Report of preliminary results from BBRO 2020 Virus Yellows variety testing

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Executive Summary

Virus yellows is understood widely to be the most significant threat to sugar beet crops in the United Kingdom and in northern Europe. Yield losses can amount to more than 50% of the crop and new controls are needed to overcome this significant threat now that neonicotinoid seed treatments are no longer widely available for control of the major vector of the virus complex, *Myzus persicae.* Therefore, starting in 2018, and further refined in 2019, BBRO began testing varieties to identify and support the introduction of VY resistant or tolerant varieties to market.

This report details the yield results from BBRO's 'Project Goliath' experiment conducted in 2020. It briefly details the method used in the experiment and key data which were generated from the experiment. It also outlines future action for this experiment and how these results will be used to develop BBRO's virus yellows variety strategy.

Method

On March 24th 2020, Project Goliath was sown at Swanton Morley Farms, Dereham, Norfolk. The field was sown with fourteen varieties of sugar beet (*Beta vulgaris* L.) with twelve replicates of each; four used as the uninoculated controls, four inoculated with *Beet mild yellowing virus* (BMYV) and the remaining four were inoculated with *Beet yellows virus* (BYV). The statistical design used was a split-split-split-plot (Mainblock/block/virus/variety) to allow appropriate analysis. This design isolates the plots of different viruses from each other to reduce likelihood of cross contamination whilst also allowing for the groups of each virus to be spread into two areas. Brassica (oilseed rape and oil radish mixture) strips were sown between the blocks to attract migrating wild aphids away from the sugar beet. Rye buffers were established alongside all plots to act as windbreaks and limit unwanted spread of the aphids used for inoculation.

Fig 1 Goliath – 22nd June 2020 –Symptoms of both viruses can clearly be seen. uninoculated plots in the foreground. BMYV mid-left & rear right, BYV mid-right & rear-left

Fig 2 Goliath – 16th September 2020 – Symptoms of viruses again clear after the site had recovered from drought in the summer.

Plots were six rows (50cm inter-row spacing) wide and seeds were sown at 17cm spacing. Populations therefore, allowing for 90% establishment, should have achieved c. 106,000 plants.ha^{-1}. Eight weeks after sowing, inoculation with virus took place when the plants were at the 4-6 leaf stage. On May 20th BMYV plots were inoculated and the BYV plots were inoculated 48hours later on May 22nd. All plants in the central four rows were inoculated with viruliferous aphids, cultured to carry either of the yellowing viruses, which were administered by hand. On both days, inoculation of all plots was completed within two hours. Biscaya (thiacloprid) was applied to the entire field on May 23rd once sufficient time for the viruses to transfer to the plants had elapsed.

The virus pressure in 2020 did result in the crop reaching threshold for control on 5th May, prior to virus inoculation, and was treated with Biscaya. This did result in several small patches (<1m⌀) of virus in some uninoculated plots. Following inoculation, the crop was treated with further aphicides on 29th May (Teppeki – Flonicamid) and Insyst (Acetamaprid) on 9th June to prevent further virus spread across the experiment. These aphicides did successfully minimise growth and secondary spread of these patches.

The trial was regularly assessed for growth and development of the symptoms on the crop using the BBRO Drone complete with thermal, multispectral and colour (RGB) imagery. Assessments of viral load using ELISA in the leaves from five plants per plot was also conducted on 8th September.

Results

Unfortunately, the site did experience problems which has negatively impacted the quality of the results. For example, the mean population was 72,000 plants but ranged from 41,000 to 96,000 plants ha^{-1}. Although this issue was seen commercially in 2020, especially on lighter soil types, interpreting the yield results has been difficult. This is because yields are known to be particularly affected by population in the range we experienced (Jaggard & Qi, 2006). The site also suffered from several patches in which no plants established (Fig 1). These plots were therefore removed from the experiment.

In addition to the low populations, the site suffered periods of drought throughout summer, again reflecting commercial reality through 2020. Swanton Morley experienced only 1/3 of the long-term average rainfall in between March and May and then 10% less rainfall than average during the summer (June to August). Consequently, drought symptoms set-in early, being visible in the crop from late June. This resulted in severe canopy damage and loss of leaf area, particularly in late August. However, the crop did recover and the canopy regenerated by mid-September (Fig 2).

