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

AUTHENTICATION

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

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GROWER SUMMARY

Headline

Planting genetically different rootstocks and inter-row cropping can both reduce severity of Apple Replant Disease.

Background

Successive planting of apples on the same location can lead previously high yielding orchards to produce reduced establishment of young trees, unsatisfactory yields and ultimate loss or removal of the tree (Mazzola and Manici, 2012). This disorder has been termed Apple Replant Disease (ARD). ARD has previously been managed using chemical fumigation of the soils to remove any pathogenic causal agents present at replanting. Legislation against many active ingredients have made us look for alternative management strategies for treatment of ARD.

Multiple non-chemical proposals have been put forward to manage ARD including anaerobic soil disinfestation and applications of beneficial microbes. These include plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF). Both can be beneficial to the tree and increase yield, growth, and disease suppression but may not be effective at treating oomycete pathogens implicated with ARD (Xu and Berrie, 2018; Shuttleworth, 2021).

Orchard management practices are also important to prevent ARD onset. Crop rotation for a period of 5 years with a non-woody cover crop can reduce ARD pressure. Often growers do not have the land, time or resources to leave their orchards fallow or cover cropped, particularly in cider orchards. Inter-row cropping is an alternative strategy previously shown to reduce the severity of ARD, with distinctly different populations between the tree rows and grass alleyways (Rumberger *et al.*, 2004; Leinfelder and Merwin, 2006; Deakin *et al.*, 2018).

Rootstock selection is important due to different relative resistance between rootstocks (Rumberger *et al.*, 2004; Leinfelder and Merwin, 2006; Fazio *et al.*, 2012). Each rootstock will have a different level of vigour and ARD tolerance. Crop rotation with a different crop such as wheat can alleviate ARD but financial restrictions and land availability make using a different crop a large obstacle for many growers (Mazzola and Gu, 2007; Winkelmann *et al.*, 2018). Alternatively replanting an orchard with a rootstock different to the previous one can be effective in reducing ARD but the genetic resistance of the rotated rootstock and its genetic relationship to the previous rootstock are also important when deciding which rootstock to choose for rotation (Xu and Berrie, 2018; Deakin *et al.*, 2019; Shuttleworth, 2021).

In this study we present a continuation of the work from Deakin *et al* (2019) and report the results of rotating successive generation of rootstocks and different planting position. Here we report on (i) whether growth in the first 5 years after replant is greater in the alleyway then the corresponding tree station (hence ARD), and (ii) whether ARD severity was worse if the same or closely related rootstock genotypes were planted as those previously planted.

Summary

In this study we present the effect of planting rootstocks in the alleyway beside the previous tree station on ARD severity. Our results suggest that planting rootstocks closely related to the previous planted genotype can increase ARD severity. M.116 (derived from MM.106 in parental cross) and MM.106 both had greater initial growth in both girth and height compared the their paired trees in the previous tree stations. Other rootstocks more genetically different to the previously planted rootstock had similar growth in both the alleyway and the tree station. The effects of ARD were also apparent by Year 1/2 for both M.116 and MM.106.

Fruit number was higher in the alleyway trees compared to the tree station trees but fruit number was low across trees. The increased fruit in the alley could be due to larger more vigorous trees but could also be interpreted as more nutritious soils allowing energy flow into fruit production. M.116 and MM.106 had the largest disparity between the number of fruit in the alley and in the tree station, again highlighting the most severe ARD in those genotypes.

This report has focused on just one aspect of the experiment whilst I concurrently conduct the following tests:

- Metabarcoding of ITS and 16S regions to compare microbial populations in soils between alley and tree station as well as between rootstock genotypes.
- Soil functional assessment to see differences in soil carbon utilisation and bacterial enzyme activity between planting positions and rootstock genotypes.

Financial Benefits

It is difficult to identify the financial impact of ARD in orchards. Our data shows that tree establishment of common genotypes used in the cider industry may be hindered by a genetic link to the previously planted genotype. In the long-term if the tree is larger and has a healthier canopy it will both produce more fruit and create a more desirable bush shape for spray applications. Planting in the alleyway is financially difficult for growers due to existing alleys and compaction in those alleyways. Growers may also have existing irrigation lines or stakes that cannot be moved without significant financial investment.

Action Points

Growers should aim to plant a genetically different rootstock to the previous planted rootstock to maximise growth in the early years following planting. This will lead to the healthiest and largest tree and best financial reward long term for the orchard. If possible, alleyway rotation should also be considered if rootstock rotation is unviable.

