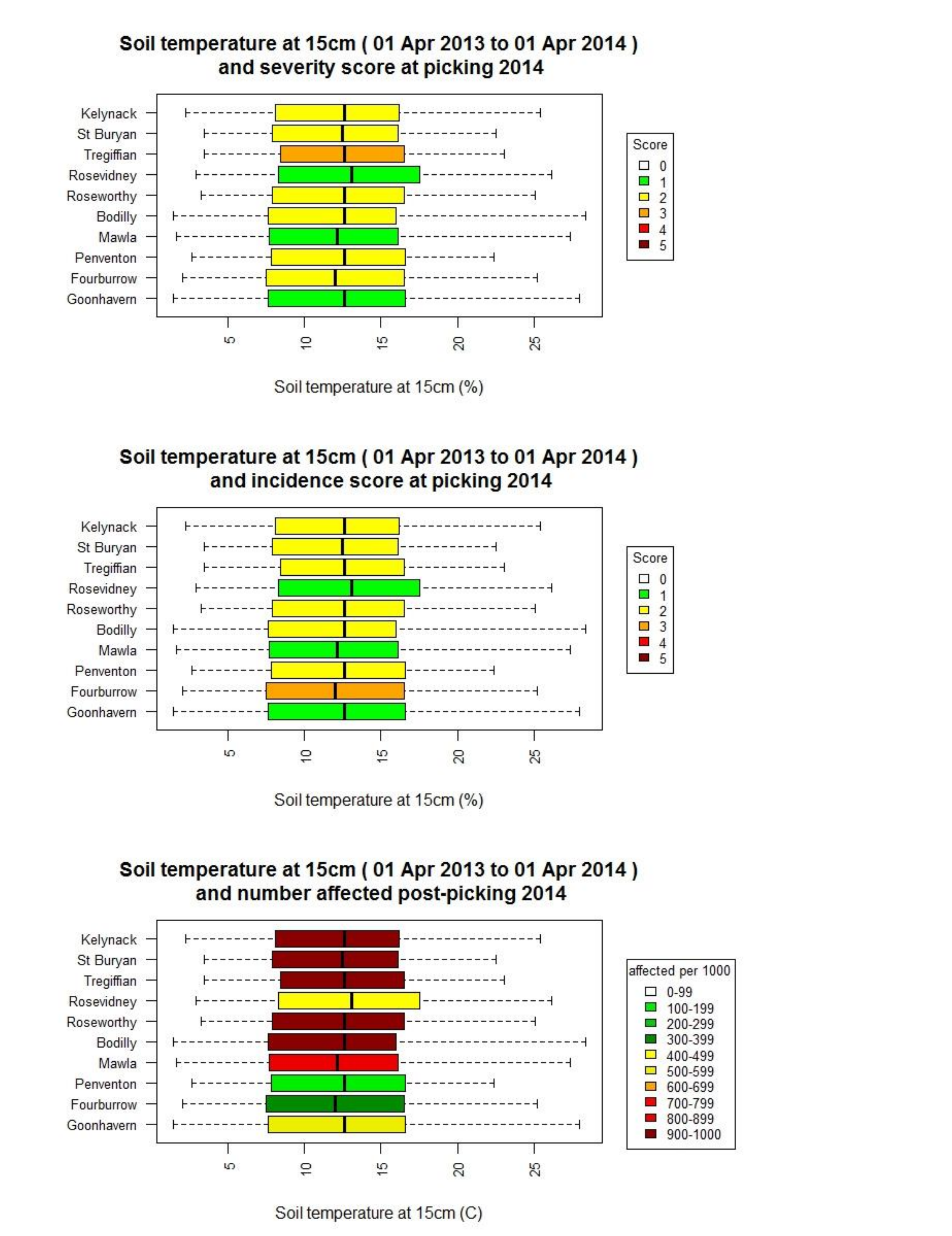


Figure 73. Box-and-whisker plots showing rust severity score at picking 2014 (top), incidence score at picking 2014 (middle) and number of stems per plot with rust

post-picking 2014 (bottom) at the ten sites in relation to soil temperature (15cm-deep) between 01 April 2013 and 01 April 2014.

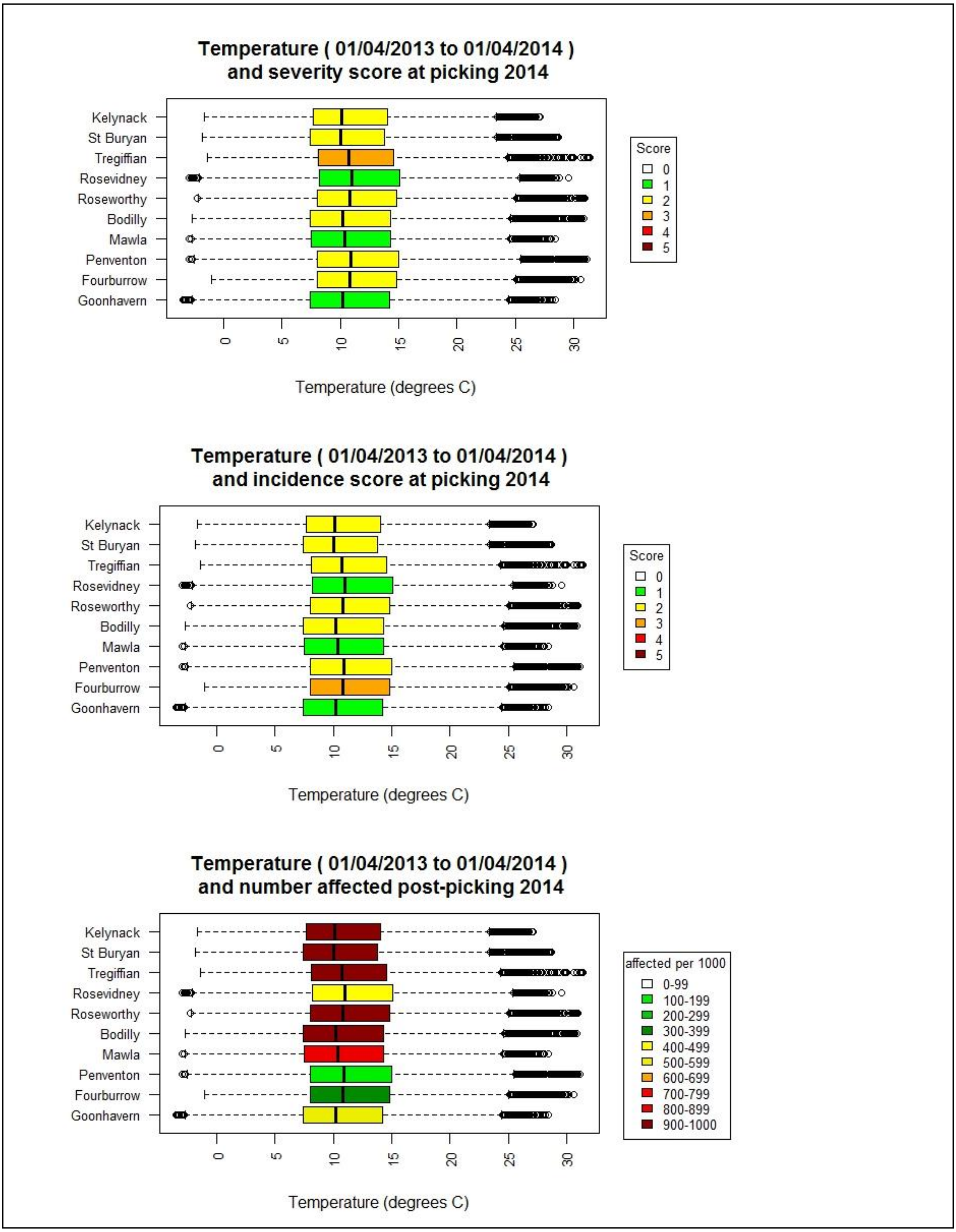
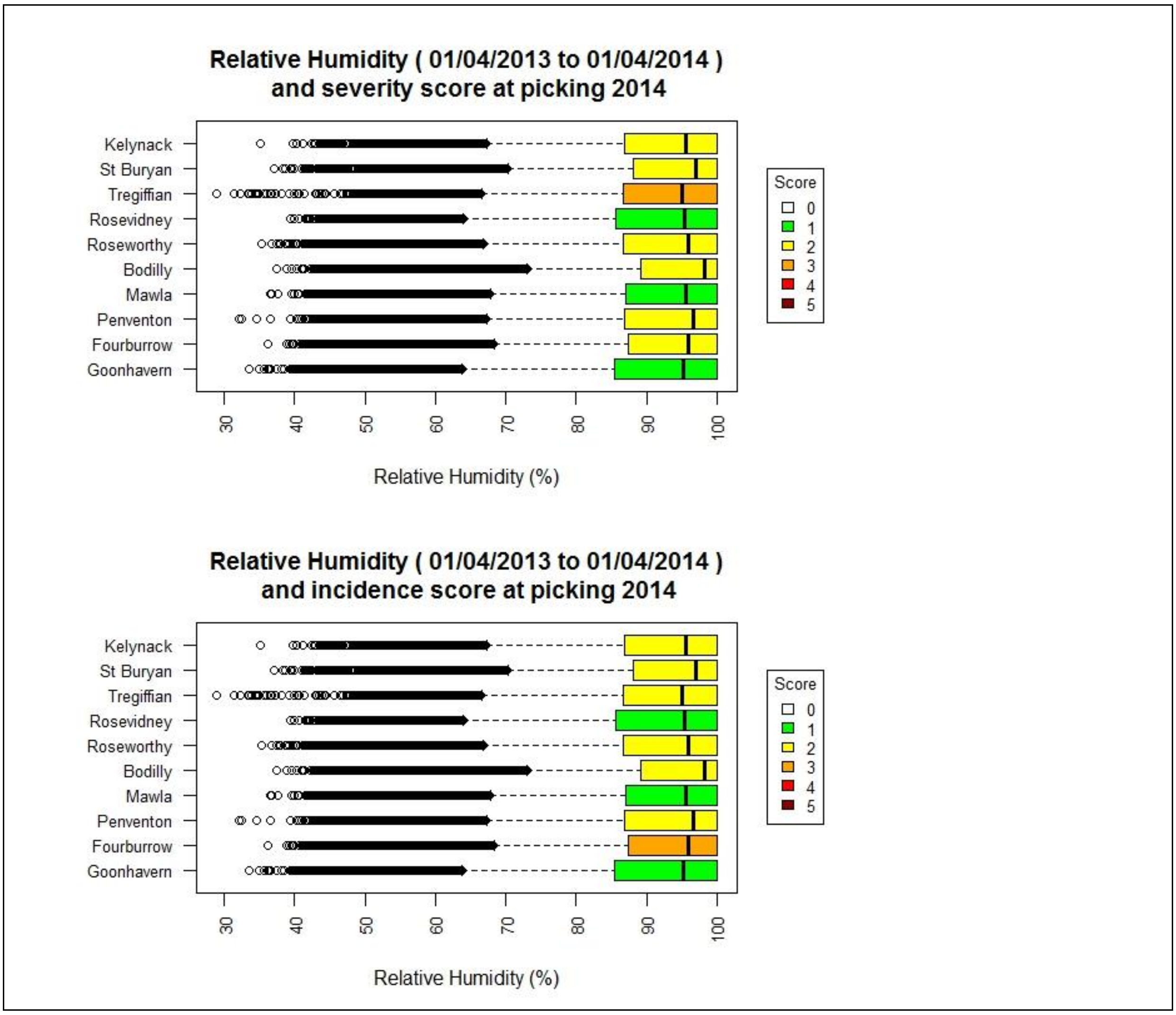


Figure 74. Box-and-whisker plots showing rust severity score at picking 2014 (top), incidence score at picking 2014 (middle) and number of stems per plot with rust post-picking 2014 (bottom) at the ten sites in relation to air temperature between 01 April 2013 and 01 April 2014.



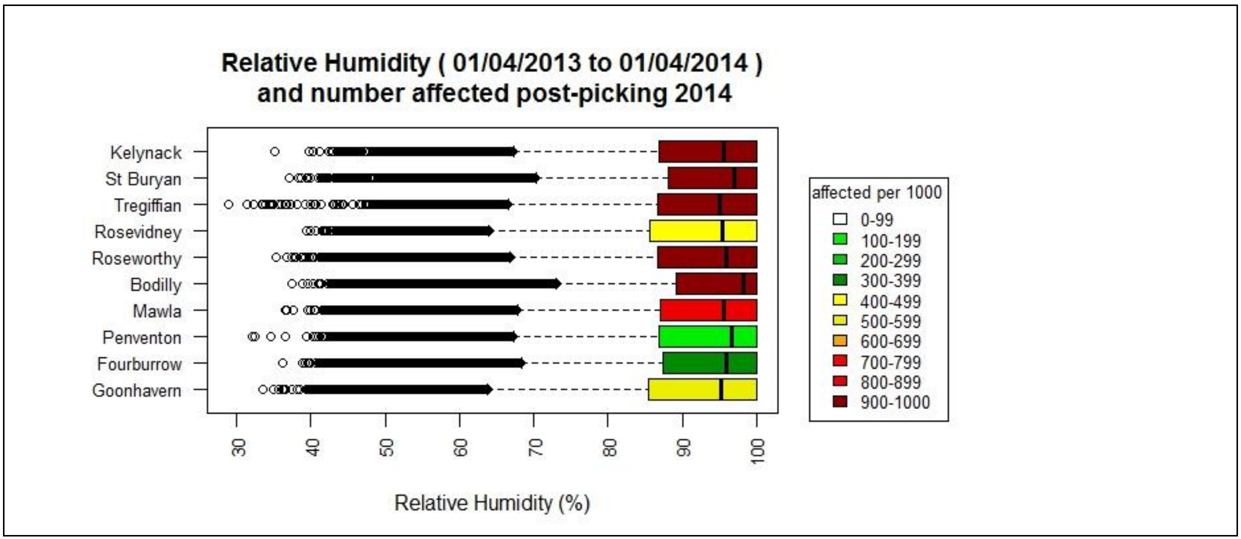


Figure 75. Box-and-whisker plots showing rust severity score at picking 2014 (top), incidence score at picking 2014 (middle) and number of stems per plot with rust post-picking 2014 (bottom) at the ten sites in relation to RH between 01 April 2013 and 01 April 2014.

Rust and geographical factors

It is likely that some effects of climate on plants could be represented by proxy measures such as longitude, latitude, altitude and distance from the sea. Scatter plots and regression analysis were used to look for relationships between these factors and rust incidence in 2013 and 2014. There was, however, no evidence of any relationship between these factors, with $R^2<0.3$ in all cases and therefore not significant (data not shown).

Rust and soil factors

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 (SSEW) (Table 4). Such factors are not easy to quantify, and Figure 76 to Figure 78 are simple histograms illustrating these factors in relation to rust incidence in 2013 and 2014, with different classes of soil texture, etc., colour-coded for easier visualisation. It is clear that a wide range of rust levels occurred in the predominant soil types just mentioned, and there is unlikely to be a relationship between rust incidence and soil texture or type.