Finally, damage of soilborne pathogens was found in some plots. Evidence of BCN and violet root rot (VRR) were recorded. Damage caused by these were recorded for each plot. VRR was scored at the BBRO plot facility once the roots had been harvested (19th October) and washed. The number of roots and the severity of infection in each root was recorded. No significant effect of variety in response to VRR was found. However, this is likely due to the uneven distribution of VRR and some plots had very severe infestation throughout the entire sample.

Therefore, due to these issues, all results in this report should be interpreted with caution, especially as no statistical significance was found between the varieties in 2020.

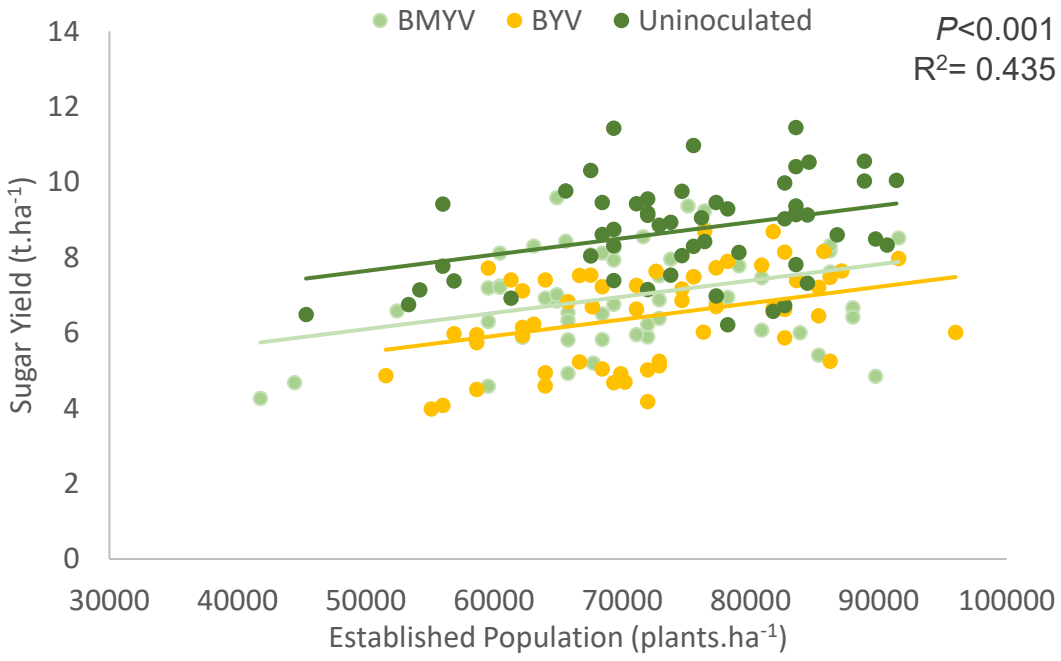

Fig 3 - Regression of yield of the plots vs. established plant population.

The data which were gathered from harvest do show a clear response to yield in response to the virus which the plots were inoculated with (Fig 3). The regression model found the best fit of the data were parallel responses, showing that BMYV and BYV reduce yield consistently across the populations but, as expected, the viruses significantly reduce the yield potential of the plants. This shows that the methodology we deployed was once again successful. Unfortunately, we could not find significant interactions between varietal yield and either virus (Fig 4a), but we include the data for completeness.

Established plant population is likely to have masked the response we expected to detect (Fig 4b) and there was found to be a significant population response (virus x variety *P*=0.04), which will have skewed the data. However, no differences between varieties alone was found. Therefore, differing soil conditions in the blocks of plots are likely the cause of this response which was exacerbated by the drought. Had the site had not suffered the aforementioned issues, we are confident that we would have detected significant varietal yield x virus interactions, since the same methodology worked well in 2019. Additionally, in 2020, in contrast to 2019, varietal yield losses due to virus (Fig 5) did not widely achieve expected levels of up to 30% for BMYV and 50% for BYV on the control varieties (2,3 and 4). (Please see Appendix Figure for 2019 data)

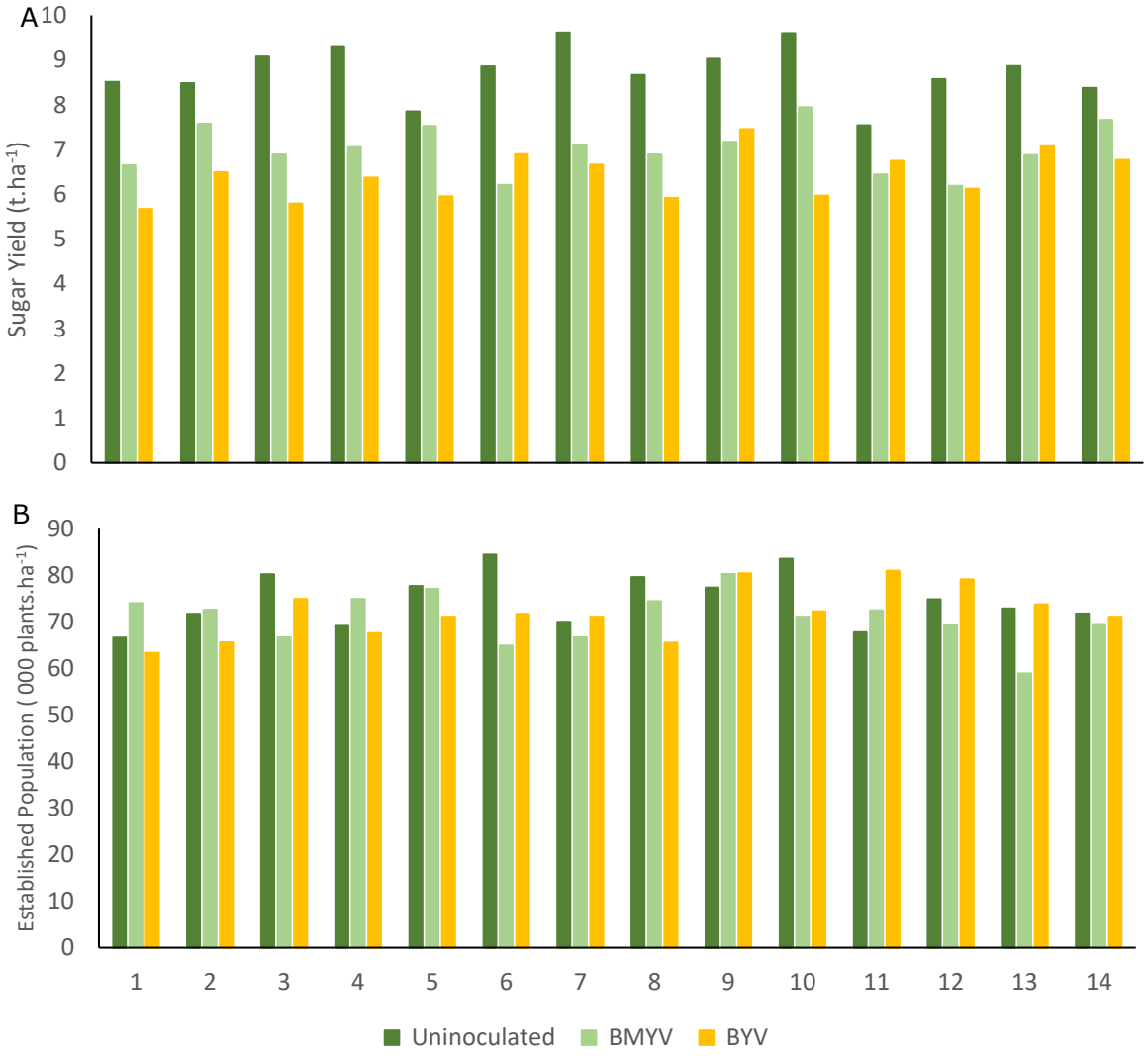

Fig 4A – Sugar yield of the individual treatments. No significant interaction of variety and virus (indicating tolerance to one or both virus) was found. Fig 4B shows the mean populations achieved for each treatment. (Mean of four replicates)

ELISA testing was able to reveal significant differences in levels of viral titre in response to BMYV (Fig 6). This shows that some varieties are hosting reduced levels of virus and are likely therefore partially resistant to BMYV. However, no varietal difference in BYV viral titres could be found (Data not shown). Both findings agree with our results from 2019.

Canopy Phenotyping: Numerous flights of the BBRO drone were conducted through the season and gathered a wealth of data. The project team are now working on combining these data, along with that gathered in 2019 to understand more about symptom development of virus on the different varieties and how phenotyping can help test varieties in the future and possibly remotely detect virus in commercial crops.