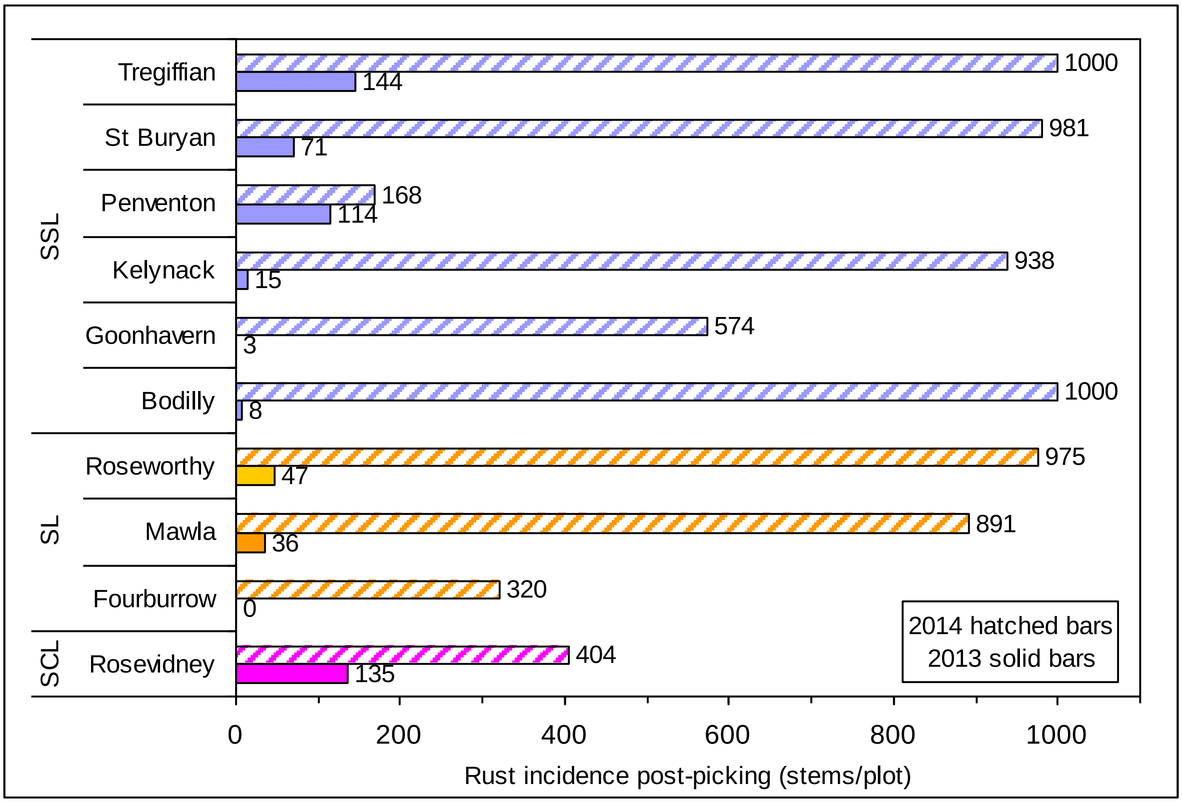


Figure 76. Rust incidence (number of stems per plot with rust post-picking) for the ten sites in 2013 and 2014, classified by ADAS soil texture (SSL, SL or SCL). The results for each soil texture are grouped together and colour-coded. Note the lack of consistency in rust levels within groups.

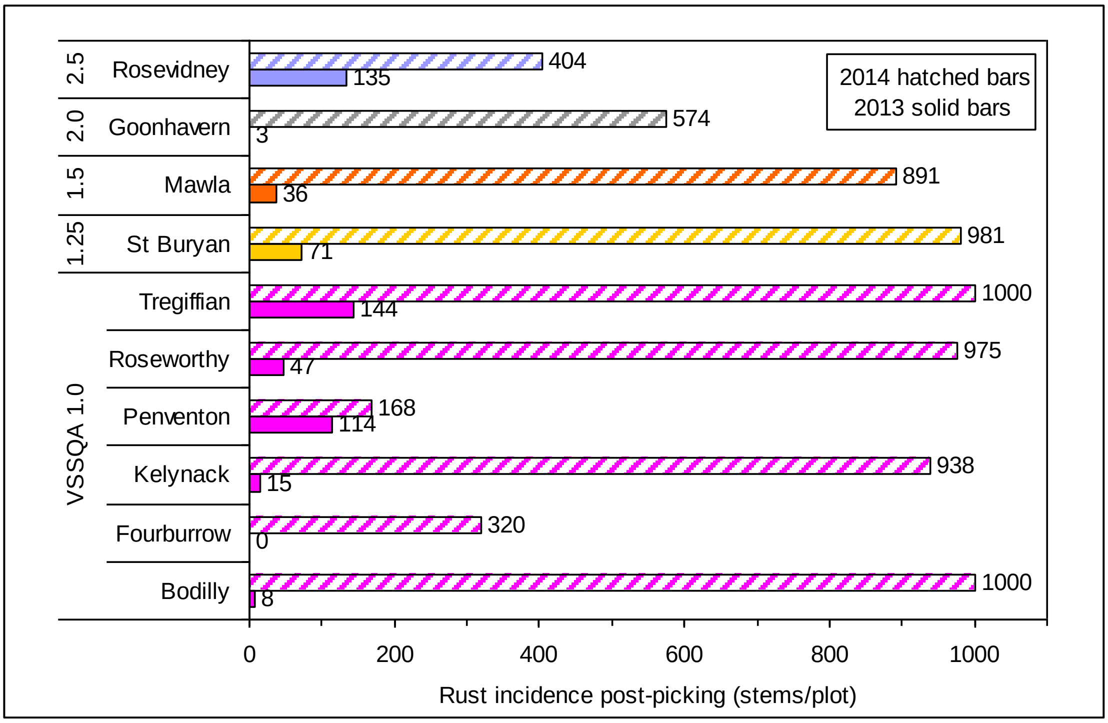


Figure 77. Rust incidence (number of stems per plot with rust post-picking) for the ten sites in 2013 and 2014, classified by VSSQA score (1 to 2.5). The results for each score are grouped together and colour-coded. Note the lack of consistency in rust levels within the main group.

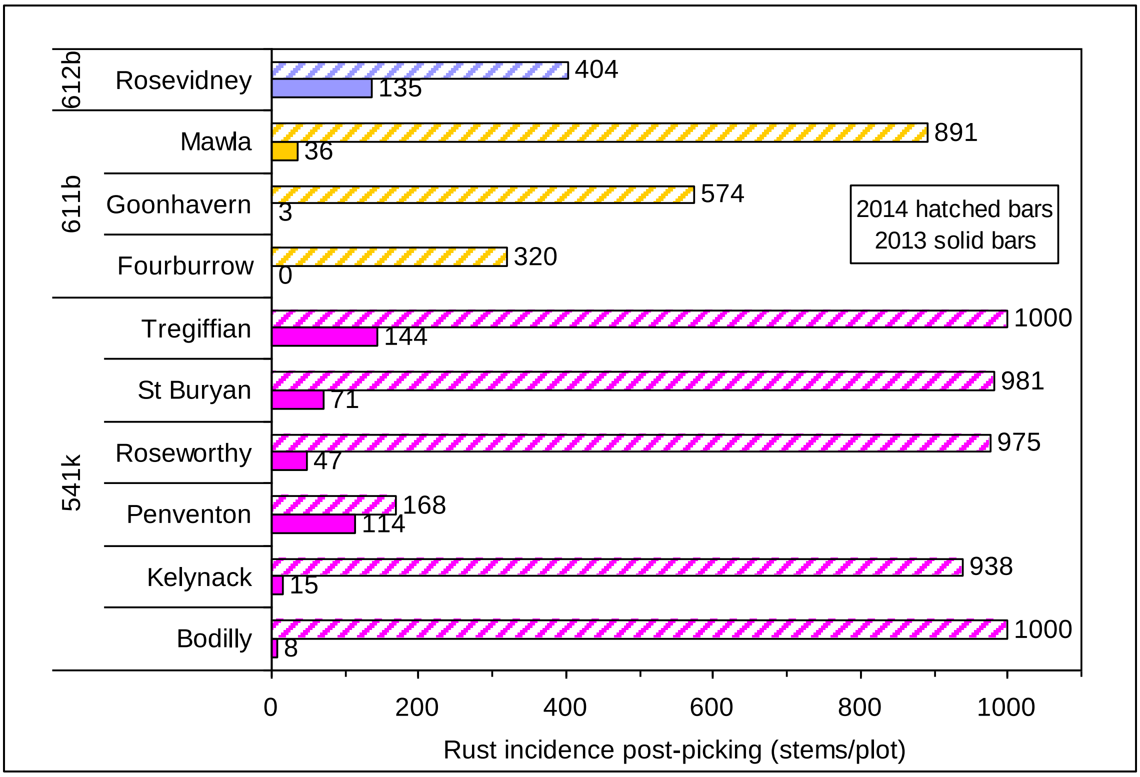


Figure 78. Rust incidence (number of stems per plot with rust post-picking) for the ten sites in 2013 and 2014, classified by SSEW soil sub-group (612b, 611b or 541k). The results for each soil sub-group are grouped together and colour-coded. Note the lack of consistency in rust levels within groups.

Some other, quantifiable soil properties – the percentage of initial soil water and of stones, sand, silt and clay (Figure 6, Figure 7) and the depth of top-soil and sub-soil (Figure 8) - also failed to show any relationship to rust levels in either year. Using simple scatter plots and regression analysis, R^2 was <0.3 (and often much lower) in all cases, and therefore not significant (data not shown).

Rust and husbandry factors

Previous cropping, type of fertiliser applied in advance of bulb planting, and the date of bulb planting (Table 3), simple histograms showed no suggestion of any relationship with subsequent rust incidence in either year. For example, a wide range of rust levels was seen in 2013 and 2014 following cereals, ley/meadow or brassicas (which would leave the soil in different states of nutrient depletion or enrichment) (Figure 79), or following the application of different fertilisers (Figure 80).

Bulb planting had to be delayed at some sites due to the very wet autumn of 2012. Although this could 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 81).

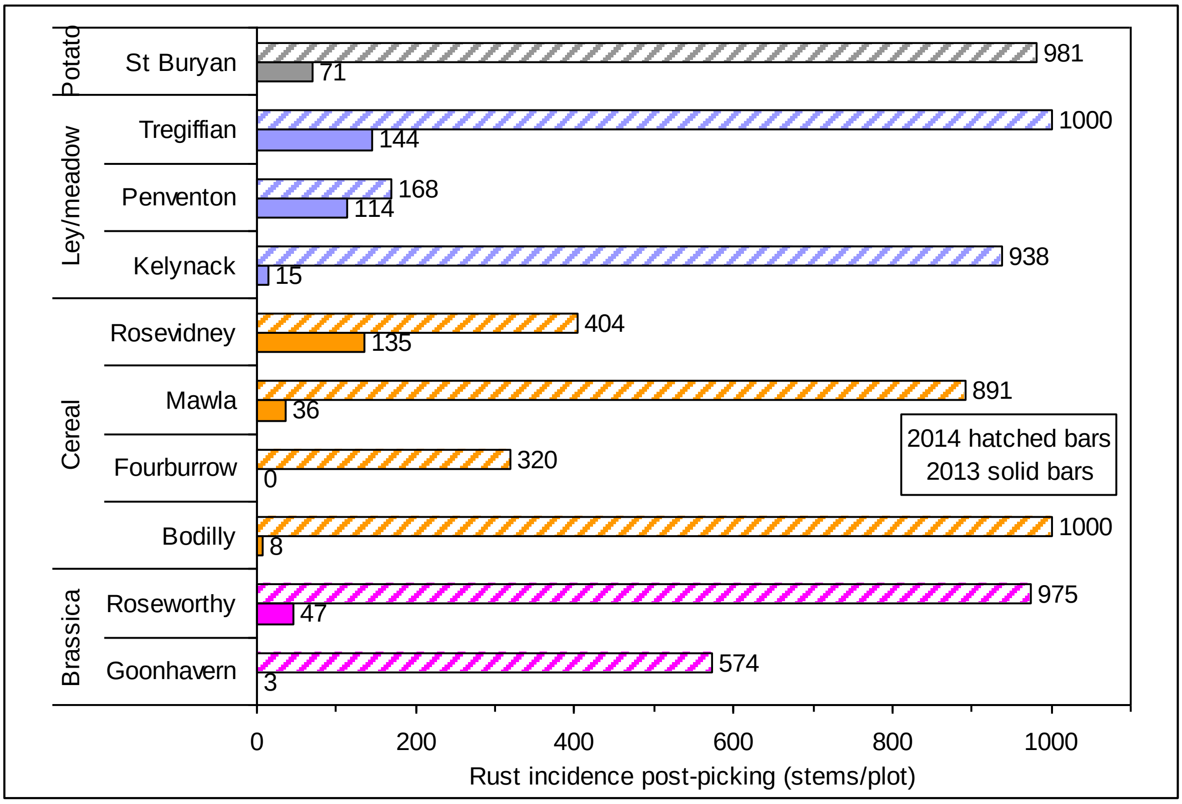


Figure 79. Rust incidence (number of stems per plot with rust post-picking) for the ten sites in 2013 and 2014, classified by previous cropping (brassicas, cereals, ley or meadow and potatoes). The results for each crop type are grouped together and colour-coded. Note the lack of consistency in rust levels within groups.

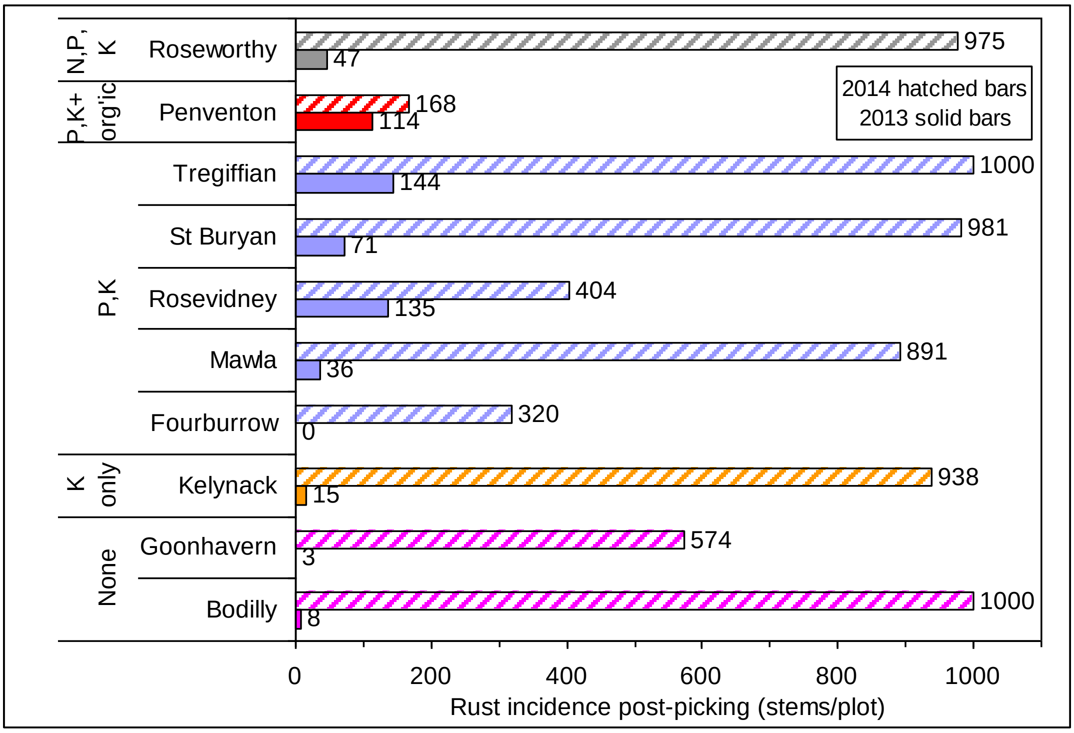


Figure 80. Rust incidence (number of stems per plot with rust post-picking) for the ten sites in 2013 and 2014, classified by fertiliser type previously applied (none, K only, P and K, P, K and organic fertiliser or N, P and K). The results for each fertiliser type are grouped together and colour-coded. Note the lack of consistency in rust levels within main groups.