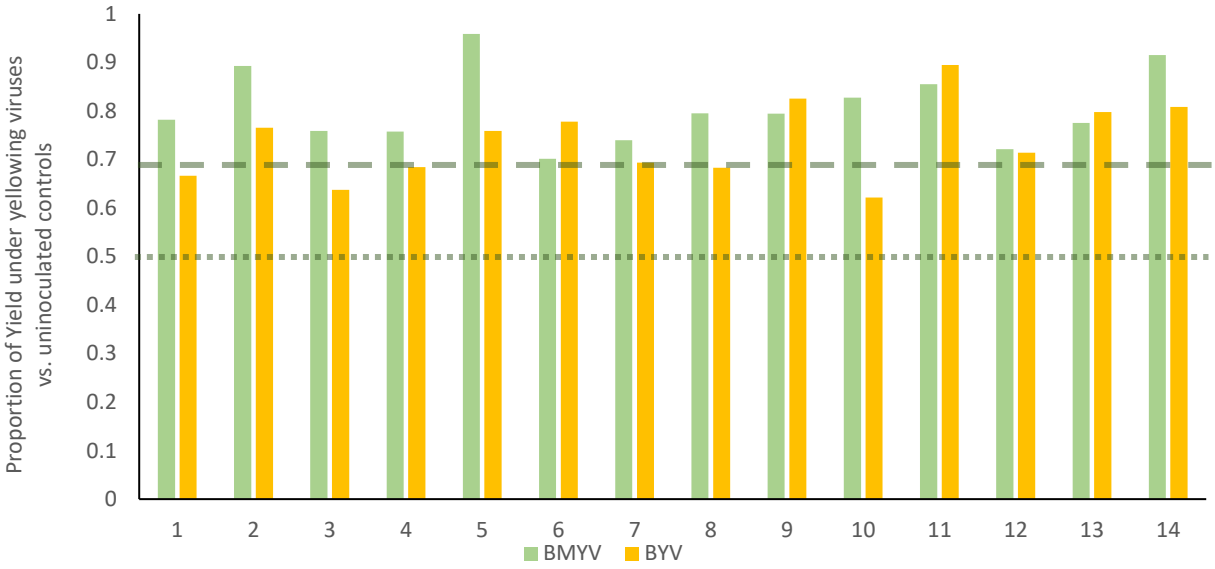

Fig 5 – Proportion of yield achieved (vs uninoculated plots) under BMYV and BYV Dashed line shows expected yield loss level for BMYV (30%) and dotted line for BYV (50%). Data have not been corrected for population, drought or soilborne pathogens.

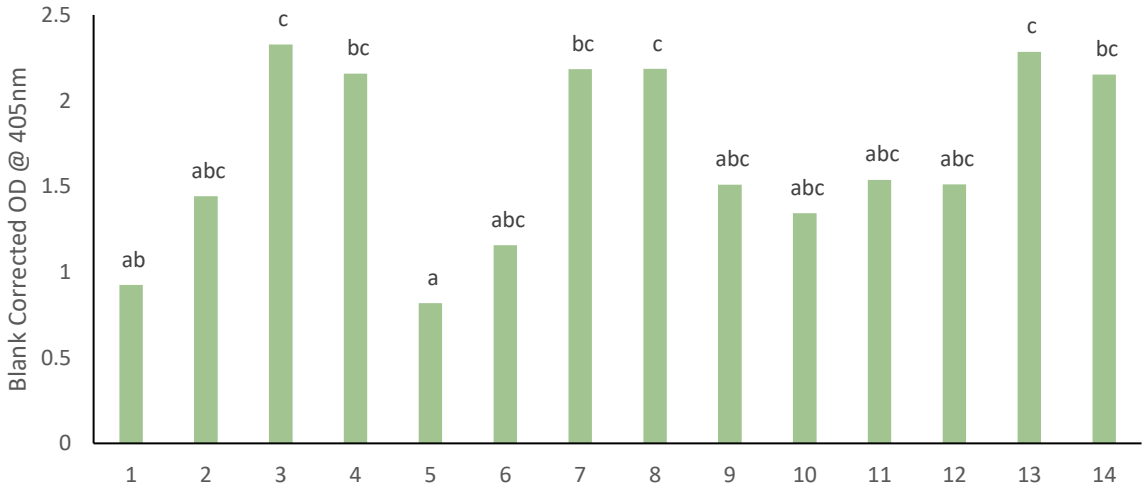

Fig 6 – BMYV ELISA absorbance data. Significant differences between the varieties were found (P<0.001, LSD = 0.7132). Different lower-case letters represent significantly different results at P = 0.05 as calculated by Tukey's Post-hoc test. Higher values represent greater levels of BMYV in the samples.