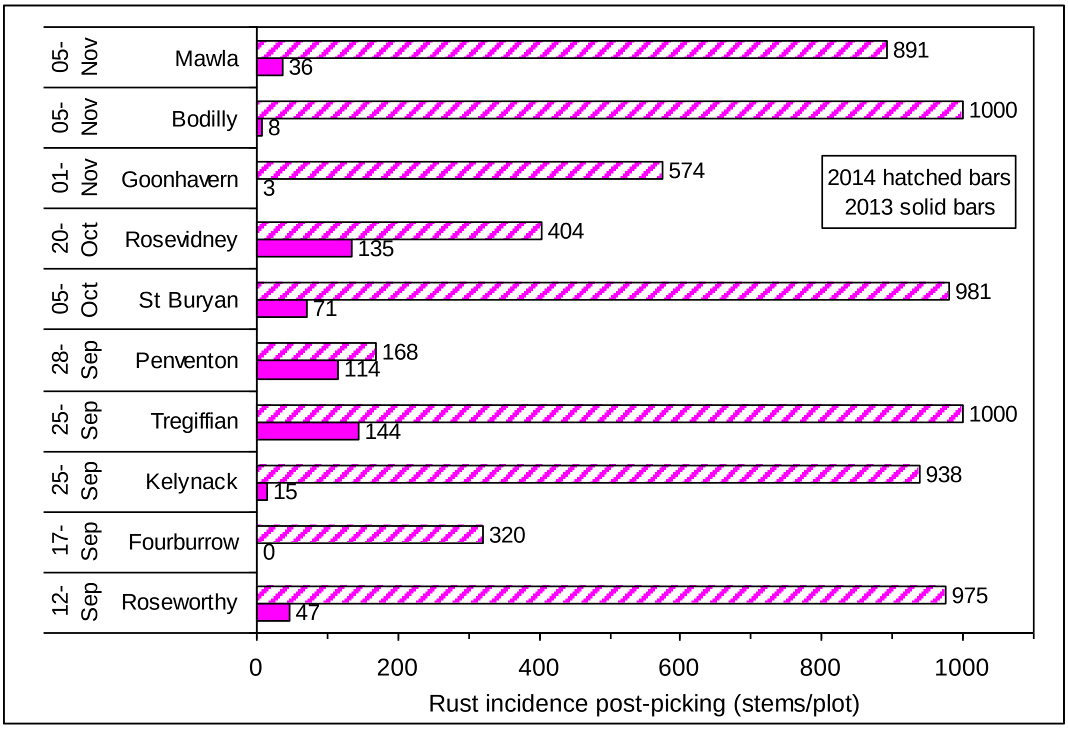


Figure 81. Rust incidence (number of stems per plot with rust post-picking) for the ten sites in 2013 and 2014, classified by planting date (ordered with earliest at top). Note the lack of any consistent trend across the planting dates.

Rust and soil and leaf nutrient concentrations

Logistic regression plots were used to look for relationships between rust levels and nutrient concentrations. Rust incidence in 2013 was examined using the soil analyses of autumn 2012 and spring 2013, and rust incidence in 2014 using the soil and leaf analyses of spring 2014. The 'R^2' values for these analyses are shown in Table 16 and are predominantly low (<0.4) and therefore non-significant. The only noteworthy relationships are for the number of stems per plot with rust in 2014 and top-soil organic matter (R^2=0.6841) and the soil concentrations of Mn (R^2=0.4885), Ca (R^2=0.4540) and SO_4 (R^2=0.4373). There appeared to be no noteworthy relationships with rust incidence in 2013 or with leaf nutrient concentrations (measured only in 2014). Linear regressions for these pairs are shown in

Figure **82**: rust incidence increased with increasing top-soil organic matter, Ca and SO_4, and fell with increasing Mn.

Table 16. Summary of linear regression analyses for rust levels (the number of stems per plot with rust at the post-picking stage in 2013 or 2014) and soil or leaf nutrients (soil concentrations measured in 2012, 2013 and 2014 and leaf concentrations measured in 2014). The more notable values of R^2 are shown in bold.

Nutrient concentration, soil organic matter or pH	**R^2 (McFadden's pseudo-R^2)**			
	Soil nutrient concentrations 2012 and rust incidence 2013	**Soil nutrient concentrations 2013 and rust incidence 2013**	**Soil nutrient concentrations 2014 and rust incidence 2014**	**Leaf nutrient concentrations 2014 and rust incidence 2014**
Aluminium	-	0.3904	0.1060	0.0547
Boron	-	-	0.0420	0.1113
Calcium	-	0.0074	0.4540	0.1743
Copper	-	-	0.1669	0.1258
Iron	-	0.0023	0.0008	0.0336
Magnesium	0.0178	0.0280	0.2058	0.1600
Manganese	-	0.0142	0.4885	0.0938
Molybdenum	-	-	0.0862	0.2254
Nitrogen	-	0.0330	0.0183	0.3389
…nitrate	0.0095	0.0500	0.0171	-
…ammonium	-	0.0201	0.2304	-
Phosphorus	0.0958	0.1274	0.0159	0.0487
Potassium	0.0236	0.0319	0.0019	0.0125
Sodium	-	0.0018	0.3526	0.3526
Sulphate	-	-	0.4373	0.3578
Zinc	-	-	0.0904	0.2833
Top-soil organic matter	-	-	0.6841	-
pH	0.0503	0.0220	0.2615	-

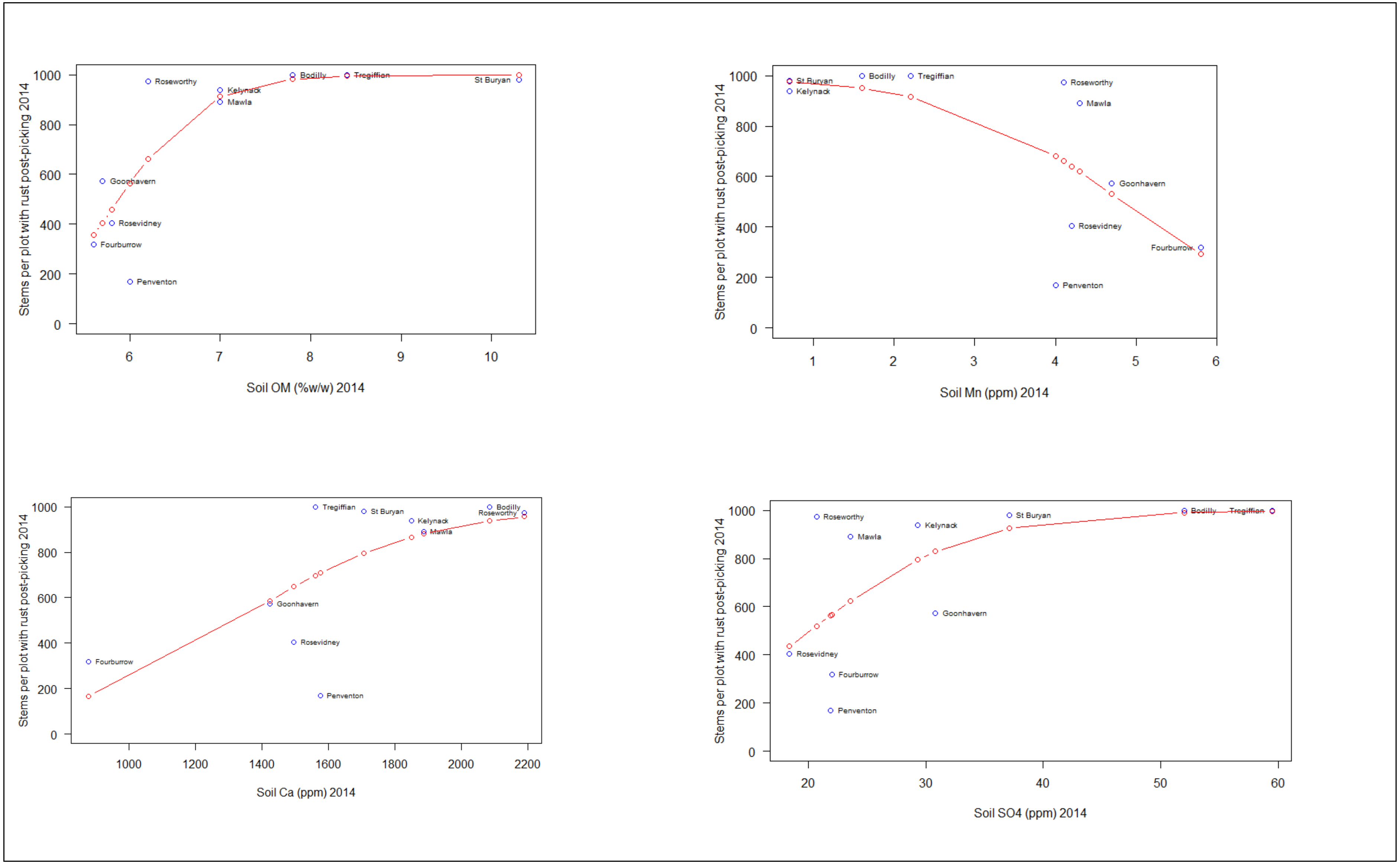


Figure 82. Logarithmic regression plots of top-soil organic matter (OM) and soil Mn, Ca and SO4 concentrations and rust incidence in 2014.

Discussion

Daffodil rust is a threat to UK horticulture

Awareness of the physiological disorder known as daffodil rust, and its recognition as a significant problem for UK daffodil growers, has come about since the early-1990s, so it is still a relatively new problem. The sporadic nature of rust, between years, locations and cultivars, has added to the difficulties of understanding rust and developing a management strategy. The present AHDB Horticulture-funded project was initiated after one of most severe rust episodes, in spring 2011, and since then rust levels appear to have been much lower. The year 2014 was, by general consensus, a year largely free of rust, but nevertheless some late-season crops were damaged to a significant degree. The flower trader's surveys of 2013 and 2014 (see Appendix 2) should serve to remind the industry of a background level of rust ready to respond to favourable conditions, while the present study has provoked a closer look for rust spots and has highlighted the insidious nature of the disorder. While some growers may be reluctant to talk about rust, rust has certainly not "gone away".

AHDB Horticulture's surveys of growers showed that rust has real cost implications for daffodil growers: in the survey for 2011 the estimated losses in flower revenue due to rust were said to vary from 1 to 15% in a serious rust year, though these years were interspersed with good years in which rust incurred no losses. Growers responding to the earlier surveys quoted an average reduction in turnover of between 2 and 3%. In the absence of more comprehensive figures maybe it would not be unreasonable to work on a 3% average annual loss or losses of 10% one year in three. Because of the way government horticultural statistics are collected in the UK, it is only possible to quote estimated values of the UK daffodil industry.[2] Using these estimates a loss of 3% in turnover of UK daffodil cut-flowers due to daffodil rust would amount to about £0.7m annually, or around £2.3m every three years. While these amounts are modest, they mask a larger threat. Daffodil production has been hailed as a truly successful sector of UK horticulture, notable for its exports, since the 1990's, but currently faces a number of issues – continuing

[2] Data from the University of Reading (Crane *et al*., 2014) give an annual value of £14m for UK cut-flower exports. Assuming that daffodils make up the bulk of these exports, and that 60% of UK daffodil cut-flowers are exported, UK production would be somewhat in excess of £23m annually. These figures are in line with other estimates used recently (Hanks, 2012).

low returns, uncertainties in the continuity of supply because of unpredictable weather, large structural changes in the industry, the loss of pesticides (particularly for use in hot-water treatment), and an inability to solve long-running problems such as base rot – even worries about supermarkets displaying daffodils by the fruit and vegetable aisle! Although not widely accepted at present, a continuing problem with rust could result in lowered customer perception of the product and a loss of sales on which UK bulb growing is dependent.

First evidence that adverse soil-water conditions can invoke rust

There is no evidence that rust results from a pathogenic or nutritional cause. Suggestions of the conditions possibly predisposing daffodils to rust have therefore concentrated on the effects of adverse environmental conditions, such as rapid changes in temperature or water availability. Such conditions can easily be envisaged as leading to problems of water uptake and translocation to the very rapidly growing stems and buds of cold-treated daffodils, the more so in large-budded double cultivars like 'Golden Ducat', doubles being forced under glass, and other double cultivars prone to flower-opening disorders such as 'Cheerfulness' and *Narcissus poeticus* 'Flore Pleno'. Such a cause would have some parallels with physiological disorders that cause physiological spotting in other horticultural and agricultural crops.

SWC, rainfall, soil temperature, air temperature and RH have been continuously monitored in trial plots of 'Golden Ducat' across west Cornwall. The changing patterns of these variables were examined for any relationships with the severity and incidence of rust in each year and at each location. From the first year's data it was clear that the patterns of soil and air temperatures and RH varied little between sites, whereas marked differences in the incidence of rust between sites were clearly evident, so these factors seemed unlikely to be responsible for favouring or discouraging rust development. SWC, however, varied considerably between sites, and at three of the four sites with relatively high rust incidence SWC was also high. In the second year of the study the differences between sites in rust incidence were greater than in the first, and there was a correspondence between high rust incidence and high levels of SWC. The extremely wet winter of 2013-2014 led to the suggestion that rust levels would be higher than in the previous year, which appears to have proved true. A different pattern of rain in winter 2014-2015 may provide a further test of this tentative finding.

Other factors may be involved too

So far little is understood of the mechanics of these SWC effects. For example, SWC has been monitored at 10, 20 and 30cm depth, and the relative water content at the different depths is by no means consistent. Variations in soil type, drainage and local rainfall patterns will all affect results, and may throw light on, for example, the failure of *all* the wettest sites to produce the highest rust incidence. In spring 2013 the 'atypical' site, Penventon, with high rust incidence but lower SWC, has some features that distinguish it from the other sites, such as a free-draining, south-facing aspect and a previous history as a longer-term ley with cattle grazing and no application of inorganic fertiliser before bulb planting.

Little is known of the water relations of daffodils. In studies by the Skierniewice, Poland, group of flower-bulb researchers, SWC was shown to have marked effects on bulb yields in daffodils (Strojny, 1975; Goniewicz et al., 1976) as well as more subtle effects on root nutrient content (Dabrowska, 1975) and root anatomy and stomatal numbers (Goniewicz et al., 1976). This group found that increasing SWC from 40 to 95% of available water capacity increased root (but not bulb) P and K content, and bulb yields were highest in soils near field capacity. In the UK, irrigation was shown to have distinct benefits on yields (Anon., 1985), and while it is rare for daffodil crops to be irrigated in the UK, it is generally accepted that a high SWC after flowering, when the new bulb units are bulking up, is beneficial to bulb yields. In developing a daffodil crop model, Wurr *et al.* (2002) showed that (a) yield of bulbs in the first crop-year was highly correlated with rainfall accumulated between planting and leaf senescence, (b) the yield of bulbs in year 2 was determined by rainfall from planting in year 1, rainfall from emergence in year 2, and by day-degrees >0°C accumulated between flowering and leaf senescence in year 2, and (c) the yield of flowers in year 2 was correlated with bulb yield in year 1 and therefore to the rainfall from planting to senescence in year 1. This raises the possibility that a high SWC might change from adverse to beneficial over a fairly small range.

Daffodils can exhibit severe but temporary wilting if bright morning sunshine follows a cool night. This may indicate that the stress physiology of daffodils is not well developed, and indeed Barton-Wright & Pratt (1931), taking porometer readings of daffodil leaves, found that the stomata took about 2 hours to open or close fully. They were relatively insensitive to stress, for example remaining open under a heavy rain shower. It is unfortunate that no more recent work on daffodil stomatal physiology has been reported.

Can pathological and nutritional causes of rust be discounted? Although previous diagnostic examinations and analysis of soil and plant nutrient levels have given negative results, there have been indications in this study that they may yet be involved.

During examination of stem samples from the ten sites, and despite other fungi being present in some samples, a *Stemphylium* sp. was consistently isolated from samples with typical rust spots. On this basis it would be tempting to suggest that *Stemphylium* is the cause of rust symptoms, especially as it was isolated from surface-sterilised as well non-surface-sterilised tissues. But *Stemphylium* spp., especially *S. herbarum*, are often associated with moribund tissues on a variety of herbaceous plants, and are considered secondary invaders. Caution should also be exercised as the stems as received in the laboratory were not totally isolated from one another, so there may have been scope for cross-contamination. On the other hand the two samples without significant rust lesions did not yield *Stemphylium*. During incubation in a humid box, the rust lesions did not appear to enlarge before the tissues as a whole senesced and rotted, suggesting that perhaps the lesions may be a limiting host-resistance response analogous to a hypersensitive reaction. We might speculate that the rust spots are the result of infection by a weak pathogen (e.g. *Stemphylium*) under certain environmental conditions, and that the infection is effectively limited by such a host-resistance response. Work on this topic is on-going as part of the project extension.

The possibility of a bacterial pathogen causing rust was all but ruled out by the present study. On the other hand the analysis of rusty and 'healthy' stem samples for viral RNA will be completed in 2015.

In the current study few associations were found between rust incidence and soil levels of major nutrients or trace elements. However, there were suggestions that higher rust levels were associated with higher soil concentrations of calcium or sulphate, and lower rust levels with lower concentrations of manganese. Higher rust levels were, however, strongly associated with higher contents of top-soil organic matter. These findings were from a single year's work, and should be treated with caution until further determinations are made in 2015. Again, from a single year's work, rust incidence was not associated with particular levels of major nutrients or trace elements measured in the leaves.