VY Boxes

A subset of six of the varieties, some chosen following results from Goliath 2019 and others chosen to represent a range of VY resistant varieties from Goliath 2020, were planted in a box experiment. The same size open-bottomed boxes as the BBRO BCN testing (1.5 x 2.0 x 0.4m) were used and filled with 'clean' soil which had not grown sugar beet for at least 15 years. Boxes were laid out in a similar fashion to Goliath, with virus infected boxes grouped to prevent unwanted virus spread and discard boxes were placed at the end of each run (Fig 7). These were sown on 24th April and thinned after emergence to ensure 36 plants established (4 rows of 9 plants) in each box. The boxes were inoculated on the 27th of May (BMYV) and 29th May (BYV) and sprayed with Biscaya on 30th May. Plants were then grown until hand-harvested on 27th November and processed through the BBRO plot facility & tare-house to determine yield of each box. Plants were irrigated to limit severe drought events.

The data obtained have found significant yield impact in response to virus, and again we can detect significant differences in BMYV viral titres in the boxes correspond directly to the titre levels measured in Goliath (Data not shown. $P<0.001$, $R^2=0.553$). Like Goliath, BYV titres did not differ between varieties. Unfortunately, no significant variety x virus yield interaction could be found which is required to support a claim of a variety being tolerant to either virus. Interestingly, both viruses also caused similar levels of yield loss. Despite this response, figure 8 has been included to show individual varietal performance.

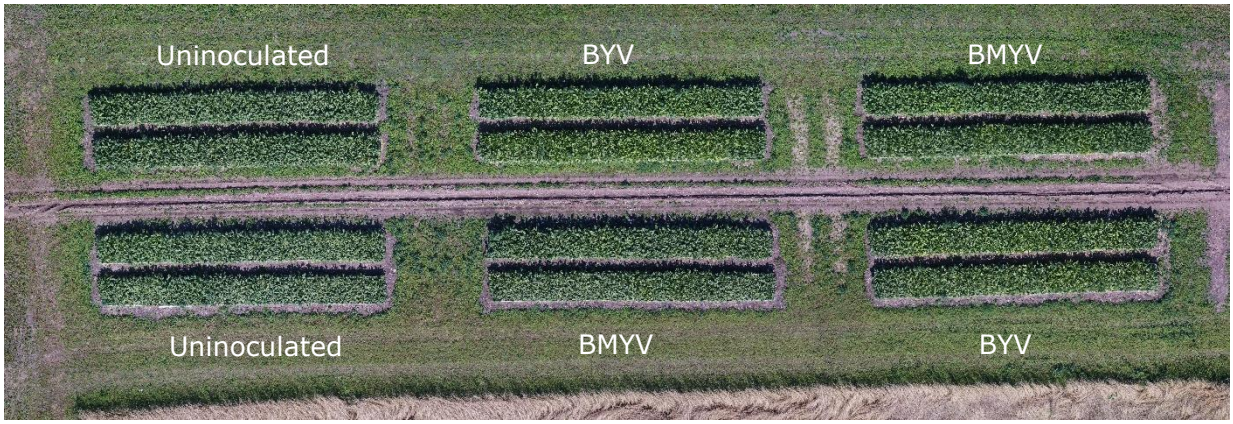

Fig 7 – Site photograph from the BBRO Drone showing the VY boxes on 7th August 2020.