Since daffodil rust is a relatively recent concern, its appearance might be a consequence of the substantial changes in bulb husbandry that have occurred since the 1970's, when daffodil growing may be said to have changed from 'traditional' to 'contemporary' practices. Principal changes include the introduction and adoption of hot-air drying, bulk handling, more efficient HWT, early planting, substantially increased planting densities, longer growing cycles (three-years-down at least) and more frequent flower picking and at an earlier growth stage. No specific connections with rust incidence have been proposed, however.

A simple genetic cause of daffodil rust appears unlikely because of the observed wide range of cultivars affected by the disorder and the sporadic nature of symptoms. However, adjacent rusty and healthy daffodil varieties can be observed growing in the same field, suggesting there may be varietal differences in susceptibility. In variety trials at Rosewarne EHS (see 'Introduction'), not all cultivars showed symptoms in both years of a study, so no inference of a genetic or transmissible basis could be made.

A final suggestion is that physiological disorders such as daffodil rust could be caused by some form of pollution, although no coherent case has been presented. Published reports about the adverse effects of likely pollutants on bulb species are sparse or dated. De Hertogh & le Nard (1993) mention ethylene and fluoride as pollutants, but give no examples for daffodils. The effects of 'illuminating gas' (containing *ca* 3% ethylene) on daffodil cultivars was reported by Hitchcock *et al.* (1932); the effects included the retardation of leaf, stem and bud growth, slight to extensive distortion or curling of the leaf-tips, and bud necrosis. Fluorine, as an air pollutant or a contaminant in superphosphate, can cause a leaf-tip scorch (Spierings, 1969; Gould & Byther, 1979). In maritime situations crops may be exposed to salt spray driven by strong winds, which has been suggested as a possible cause of damage to daffodils. At the two 'Golden Ducat' plots closest to the sea, the concentrations of sodium in the leaves were higher than at the other sites, and although rust incidence was also high at these two sites, it was equally high at three other sites away from the sea and where leaf levels of sodium were low.

Rust symptomatology

The first good description of early rust symptoms on stems was provided by Andrew Tompsett, who perceptively observed that the mildest or earliest stage of rust was a "slight blistering of epidermis only - not really rust[-like]". This form of 'slight blistering' of the epidermis was widely seen on stems in the trial plots, perhaps most clearly when found in association with one or two typical, small rust lesions. Small, slightly sunken tracts are also seen along the stems, which may also be connected with rust. Such observations raise the possibility that rust is always present to some degree in daffodil crops, or at least in 'Golden Ducat'. In the trial plots rust lesions appeared to be present from soon after the top of the stem had become visible between the shoots, and to increase slowly up to and beyond the flowering stage. It is possible that the early-stage lesions are already present in the lower part of the stem, the part growing through the ground or in the bulb itself. In commercially significant cases of daffodil rust, presumably the increase in lesion incidence and severity is much more explosive. The length of time the lesions will be visible in a commercial crop will depend on the rate of growth and development of the stem from soon after shoot emergence to flower picking, a window of only about three weeks. The question to ask may be "what factors cause daffodil rust to develop quickly between shoot emergence and flower picking, producing unmarketable stems?"

Managing rust

Assuming progress continues to be made on the effect of SWC on rust, what are the likely prospects for managing rust? While risk-avoidance, such as avoiding unsuitable sites and replacing rust-prone cultivars, is an obvious answer, pressures on suitable farming land might make this impractical on any significant scale. Knowing the environmental cause(s) of rust would at least lead to better risk-assessment regarding suitable sites for rust-prone cultivars. On the other hand, some practical steps might be worthwhile, such as attention to improving drainage in potentially waterlogged fields or low-lying parts of fields by digging temporary ditches or running tines along the furrows before winter rains. It has also been suggested that daffodils may have relatively poor water-use efficiency, and if this were to be confirmed the water use of daffodils might be manipulated at key growth stages through the use of anti-transpirants and practical measures to change stomatal frequency.

Technology transfer

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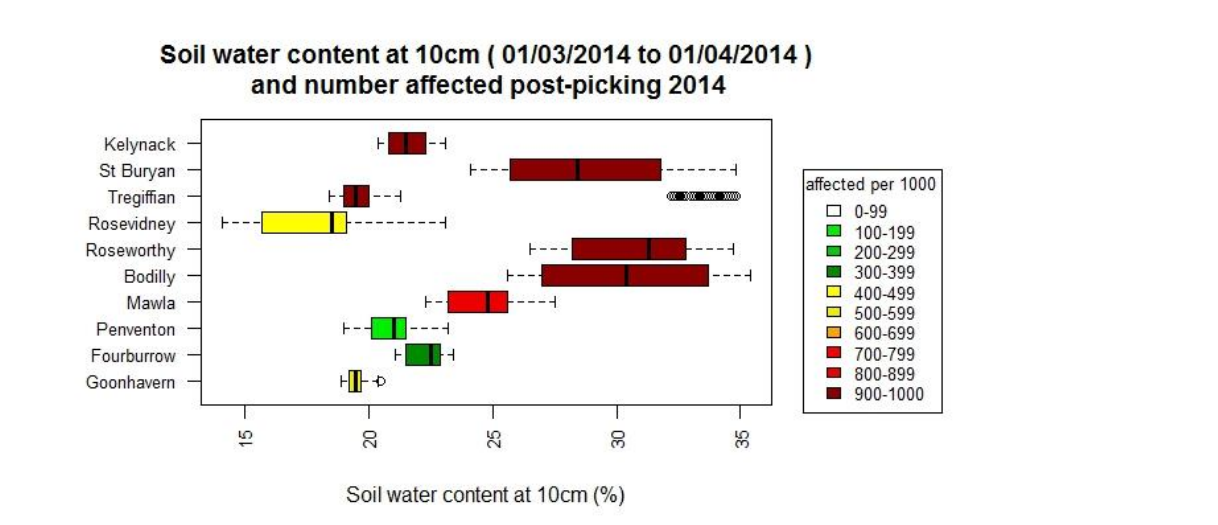
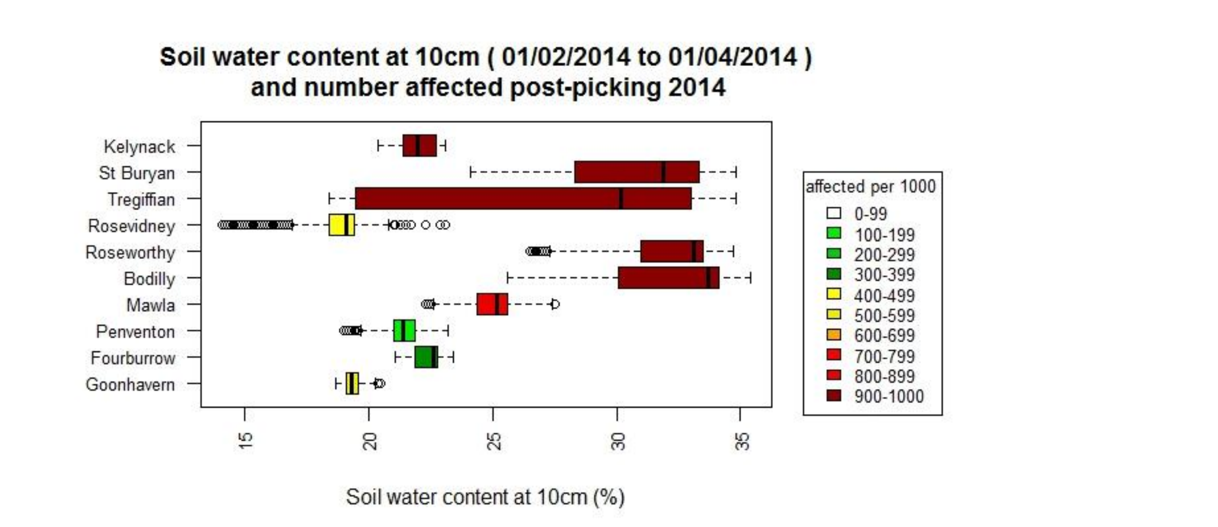
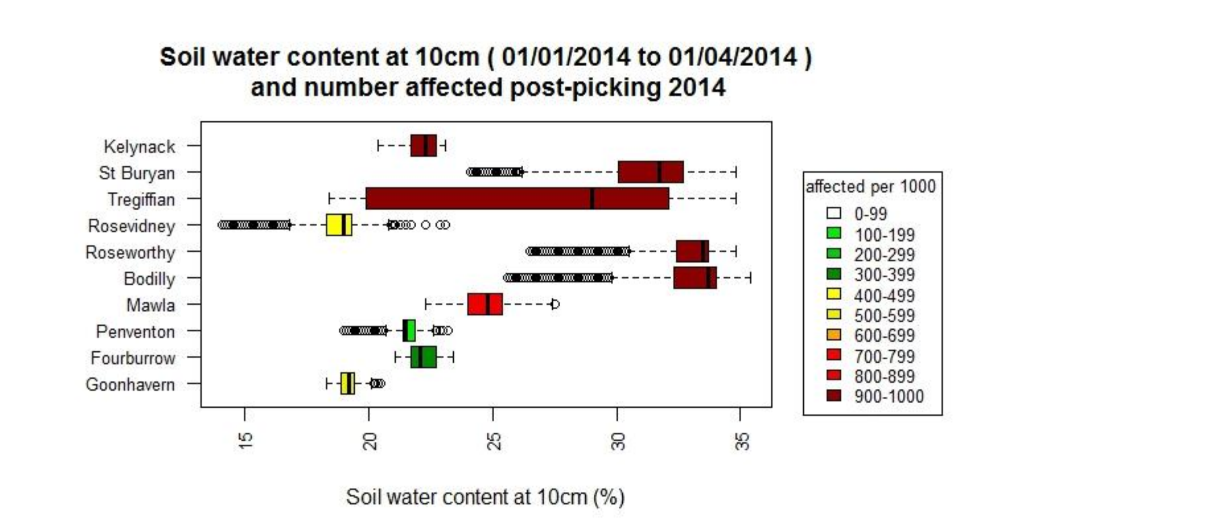
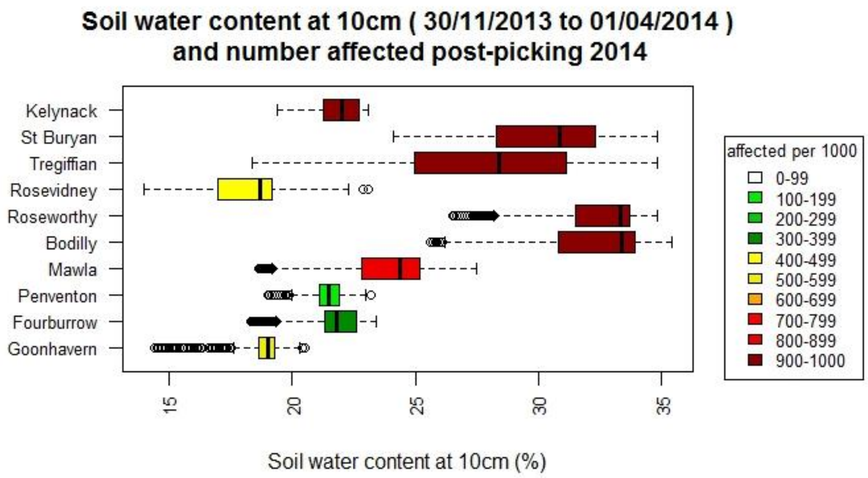
Appendix 1: Further results

Box-and-whisker plots showing the number of stems per plot with rust at the ten sites post-picking 2014 in relation to SWC 10cm deep over the periods stated, from 01 March 2014 to 01 April 2014 (top) through to 01 November 2012 to 01 April 2014 (bottom).





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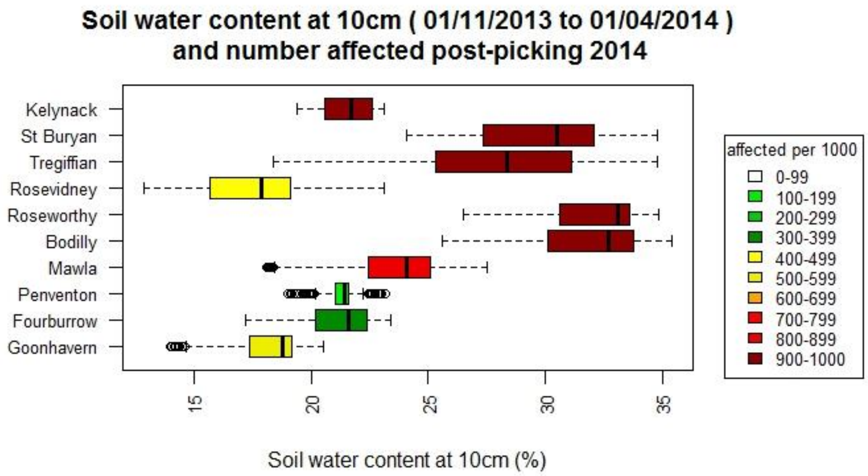
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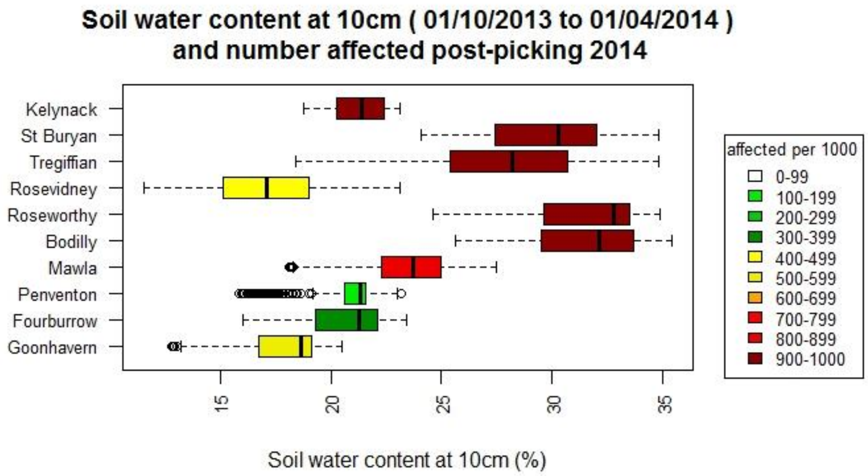
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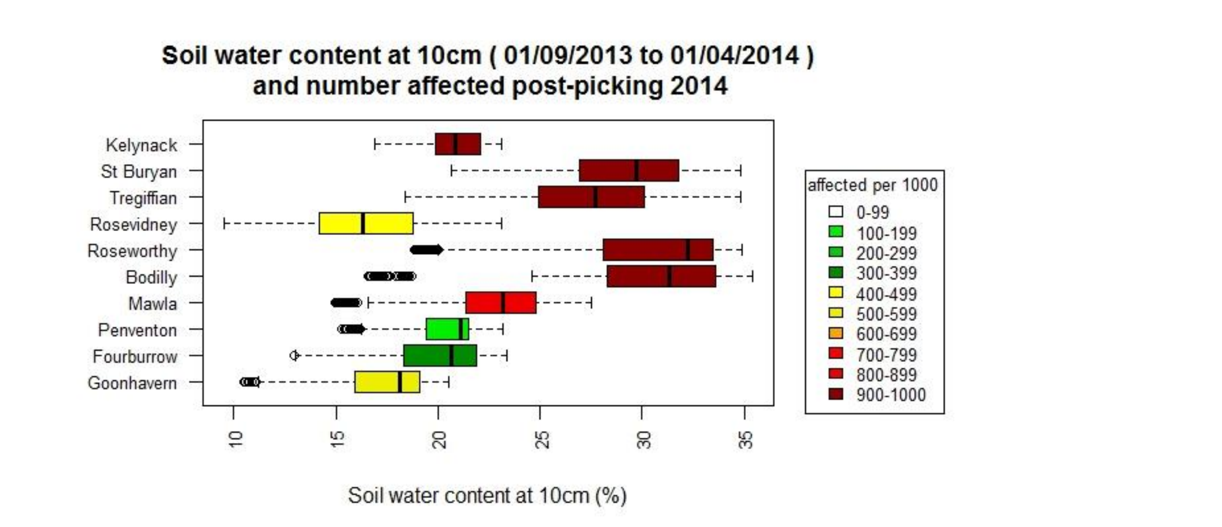
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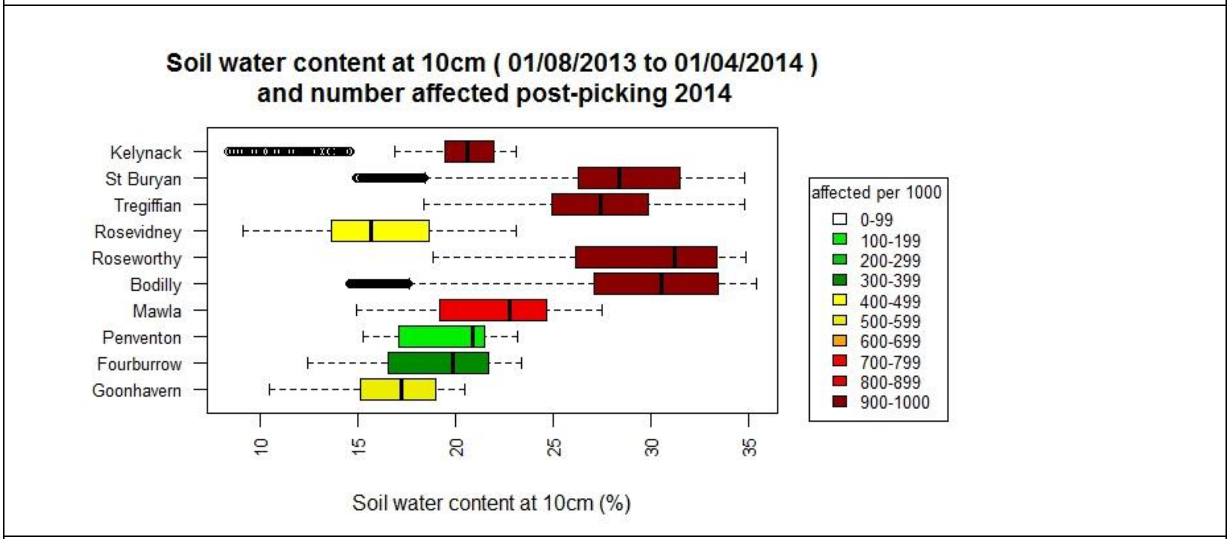
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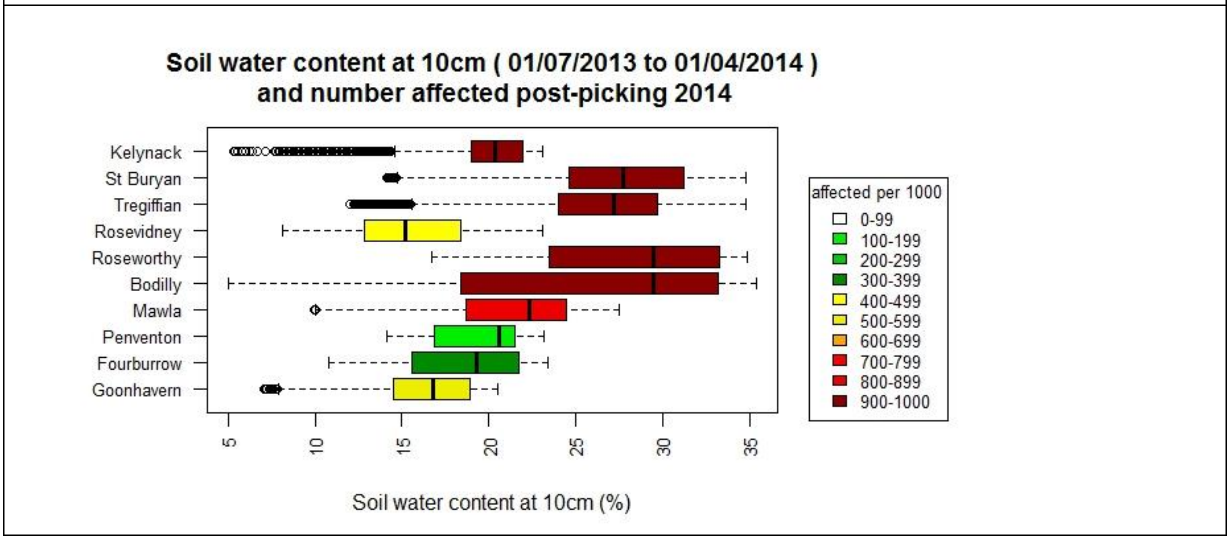
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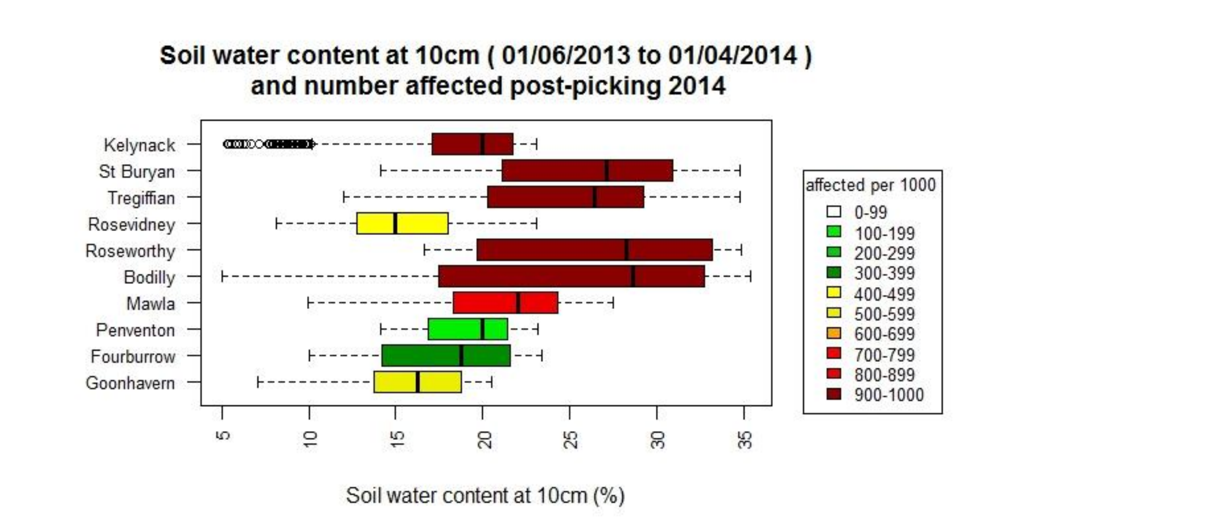
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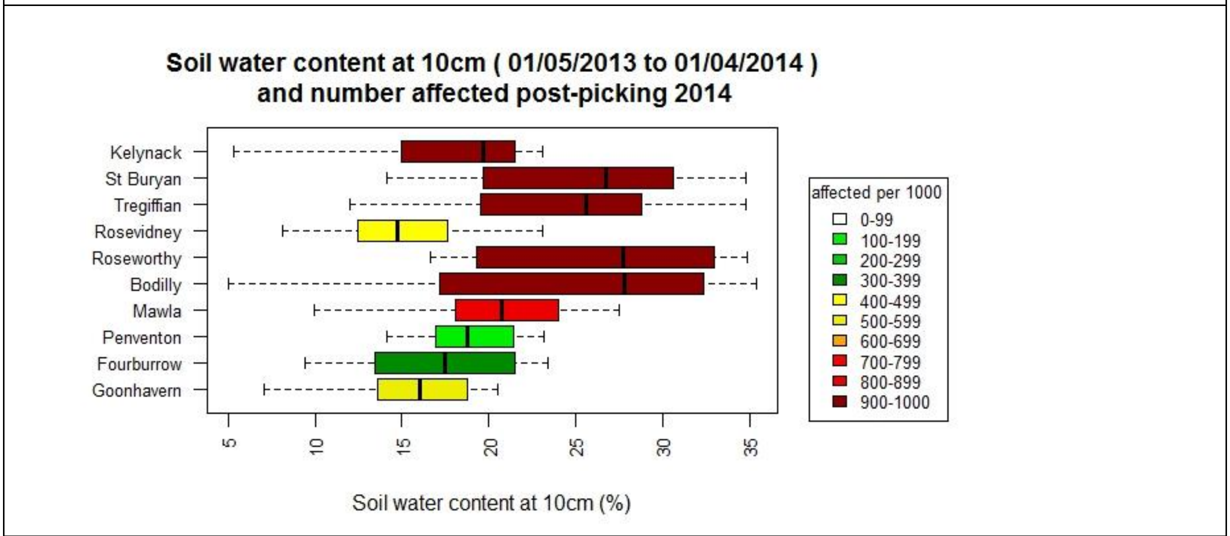
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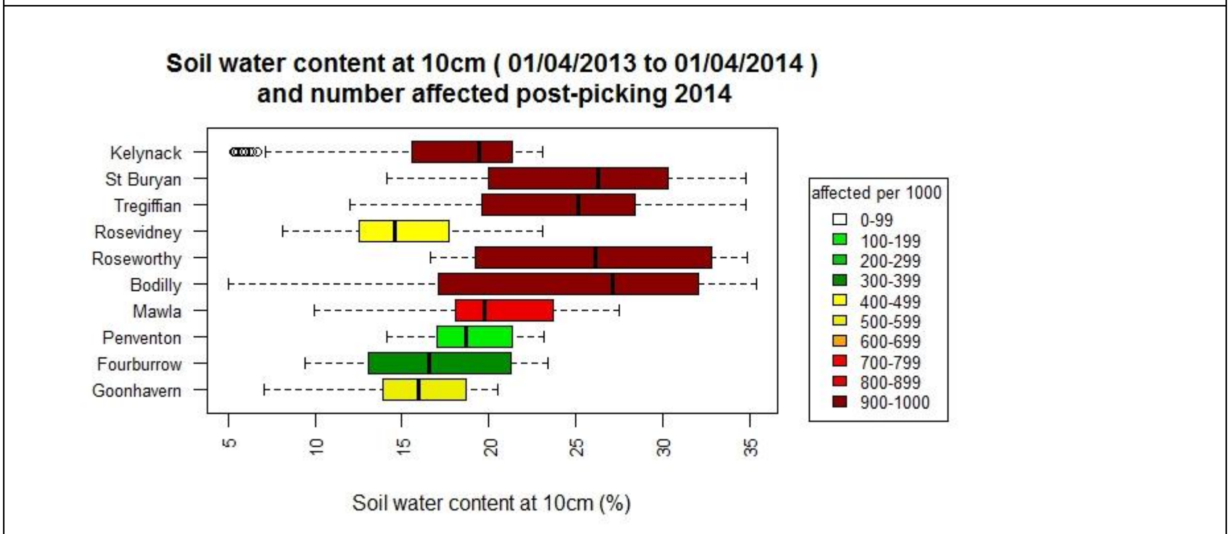
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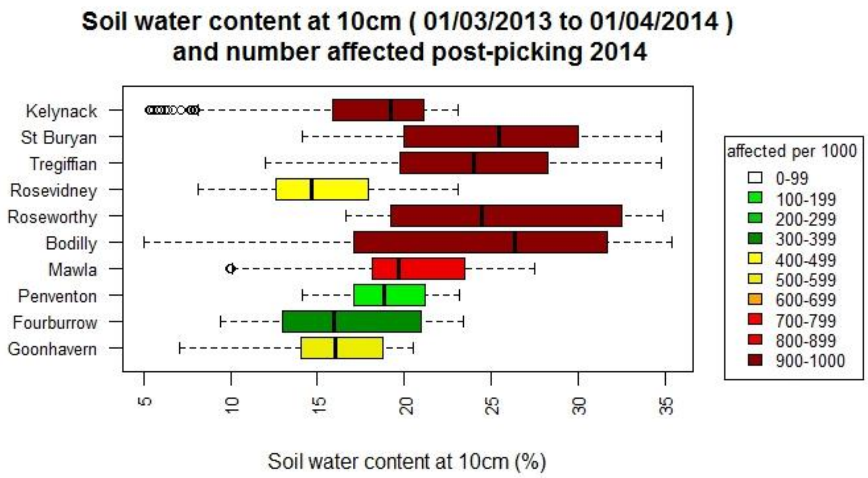
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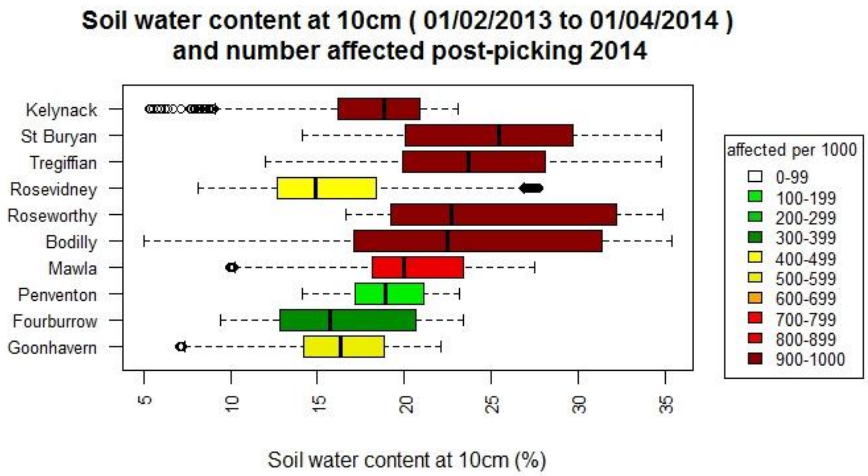
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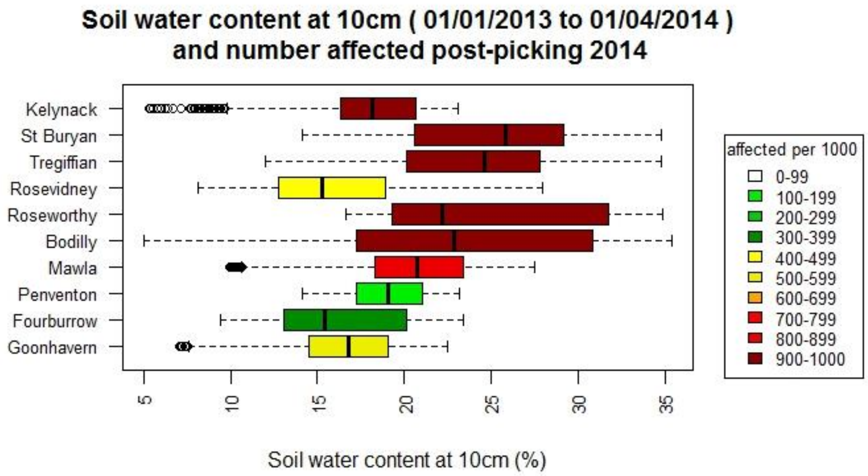
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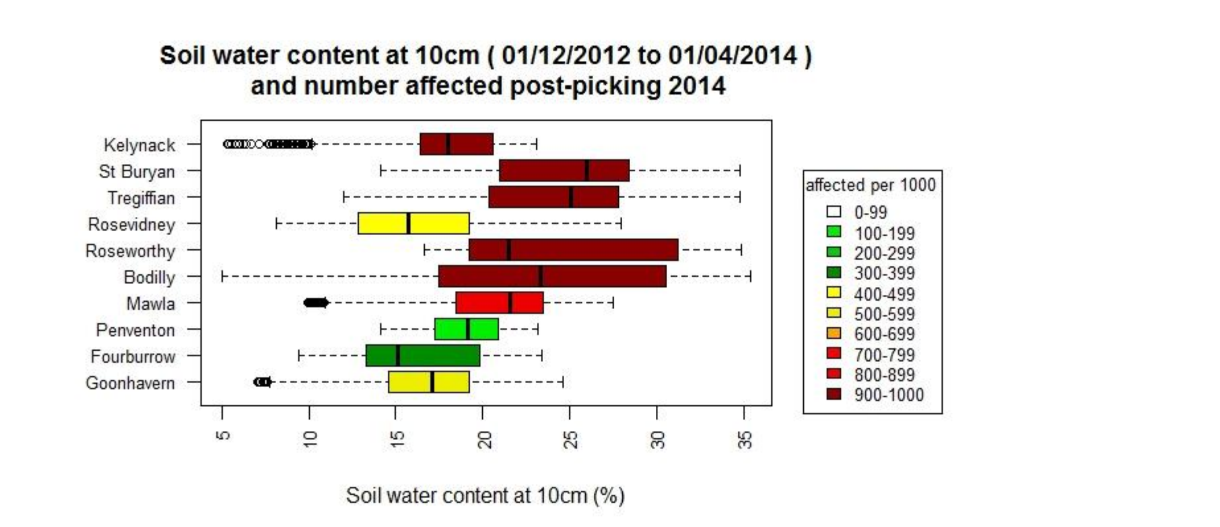
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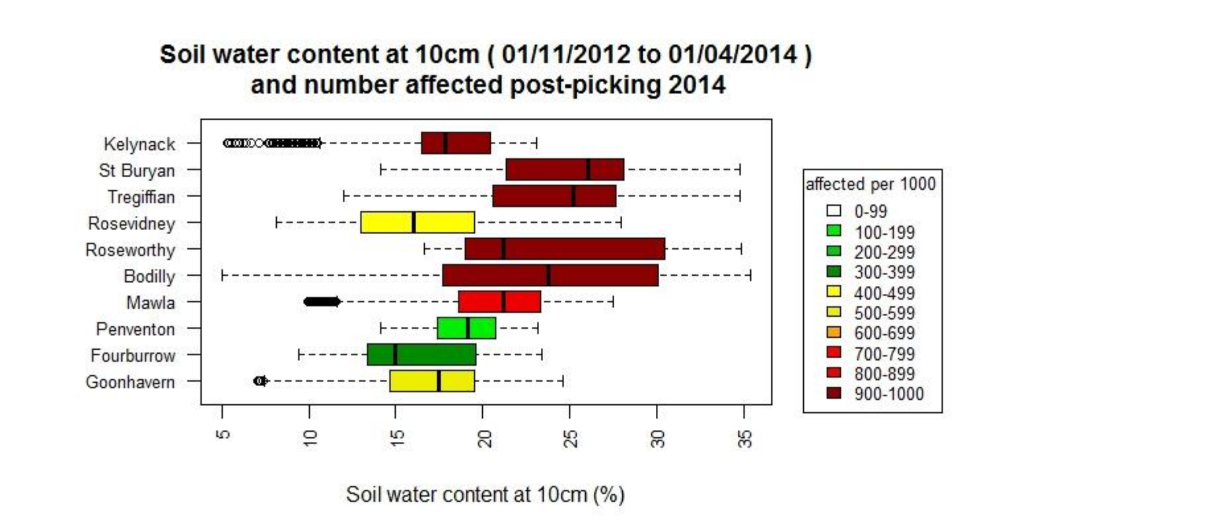
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Appendix 2: Survey of growers