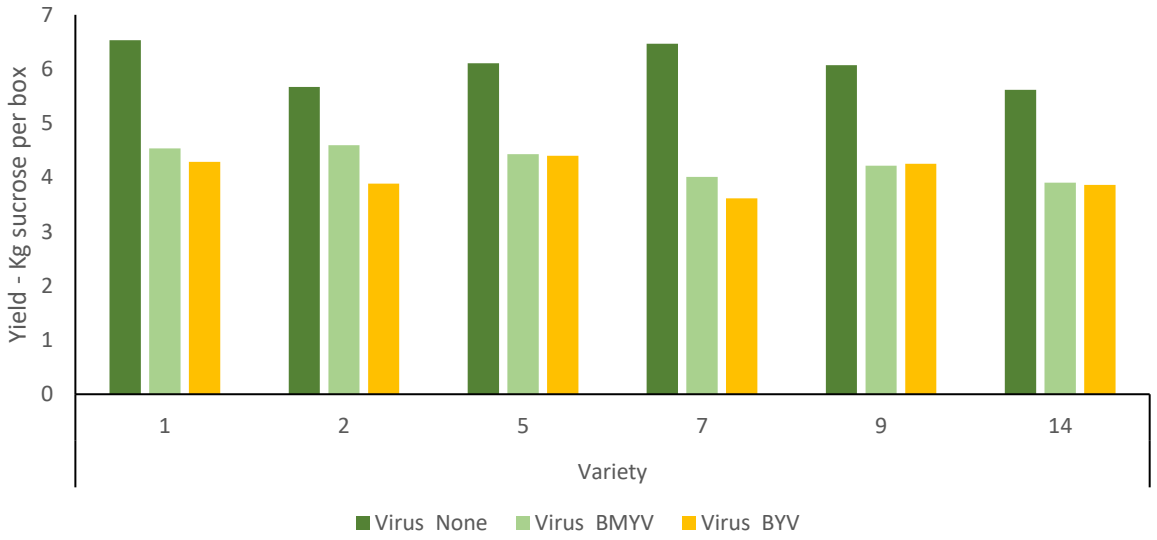

Fig 8 – Mean yields of the six varieties tested in the VY box experiment in 2021. No significant variety interaction was found, although virus did have a significant overall impact on yield ($P=0.002$ – Data not shown) but all varieties were found to respond similarly to the viruses. Additionally, there was no significant difference in yield between the plants inoculated with BMYV or BYV.

Significant relationships have been found between the six varieties tested in the boxes, virus levels (BMYV) and canopy indices measured by the BBRO Drone. For example, BMYV ELISA samples collected on the 22nd of September were accompanied by an aerial survey of the experiment. From this, we have found a significant relationship regarding the proportion of the box which has turned yellow, which was determined by measuring the proportion of the image of each box covered by yellow pixels, and measurement of the same box using the multispectral camera, and specifically, calculation of the mND_{blue} vegetation index (Jay et al. (2017). This index measures levels of chlorophyll in the crop canopy and it is clear that canopies with less yellowing show less negative levels of mND_{blue} (i.e. they have a greater level of chlorophyll (Fig 9).

The analysis of these data can also be taken one step further, since significant relationships were found against the six varieties trialled, their viral titres measured in the ELISA test and the mND_{blue} value of the canopies of the boxes (Fig 10). From our data it appears that varieties 1,2, 9 and 14 show more intense chlorophyll loss in boxes with increased BMYV titres but varieties 5 and 7 show lower levels of chlorophyll across all titre levels observed. This could be a response to the virus, but could also alternatively be an inherent trait of these two varieties to have less chlorophyll-rich canopy types. Further analysis will be conducted on the range of measurements taken throughout the growing season and to look at possible relationships to predict yields and/or VY tolerant varieties.