A survey of daffodil growers was undertaken by AHDB Horticulture in 2002 and 2003 to discover the impact of the disorder on production. Those responses were analysed as part of an earlier AHDB Horticulture-funded daffodil rust project (BOF 62). Subsequently, to assess whether the grower perception of rust had changed over the intervening decade, AHDB Horticulture carried out follow-up surveys in 2011, 2012 and 2013, to be summarised as part of the present project. The results were included in full in the previous, 2013, annual report. Since then, a further year of results for the 'flower trader's report' has been added. To keep a complete set of results in one place, they are all included in this appendix.

Survey methodology

Questionnaires were developed by AHDB Horticulture technical staff and BOF Panel members. They followed a similar pattern over the two survey periods, though details varied as experience was gained. In 2002 and 2003 the questionnaires were posted to all daffodil growers on the AHDB Horticulture database, while in 2011 to 2013 they were sent by broadcast email. In 2013 the process was further adapted and two questionnaires were sent out, the first asking for the "dates of first occurrence of [rust] symptoms", and secondly, requesting a "summary for 2013". In addition, in 2013 growers were asked if they had previously submitted rust-affected plant and soil samples for disease diagnostics or nutrient analysis and, if so, whether they would be prepared to share this information. Responses were encouraged through reminders in the broadcast AHDB Horticulture Weekly Newsletter and by personal contacts.

In addition to these surveys of growers, in 2013 and 2014 a major flower trader was able to make available a record of the total number of flower 'lots' received each season, and the number of them free from, or affected by, rust. A lot was defined as one variety received from one grower, irrespective of the number of deliveries of each received. For each lot, a random sample of two full flower trays or boxes (160 to 200 bunches of ten stems) was examined and scored for rust severity, recording the maximum score seen for each lot.

While efforts were made to make these surveys representative and interpret the data fairly, they were essentially random spot-checks limited by commercial sensitivity. The results should therefore be interpreted with caution. Unfortunately the survey responders provided little information on stocks or cultivars that did *not* show rust symptoms.

A key element of all assessments was the scale of 0 to 5 used for scoring the severity of rust. In 2002 and 2003 the scores and guideline illustrations used were those provided by Andrew Tompsett (personal communication, 2002; Table 17). In 2011, 2012 and 2013 these descriptions were modified in the hope of providing clearer criteria for the ‘very slight’ or ‘slight’ levels of rust below a ‘commercial’ level that were becoming evident (Table 18). Both scales were intended for recording rust in a commercial situation, with a score of 3 indicating rust severe enough to downgrade the stems, and scores of 4 and 5 indicating stems that were unmarketable because of more extreme damage.

Table 17. Rust severity scores developed by Andrew Tompsett and used in AHDB Horticulture surveys of 2002 and 2003.

Score	Description
0	No symptoms
1	Slight blistering of epidermis only - “not really rust”
2	Slight cracking and rusting
3	Moderate cracking and rusting – becoming disfiguring
4	Severe rust, very disfiguring, flowers unmarketable
5	Very severe rust, cracking and stem bending, flowers unmarketable

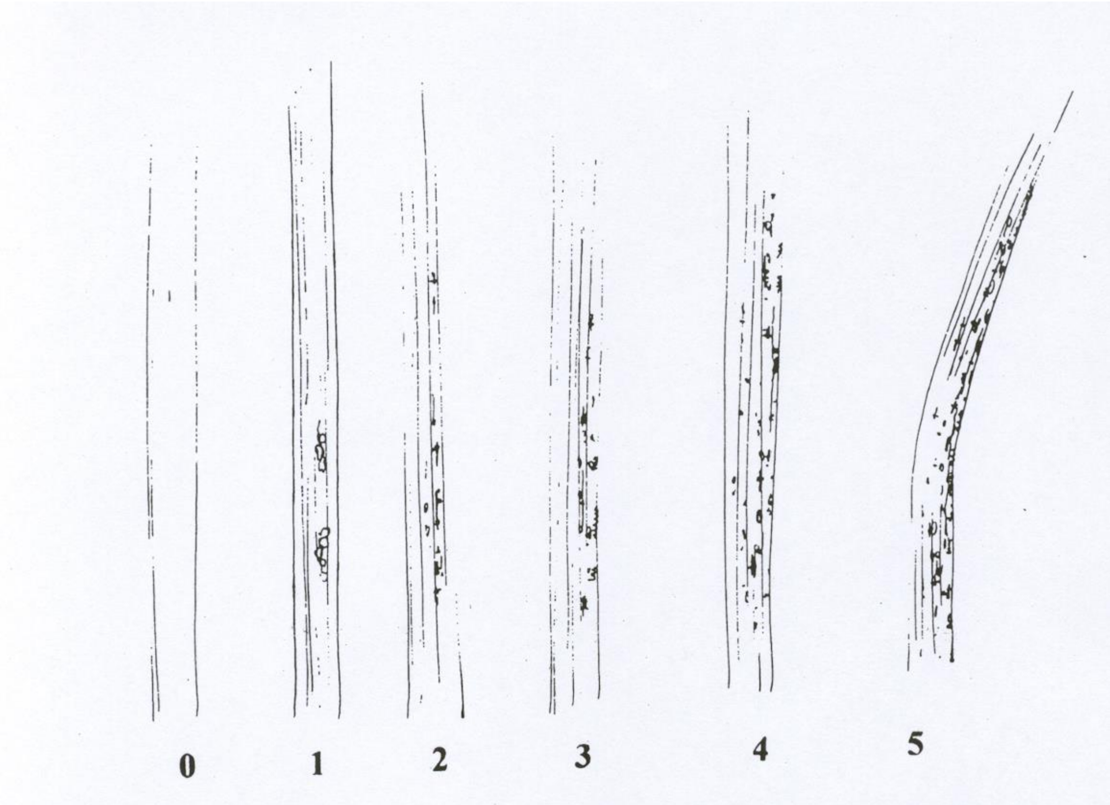


Table 18. Rust severity scores modified for use in AHDB Horticulture surveys of 2011 to 2013.

Score	Description
0	No symptoms or slight blistering of epidermis only
1	Very slight rust (only one or two small lesions per stem, or small lesions that are not fully rust-coloured)
2	Slight rust, not disfiguring, no effect on sales
3	Moderate rust, downgrade or find other buyers
4	Severe rust, some cracking across stems, unmarketable

5	Very severe rust, obvious cracking and bending of stems, unmarketable

Results and discussion

2002 survey

One hundred and eighty-three survey forms were posted by AHDB Horticulture in January 2002 and 44 replies were received, a response rate of 24%. The respondents accounted for 1715ha of daffodil production, some 43% of the total area grown in England and Wales at the time. The main findings are shown here.

- Sixty-eight per cent of respondents had seen rust in the previous four or five years.
- Respondents estimated their loss of turnover due to rust varied between 4 and 15% in their worst year, and between 0 and 3% in their best year.
- In 2002, 36% of respondents had seen flower sales reduced through rust, 36% had seen flowers down-graded, 25% had been unable to supply their preferred customers or had had to seek alternative outlets, and 25% had experienced totally unmarketable flowers.
- Twenty-three cultivars were included as exhibiting rust. ‘Golden Ducat’ was mentioned 13 times, ‘Carlton’ and ‘Golden Harvest’ each nine times, and ‘Mando’, ‘Saint Keverne’ and ‘White Lion’ each five times. ‘Golden Ducat’ and ‘Mando’ were cited a disproportionately large number of times compared with the relatively small areas of these cultivars grown at the time.
- Nine of these cultivars were reported to have shown rust in their first crop-year, but surprisingly they did not include the rust-prone ‘Golden Ducat’. All 23 cultivars were reported to have shown rust to some level in their second crop-year.
- The numbers of cultivars showing rust scores of 1 through 5 were 14, 8, 6, 9 and 5 out of 23, respectively (different sources or stocks of a variety would have different scores, so the numbers did not add to 23). The cultivars with the highest scores were ‘Carlton’, ‘Ice Follies’, ‘Saint Keverne’, ‘Kerensa’ and ‘White Lion’ – but not ‘Golden Ducat’ or ‘Mando’.

Some respondents mentioned that flower sales were then of increasing importance at a time of falling bulb prices, so research on the problem would be welcome. Other comments made included the following.

- “Symptoms usually occur with repeated mild & damp/cold/mild & damp conditions. The belief is that the crop grows too fast after a cold frosty spell and that the flower stems are brittle. Signs of rust can be traced on the stems almost to the first day of the cold period."

- "The 2002 season was good considering it was a wet mild winter." "Rust not a problem in 2002 (in Scotland) as weather has been dry and cool."
- "Rust does not appear to affect the whole field – often only parts and not the same part each year."
- "Suggest that the problem is due to boron deficiency."
- "Only seen rust in a two year down crop, ex forced stock and mainly in waterlogged areas of the field."
- "If rust is present in the first year, it always occurs in the second year." "Crops more prone to stem rust in third year down."
- "Gannilly [sic], St Keverne, Karenza [sic] and Golden Ducat show problems almost every year." "Rust is a severe problem on Golden Ducat…" "Golden Ducat seems to get rust every year." "Golden Ducat is the only variety that causes concern." "Generally find that Golden Ducat and Carlton are worst affected." "Tamara… is susceptible."
- "Not really a problem on Isles of Scilly although there was one case reported for Soleil d'Or."

<u>2003 survey</u>

A similar number of survey forms were posted by AHDB Horticulture in early-2003. Eighteen responses were received, a response rate of only 10%, and two of the respondents grew daffodils exclusively in pots so they were excluded from further assessments. The remaining 16 growers accounted for 872ha of daffodil production, some 22% of the total area grown in England and Wales.

- Seventy-five per cent of respondents had seen rust in the previous four or five years.
- The proportions of respondents who had seen rust in 2001, 2002 or 2003, were 69, 75 and 63%, respectively.
- For the respondents providing actual figures for the periods before 2001 or in 2001, 2002 or 2003, the average percentage area affected by rust varied between 6 and 9% across the four periods, average area where flowers were downgraded between 3 and 5%, and average area where flowers were unmarketable between 1 and 3%, with an average reduction in turnover between 2 and 3%.
- 'Golden Ducat' was again the cultivar with most incidences of rust recorded.

2011 survey

In 2011 seven responses were received in response to the broadcast email (included in Table 19). Respondents were growing between 15 and 950ha of daffodils each, a total area of 1771ha, representing about 38% of the total area of daffodils grown in England and Wales at that time. Despite the low response rate, the fact that two of the respondents were together growing 1350ha of daffodils, about 34% of the total grown, means that these observations can hardly be dismissed. In 2011 growers were also asked about their overall impressions of rust, and how these had changed since the previous surveys.

- Six growers replied they had seen more rust in the past 6 years than before.
- Five had had to downgrade flowers, of whom four had seen overall flower sales reduced, had not been able to supply their preferred customer, or had had to seek alternative markets, while three had experienced completely unmarketable flowers. Some had been able to find alternative and less particular markets for poorer or downgraded stems, while others thought this practice was unacceptable and considered it better not to pick poor stems, one commenting that “stems can split after picking…”
- Estimated losses in revenue due to rust had varied from 1 to 15%, or £100k to £221k, in their worst year, while in the best years there were no losses.
- Some respondents mentioned that 2010 or 2011 were the worst years for rust so far encountered.

Grower comments included ideas on the factors thought to favour the development of rust.

- “In years when rust is a problem, it can usually be traced back to a gap in the spray programme.”
- “The winter of 2011 started very cold and was 4 weeks late [resulting in] the crop growing very fast once it got moving, to the point that we were cropping 4 weeks of flowers in just over 2 weeks. Winters prior to this were considerably milder and although we see rust every year… the actual losses were considerably less. However, I also believe that post 2001, we started to use more advanced fungicides on the crop than in the 1990s and I think that the use of Amistar and in particular Folicur, which has cell wall shortening properties has had an effect on the levels of rust. Prior to 2001 my memory tells me that outbreaks were more frequent and severe (similar to this year), and were certainly linked to the prevailing weather patterns. In particular, repeating periods of frost, followed by rapid thaw and heavy rain would induce outbreaks. There was a theory… that you could estimate the date of the outbreak by measuring the position of the rust on the stem/leaf compared to the growing tip and dividing the amount by 1cm.

This would give you a date in time that would invariably relate to a hard frost being broken by a rapid rise in temps and heavy rain."

- "Rust can appear after a first picking where the crop has been clean. Second and third picks can be more affected following a clean first pick. Second and third picks can be stunted and twisted particularly in Ganilly. Golden Ducat can exhibit symptoms from the outset. Seemingly good flowers can deteriorate in the tray and rust marks lead to brittle stems and cracking. Varieties that have two sharp spines [keels] to the flower stem [are] very badly affected e.g. Dellan and St Keverne."
- "Rust seems to be worst during/after a period of adverse weather e.g. cold/frosts, winds."
- "Carlton and Golden Ducat are the two varieties which show symptoms of rust on an annual basis. The severity of the symptoms [is] very dependant on climatic conditions. The expression of the disorder is usually with distortion to the flower stems and leaf in Golden Ducat, and with brittle breaking flower stems in Carlton. The symptoms are often seen after a cold spell being followed by a milder [growing] spell."
- "We have trialled various trace element treatments, inc copper, boron and manganese - with no obvious reduction of rust."
- "The rust in 2011 seemed to develop after rapid growth after the snow melt. Later varieties grew slower while it was dryer and we saw no rust. The Year 3 is more susceptible[,] I think we never see it much in 2 year down crops."

<u>2012 survey</u>

In 2012 only three responses were received (included in Table 19). The respondents were growing between 10 and 135ha of daffodils each, a total of 156ha, and, as this represents only about 1% of the total area of daffodils grown in England and Wales the data should be treated with caution. Of the seven stocks reported on, the highest rust scores were 2, occurring in stocks of 'Martinet' and 'Golden Ducat', and 1.5, occurring in one stock of 'Saint Keverne'; the other four stocks scored 1. Grower comments included the following.

- "…very little rust this season in complete contrast to 2010/11."
- "…the worst variety was 'Golden Ducat'… [but this] did not cause problems this year as the doubles were wanted [i.e. strong demand ignored the rust symptoms] but in some years the extent of rust on the last 2 picks could [have] created problems."
- "[rust] seemed to start after rapid growth following two cold weeks."

2013 survey

A questionnaire asking about the dates of first rust occurrence in 2013 was sent by broadcast email on 8, 15 and 22 February and 1 and 8 March to levy-payers in the BOF sector (1,418 recipients, including 380 daffodil growers). The broadcasts were opened by 266 recipients (19% of the total) and 20 recipients (1% of the total) also clicked on the link in the broadcast. Only three growers – corresponding to <1% of the daffodil growers - returned completed questionnaires. A second questionnaire, asking for a summary of rust damage, was sent by broadcast email on 24 May specifically to daffodil growers (380 recipients); it was opened by 89 recipients (23%) and just one returned a completed questionnaire. The four respondents were growing between 10 and 900ha of daffodils, a total of 1160ha, representing about 29% of the area grown in England and Wales. As in the 2011 survey the fact that one of the respondents was growing 900ha of daffodils, about 22% of the total area, means that the observations, though derived from so few growers, cannot be dismissed (included in Table 19). A member of AHDB Horticulture's erstwhile BOF Panel who telephoned other growers over this survey period to seek further responses, concluded there was reluctance to discuss the issue, perhaps because of commercial sensitivities and the possibility of giving the product a bad name.

Of the 27 cultivars or stocks reported on (some cultivars were represented by more than one stock), the highest rust scores were 4 to 5 which occurred in one stock (third-year-down 'Dellan') and 3 which occurred in two cultivars, one of which worsened substantially over the observation period and was then downgraded to a lower specification. The other cultivars showed only mild rust symptoms, mostly scoring 1.

- "[There was] low level rust from our own stocks [and locally bought-in stocks] across the board… all crops exhibiting very brittle stems when cropping… [There was] very high rainfall… mild January… early season… The incidence of rust was very widespread this winter but at a low level and certainly not at a level to affect sales apart from one incidence [where the product] was withdrawn from the packing line but subsequently sold to a lesser specification customer…"
- "[Rust was] terrible in 3rd-year-down 'Dellan' [but] not a problem in others". This grower estimated that losses ranged from 2% to 30% across the different stocks.

Summary of 2002 to 2013 surveys

- Despite a poor response to later surveys, the data received indicated that rust has remained a problem over 2002 to 2013, and has probably increased. The decline in responsiveness to surveys may have been a result of administrative overload, commercial sensitivity (including the possibility of giving the product a bad name) or perhaps acquiescence that "rust happens" but is severe only in some years and in certain varieties and "nothing can be done about it even if you knew what caused it".

- Rust can occur in many cultivars, but only a few cultivars are especially susceptible to it; unfortunately these include two varieties very much in demand, 'Golden Ducat' (a popular, late-flowering double) and 'Carlton' (out-dated and base rot-susceptible, but nevertheless a popular and classic variety).
- Rust can occur in the first, second and subsequent crop-years. There is no evidence that it does not appear in first-year-down crops.
- Theories about the causes of rust appear to centre round (a) wetness and (b) marked changes in temperature.
- Growers may think of rust as a fungal disease and therefore implicate spray programmes as affecting its severity.
- Samples sent by growers for analysis do not appear to have suggested that a pathogen or nutrient imbalance is responsible for the occurrence of rust.

The between-year difference in rust severity is evidently an important feature of the condition, and some information for 2011-2013 is illustrated in Figure 83. Rust severity scores varied greatly across the three years, with 2011 showing the highest severity scores. Among the 18 stocks reported on in this period, scores of 4 or 5 (meaning the flowers were virtually unmarketable) were reached by nine stocks, with four more reaching 3 (meaning downgrading the flowers). These high scores covered eight cultivars and five of the six growers who had provided varietal information. In cases where crops of a cultivar in different crop-years were included, not all crop-years exhibited rust to the same degree, and, generally, it was less severe in the younger crops.

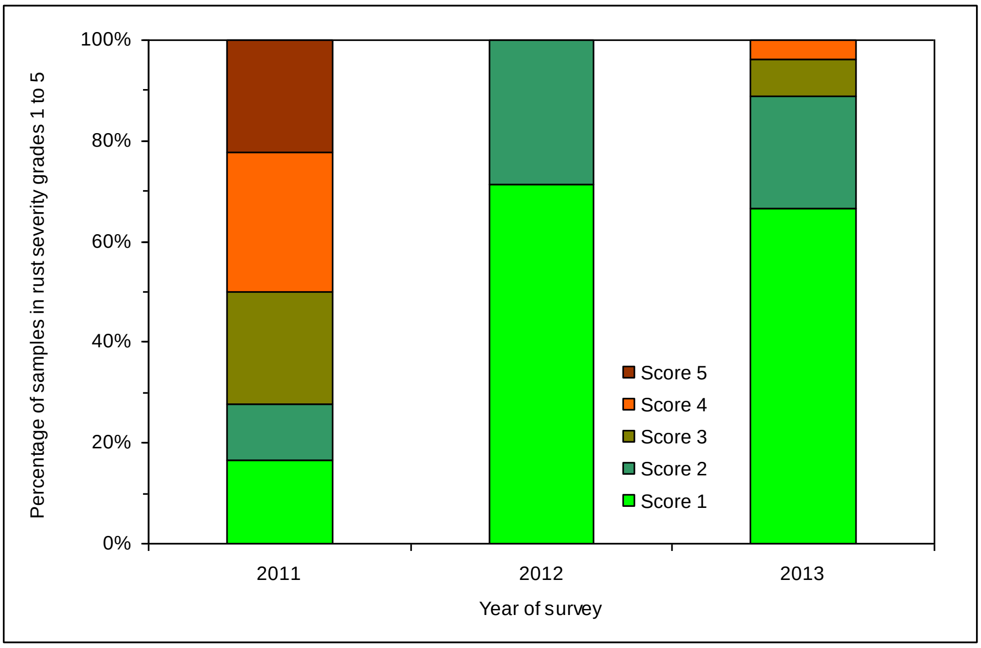


Figure 83. Frequency of rust severity scores in 2011, 2012 and 2013 surveys.

Table 19. Incidence and severity of rust reported in the 2011 to 2013 surveys of growers.

Grower number and total area grown	No. of stocks and crop-years having rust	Dates when rust first seen	No. of stocks and cultivars with different rust severity scores				
			1 or 1.5	2	3	4 or 4.5	5
Survey year 2011							
1 na	1 All	na	1 'Grand Soleil d'Or'	0	0	0	0
2 950ha	4 3rd, 4th	na	0	0	0	4 'Golden Touch'; 'Golden Ducat'; 'Standard Value'; 'Saint Patrick's Day'	0
3 30ha	8 2nd upwards	na	2 6th-year 'Saint Patrick's Day'; 2nd- and 4th-year 'Camelot' but not some other years	1 6th-year 'Carlton' but not younger crops	2 2nd-year 'Ganilly' and 3rd-year 'Golden Ducat' but not some other years	1 3rd-year 'Ganilly'	2 2nd-year 'Dellan' and 6th-year 'Golden Ducat' but not 1st-years
4 400ha	2 1st to 3rd	na	0	0	0	0	2 2nd-year 'Golden Ducat' and 2nd- and 3rd-year 'Carlton' but not 1st-year crops
5 22ha	2 2nd	na	0	1 'Carlton'	1 'Golden Ducat'	0	0
6 15ha	na	na	-	-	-	-	-
7	1	na	0	0	1	0	0

100ha	1st to 3rd				3rd-year ‘Tamsyn’ but not 1st- or 2nd-year		
Survey year 2012							
8 10ha	3 3rd and 4th	December (‘Martinet’) to 2 February (‘Saint Keverne’)	2 3rd-year (but not 2nd-year) ‘Mando’; ‘Saint Keverne’	1 ‘Martinet’, with some down-graded	0	0	0
9 12ha	3 2nd	3 March (‘Carlton’) to 20 March (‘Standard Value’)	2 ‘Carlton’; ‘Standard Value’	1 ‘Golden Ducat’	0	0	0
10 135ha	1 3rd	27 February	1 ‘Tamsyn’	0	0	0	0
Survey year 2013							
11 900ha	11 2nd and 3rd	8 January (‘Tamara’) to 5 February (‘Golden Harvest’ and ‘Investment’)	9 (wide range of cultivars	1 3rd-year ‘Jedna’ (score 1 for 2nd-year ‘Jedna’)	1 ‘Mando’, (score in-creased from 1 (15 January) to 3 (3 March) when product down-graded)	0	0
12 10ha	3 3rd and 4th	“At flowering”	1 ‘Standard Value’	1 ‘Saint Keverne’	1 ‘Martinet’	0	0
13 100ha	3 2nd and 3rd	10 January (3rd year ‘Tamsyn’) to 18 March (‘Dellan’)	0	3	0	0	0
14 150ha	10 1st to 3rd	na	8 including 1st-	1 2nd-year	0	1 3rd-year	0

	year ‘Dellan’	‘Dellan’	‘Dellan’

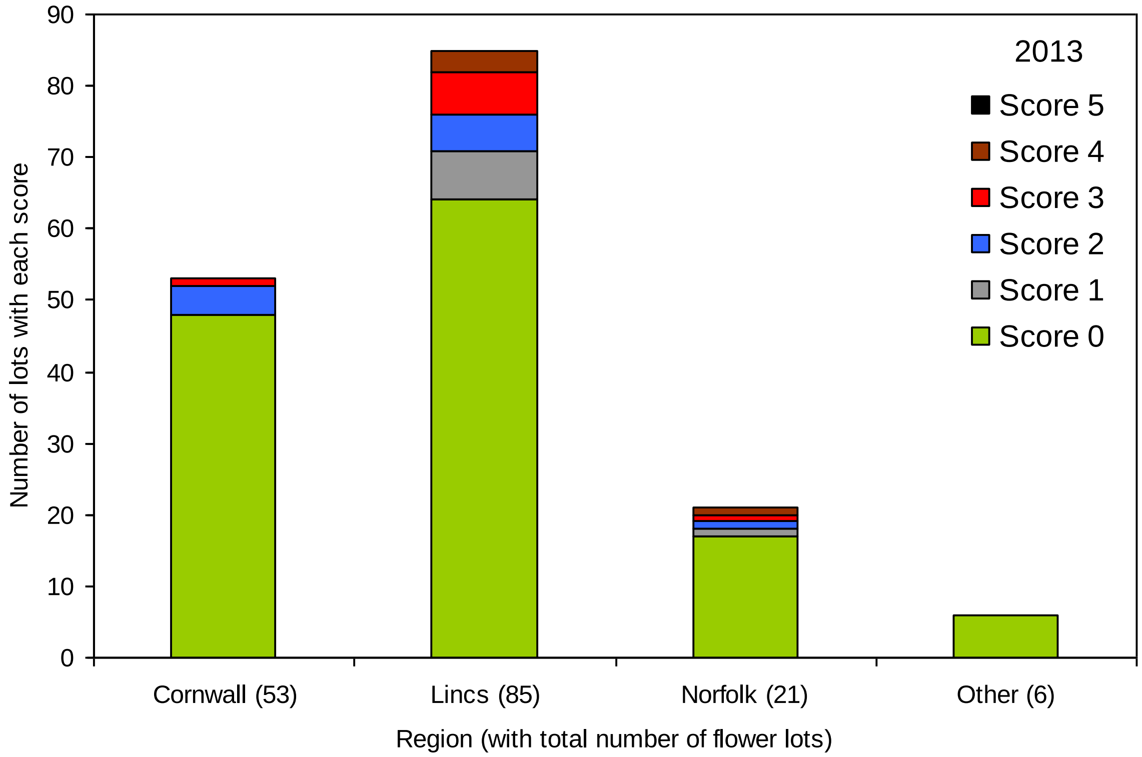
na = not available

Flower trader's survey 2013 and 2014

The survey covered a trader's intake of flowers in 2013 and 2014, comprising 165 and 163 'lots', respectively. A 'lot' was defined as the flowers of one variety received from one grower, irrespective of how many times that variety was delivered to the trader by that grower. The severity of rust symptoms of each lot was assessed on a scale of 0 to 5 (Table 18).

Overall, 135 lots of the 165 were free of rust symptoms in 2013 and 149 out of 163 in 2014, 82 and 91%, respectively. The percentage of lots from each region with maximum rust scores of 0 to 5 are shown in Figure 84. In 2013, 75, 81 and 91% of lots from Lincolnshire, Norfolk and Cornwall, respectively, were free of rust. Only six lots were from other regions, and none of these had rust symptoms. In 2014 the respective lots free of rust were 94, 93 and 100%, substantially higher, while of the just twelve lots from other regions 50% had rust symptoms. In 2013 'Golden Ducat', 'Kerensa', 'Mando' and 'Tamara' had rust symptoms a disproportionate number of times, and although these cultivars are known to show the symptoms regularly, the results were probably skewed because (apart from 'Kerensa') they are widely grown. The smaller number of cultivars affected in 2014 meant that no conclusions could be drawn about cultivar susceptibility to rust, while the cultivars with the highest rust severity scores were different in each year. Of these varieties 'Golden Ducat' probably has the most economic significance, because of its longer stems and heavier buds, though these probably contribute to stem breakage during handling, itself accentuated by the effects of rust which include cracking across the stems and brittle stems.

As the survey included widely divergent numbers of lots per region, these results should be used with caution, though the notion that the disorder is more common in Cornwall than in other bulb-growing areas was not supported. The data confirmed the unpredictability of rust occurrence from year-to-year.



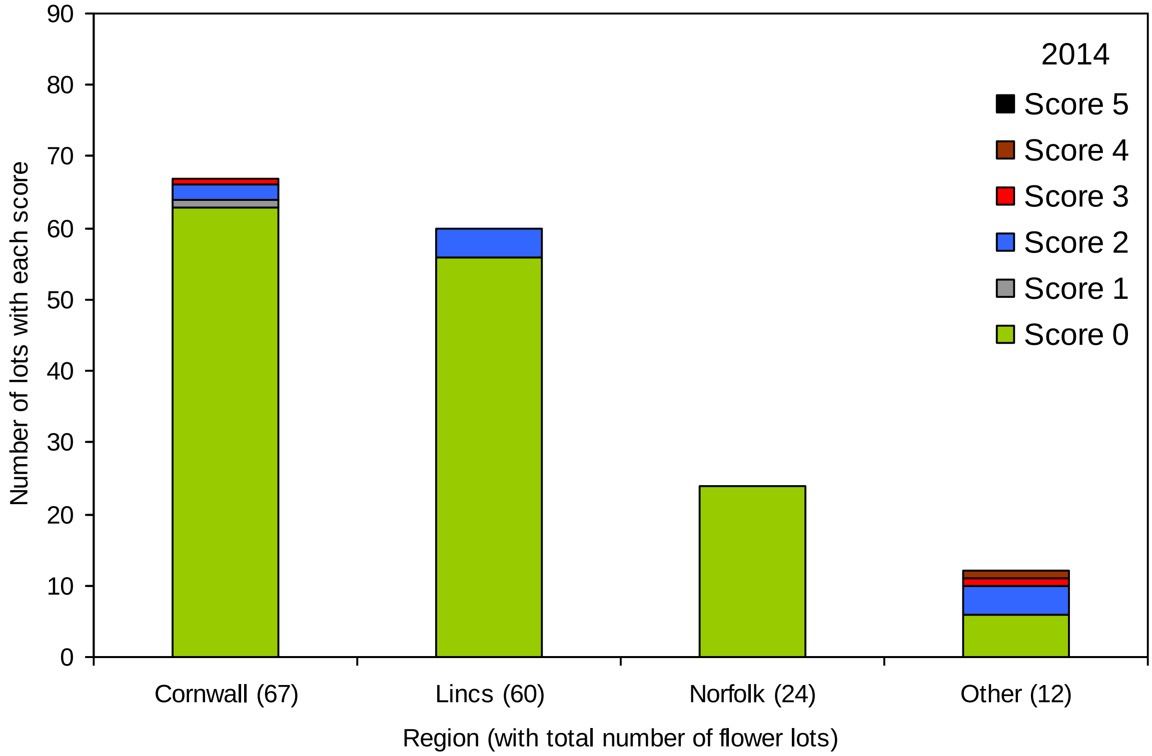


Figure 84. The number of flower lots having maximum rust scores of 0 to 5, classified by region of origin, from a flower trader's survey of sendings received in 2013 (top) and 2014 (bottom).