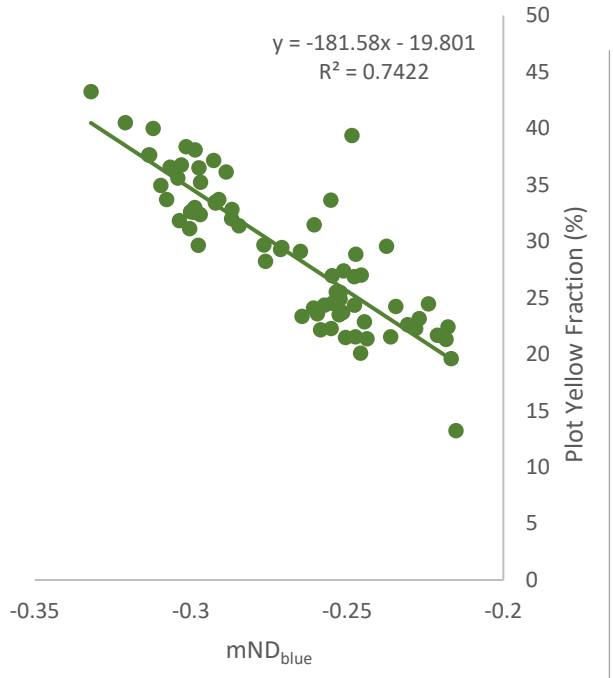

Fig 9 – Relationship between Area of sample with yellowing (as determined by image colour thresholding) and mND_{blue} vegetation index, which measures chlorophyll levels in sugar beet canopies. Data obtained from the BBRO drone cameras on 22 September 2020 from all boxes used in the virus box experiment.

N.B. less negative mND_{blue} levels correspond to greater chlorophyll levels in the canopy

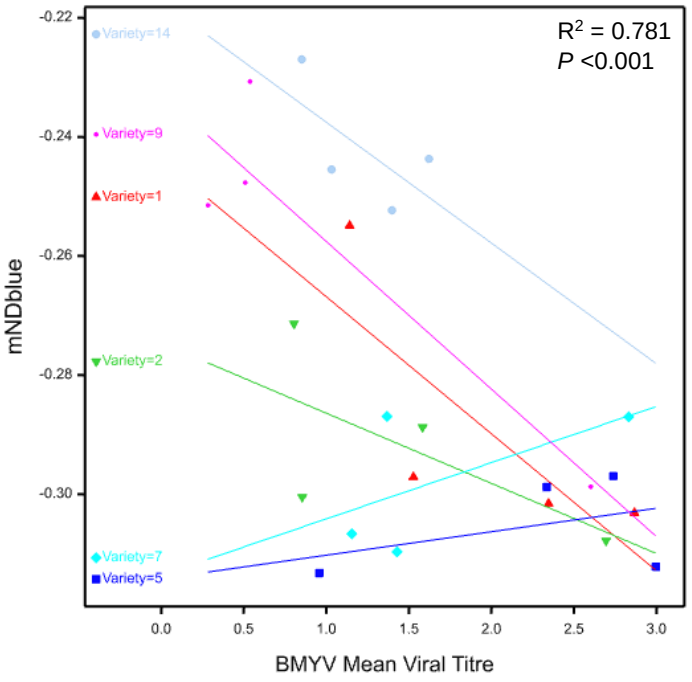

Fig 10 – Significant relationship between viral titres (measured by ELISA) and mND_{blue} measurement of canopies infested with BMYV. The results show that some varieties appear to suffer from heavy chlorophyll reduction even when viral loads are low, whereas other varieties show a much more severe level of chlorophyll reduction at high virus levels.

To overcome the challenges faced this year by this most crucial experiment, BBRO is implementing a new range of controls to reduce the likelihood of these problems occurring again. In 2021, varieties will be double sown and then thinned to a stand to improve establishment. We are also going to situate the experiment on a site which is less prone to water stress and if a long period of drought is forecast, irrigation will be deployed by BBRO. Sites without recent cropping of sugar beet and other host crops of soilborne pathogens will be used in the future in order to minimise the risk of root rots occurring. Whilst the field in 2020 was checked for BCN by an independent laboratory, greater sampling resolution will be used in 2021 to rule out this threat also.

However, data obtained in 2020 supports the methodology we have developed and deployed since 2019 and that we can reliably infect and test varieties for VY tolerance. Therefore, the BBRO stakeholder board recently signed off on three more years of variety testing which is also going to be thoroughly expanded. This also means that how we test varieties will change in line with the new trials.

In 2021 we are launching 'Operation Emerald' – BBRO's strategy to test, validate and deploy novel traits against VY:

1 – Project Goliath 2021 – Testing varieties with claims of VY tolerance and/or resistance

Much the same as 2019 and 2020, in 2021 we will be testing forthcoming new material for VY resistance against a range of existing elite varieties. We will however be changing the nature of the testing. All varieties included in this trial should have tolerance or resistance to BMYV and/or BYV. Testing of elite RL varieties for their response to VY will no longer be part of the scope of Goliath.

2 – Project Verde - Screening all 2022 RL varieties (one year ahead of commercial sowing)

In 2021, all varieties which will be marketed for sowing in 2022 will be sown in an experiment which will implement the methodology developed in Goliath. This allows us to screen all varieties which growers will be using and provide information to tailor their deployment.

3 – Project Titan – Testing for variation in susceptibility of varieties to VY at different growth stages. This will be our most complex experiment. A subset of eight varieties chosen from the RL and Goliath will be sown at three different times (early, mid and late drilling) and inoculated with either BYV or BMYV. We will investigate whether plant age significantly impacts the resistance or tolerance of varieties against VY.

All experiments will be completely artificially inoculated with either BMYV or BYV carrying viruliferous aphids. Goliath, as with 2019 and 2020 will be comprised of six-row plots. Whereas Project's Verde and Titan will use three row plots. Methodology will follow the same as Goliath in previous years, with plots being protected by brassica and rye buffer strips. All sites will also be regularly monitored for wild aphids by checking plants and also using yellow water pans.

We have also recently invested in new state-of-the-art LED growth facilities, so aim to also develop a VY screening assay to harmonise resistance screening by ELISA. These results will support the field data and findings which are published to the industry.

2021 will also see expansion of the VY box trial which was set-up in 2020 for virus yellows testing. However, the number of replicates (from four to six) and the size of the boxes (from 1.5 x 2.0m to 2.0m2) should improve the quality of data this trial generates and therefore the opportunity this method has to support field experiment data.

Acknowledgements

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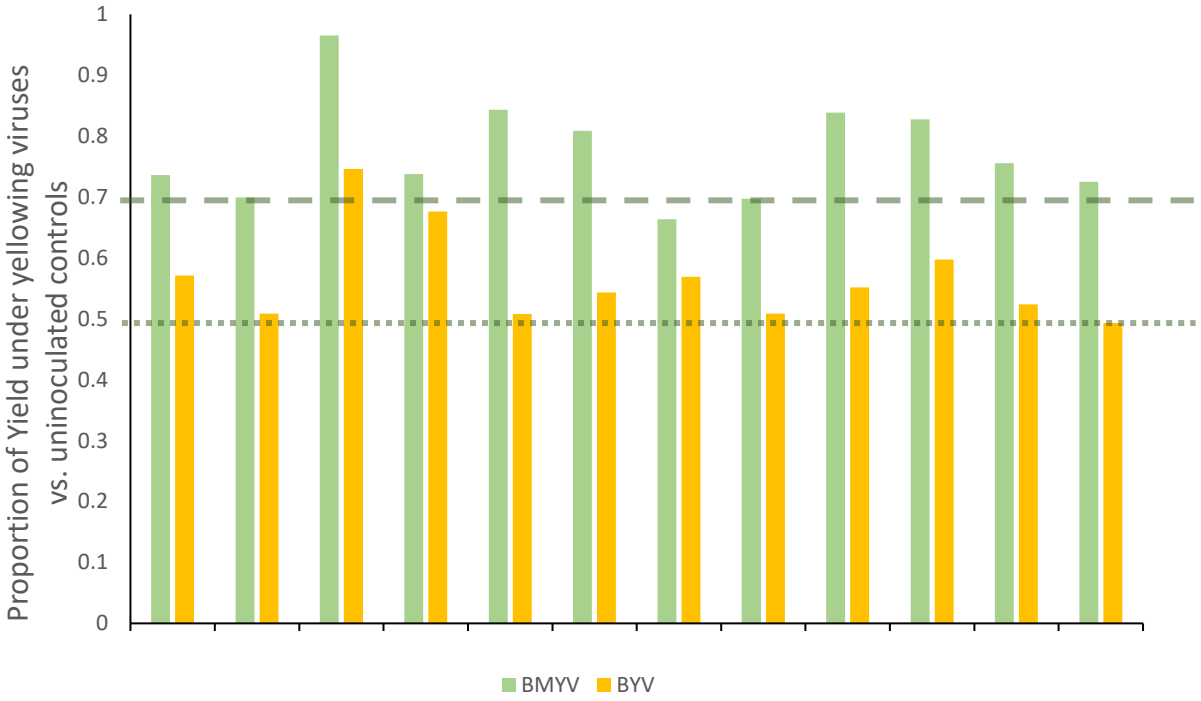

Appendix Figure 1 – Data from Goliath 2019 showing the range of results gathered from the experiment and how losses were closer to expected levels of 30% for BMYV and 50% for BYV. Variety has been anonymised.