Soil and plant analysis undertaken by growers

In the 2011 survey three growers commented on sending soil or plant samples associated with rust symptoms for mineral analysis or disease diagnostics. One commented "we have sent many samples for analysis including soil, bulb and leaf tissue… all of the tissue and soil samples have all come back from the lab over the years showing normal or near normal levels of nutrient and no deficiencies. There have never been any pathogens found in any of the samples sent that would/could cause the deformities that are associated with rust…" The other comments were "I have had flower stems and the soil sampled in the past… Results were inconclusive and remedial action taken did not solve the problem", and "Sample sent to Netherlands for analysis, no organisms present…"

Two cases were reported in further detail.

Case 1. In March 2003 a Lincolnshire grower experienced severe rust in some areas of his crop and sampled plants and soil for analysis. Samples were taken from normal crops of 'Golden Ducat' and 'Saint Keverne' and from crops of the same cultivars that had severe rust with unmarketable stems. In the case of the normal 'Golden Ducat', samples were taken from (a) a uniformly normal area of the crop and (b) by selecting normal plants from an area of mixed quality, these being referred to as 'random' and 'selected' samples, respectively. The crops were in their second or third crop-years and none had received fertilizer in the base before planting or subsequently as top-dressing.

Soil samples were analyzed for pH and for concentrations of P, K, Mg and, because it has been associated with physiological browning and tissue breakdown in a wide range of crops, calcium. The results are given in Figure 85. The graph showed that it was not possible to differentiate normal (marketable) and unmarketable samples by their having distinctive pH or P concentrations, the values for marketable (blue symbols on graph) and unmarketable (red symbols) samples being similar. However, in the case of K and calcium concentrations the unmarketable samples had lower levels than the marketable samples, whereas for Mg the reverse was true. According to the recommended levels given in 'RB209', both soil samples that yielded unmarketable samples were deficient in K.

Bulb and stem samples were analyzed for major and trace nutrients, with results shown in Figure 86 and Figure 87, respectively. In all but three cases the values for marketable and unmarketable samples were intermingled, showing no characteristic high or low levels dependent on the rust status of the plants. The exceptions occurred in bulb samples only, with unmarketable samples having lower concentrations of calcium and iron and higher concentrations of Mg.

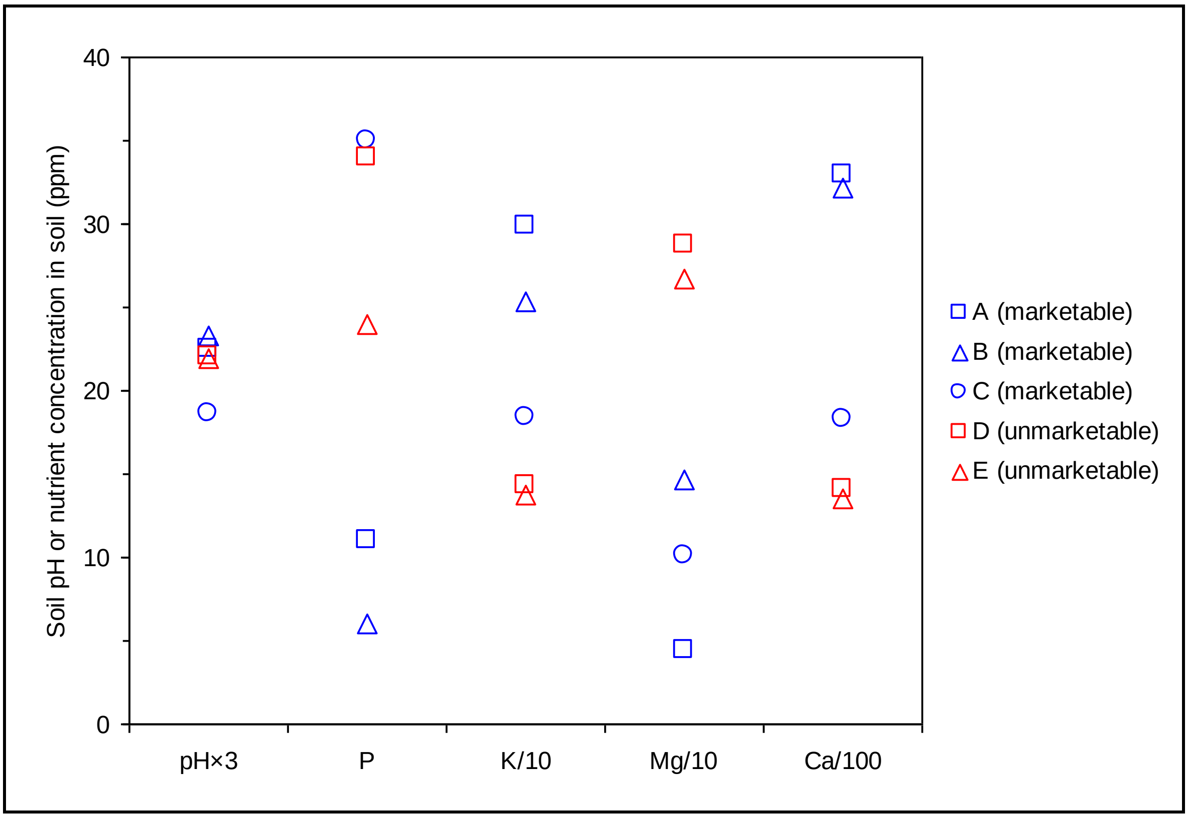


Figure 85. Soil pH and concentrations of P, K, Mg and Ca at three sites producing rust-free, marketable stems and two sites yielding unmarketable stems due to rust (referred to as Case 1 in text). Samples A, B and D are ‘Golden Ducat’ (A is ‘random’ sample, B is ‘selected’ sample, see text) and samples C and E are ‘Saint Keverne’. For convenience, the values for pH, K, Mg and Ca have been scaled up or down in order to bring them onto one convenient scale (indicated by ×3, /10, etc.).

It is tempting to highlight the lower levels of soil and bulb calcium in unmarketable plants (and perhaps the antagonism with Mg), but there were no indications of any other associations. This suggestion should be treated with caution, since the results were based on only five samples and the marketable and unmarketable stems were from crops in different locations. Further, some calcium-related disorders are due to the lack of mobility of the calcium ion and the availability of soil water, not to simple concentrations; finally, early experiments on bulb nutrition showed that nutrient reserves within the bulb could delay the onset of nutrient deficiencies due to any inadequacies of the growing medium for a period of years. Interestingly, the analyst in the current case noted that the results of analysis might be more meaningful if soil moisture variations at the sites were also taken into account.

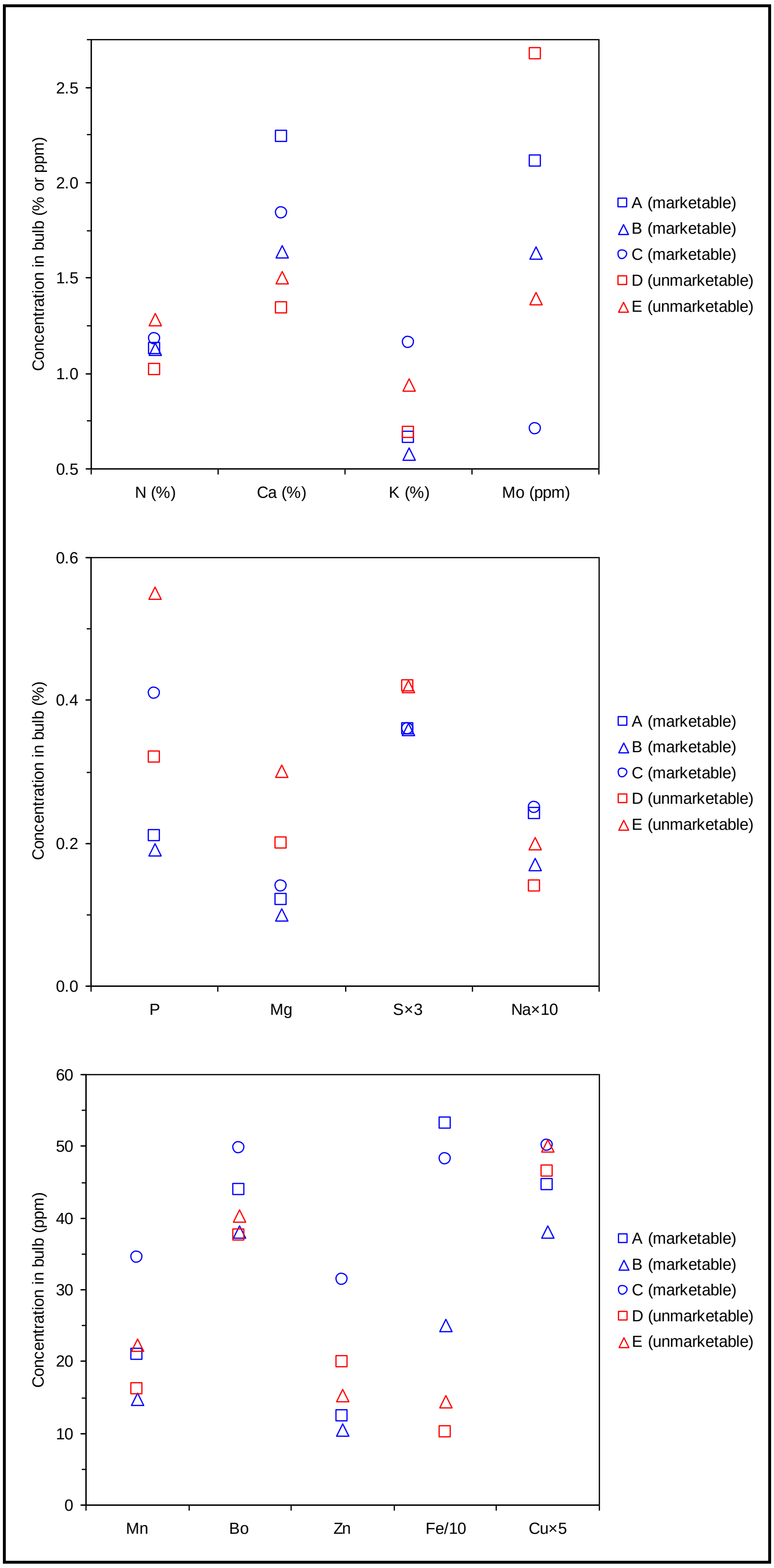


Figure 86. Concentrations of nutrients in bulbs at three sites producing rust-free, marketable stems and two sites yielding unmarketable stems due to rust (referred to as Case 1 in text). Samples A, B and D are 'Golden Ducat' (A is 'random' sample, B is 'selected' sample, see text) and samples C and E are 'Saint Keverne'. For

convenience, the values for S, Na, Fe and Cu have been scaled up or down to bring them conveniently on-scale (indicated by ×5, /10, etc.).

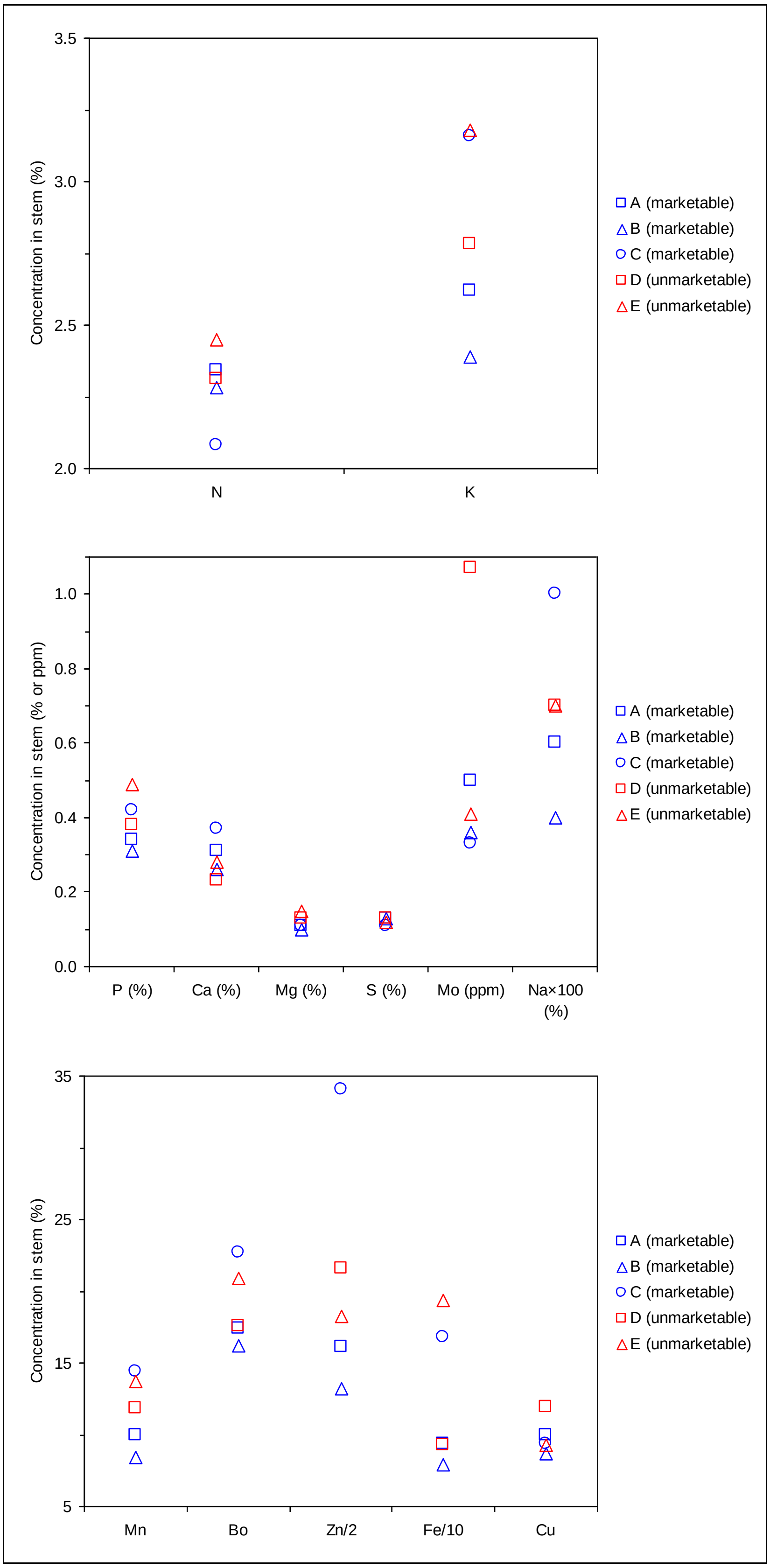


Figure 87. Concentrations of nutrients in stems at three sites producing rust-free, marketable stems and two sites yielding unmarketable stems due to rust (referred to as Case 1 in text). For explanations of A to E and scaling of some values see legend of Figure 86.

Case 2. In June 2012 a grower in Cornwall experienced damaging levels of rust in one area of a field, though stems were of a high quality in the remainder of the same field. Soil samples were therefore taken and analysed from both the ‘good’ and ‘rusty’ areas.

The only specific problem with the soil from the rusty area was a high calcium content: high calcium would be expected to restrict Mg availability. On the other hand the peculiarity of the good site was a very high level of iron (suggested as possibly the result of poor drainage). Both sites were low in boron and manganese and high in copper, zinc and molybdenum. There were no other substantial differences between samples in the levels of macro- or micro-nutrients or soil pH.

These results might appear to suggest low Mg availability as a possible cause of rust (though in Case 1, above, soil Mg levels were *higher* in the rusty areas), and also that low levels of boron or manganese are unlikely to be a cause of rust. Unfortunately this is a report of a single example which may mean little in general.

It would be useful to see the results of similar analyses which growers are likely to have obtained from time to time.