SCIENCE SECTION

Introduction

Successive planting of apples on the same location can lead previously high yielding orchards to produce reduced establishment of young trees, unsatisfactory yields and ultimate loss or removal of the tree (Mazzola and Manici, 2012). This disorder has been termed Apple Replant Disease (ARD). The average cider orchard productivity lasts approximately 50 years so long-term growers will have to consider replanting and risk ARD onset at least once. ARD can cause stunted growth, poor fruit appearance, root tip necrosis, reduction in root biomass and delay in fruit cropping 2-3 years after expected years (Mazzola and Manici, 2012; LIU *et al.*, 2014; Zhu, Fazio and Mazzola, 2014). 50% reduction in orchard profitability has been reported on some sites, which with the already fine profit margins in the cider industry could cause the loss of multiple cider orchards in the UK. The causal agents of ARD include the fungal pathogens *Cylindrocarpon*, *Rhizoctonia* and *Fusarium* and the oomycetes *Pythium* and *Phytophthora* (Braun, 1995; Tewoldemedhin *et al.*, 2011; Mazzola and Manici, 2012; Manici *et al.*, 2013). Root lesion nematodes such as *Pratylenchus Penetrans* can exacerbate ARD caused by other causal agents by creating an entry point via root lesions (Mai and Abawi, 1981; Mazzola and Manici, 2012).

ARD has previously been managed using chemical fumigation of the soils to remove any pathogenic causal agents present at replanting. Products such as methyl bromide and chloropicrin were once useful in treating ARD but have since been banned due to their damaging effect on the environment and the chemical broad-spectrum fumigants that remain are not as effective in controlling ARD as their predecessors (Xu and Berrie, 2018). Multiple non-chemical proposals have been put forward to manage ARD including anaerobic soil disinfestation for disease management by either addition of plant-based-products followed by covering with plastic or application of brassica seed meal shown to increase tree growth and suppress ARD (Rosskopf *et al.*, 2015; Xu and Berrie, 2018; Wang and Mazzola, 2019). Applications of beneficial microbes such as plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) can be beneficial to the tree and increase yield, growth, and disease suppression but may not be effective at treating oomycete pathogens implicated with ARD (Xu and Berrie, 2018; Shuttleworth, 2021).

Orchard management practices are also important to prevent ARD onset. Crop rotation for a period of 5 years with a non-woody cover crop can reduce ARD pressure and short-term rotation with *Allium fistulosum* mixed with *Trichoderma* was able to increase *Malus hupehensis* seedling growth compared to ARD soils but were not as effective as sterile soil (Pan *et al.*, 2017; Hewavitharana, Mazzola and DuPont, 2019). Often growers do not have the land, time, or resources to leave their orchards fallow or cover cropped particularly in cider orchards. Inter-row cropping is an alternative strategy previously shown to reduce the severity of ARD, with distinctly different populations between the tree rows and grass alleyways (Rumberger *et al.*, 2004; Leinfelder and Merwin, 2006; Deakin *et al.*, 2018). Weed management must be included when replanting in alleyways due to the detrimental effect of weed competition on young trees being more severe than ARD symptoms in some cases (Xu and Berrie, 2018). Planting trees in the alley is more viable in dessert orchards that do not rely on mechanical picking like in cider orchards and trees are rotated more frequently.

Rootstock selection is important due to different relative resistance between rootstocks (Rumberger *et al.*, 2004; Leinfelder and Merwin, 2006; Fazio *et al.*, 2012). Cider orchards tend to use semi-vigorous rootstocks such as MM.106 that is more susceptible to ARD and M.116 that is more tolerant to ARD with more generally more vigorous rootstocks/varieties less likely to be affected by ARD (Auvil *et al.*, 2011; Wang and Mazzola, 2018; Xu and Berrie, 2018; Deakin *et al.*, 2019). Important dwarfing dessert orchard rootstocks are very susceptible to ARD (Auvil *et al.*, 2011). Geneva rootstocks (G.16, G.30, G.41, and G.210) have been shown to be more tolerant to ARD than Malling rootstocks (M.7, M.9, M.26, and MM.106) and have different bacterial rhizosphere species composition between the Geneva and Malling rootstocks (Rumberger *et al.*, 2004; Leinfelder and Merwin, 2006; Wang and Mazzola, 2019). Crop rotation with a different crop such as wheat can alleviate ARD, but financial restrictions and land availability make using a different crop a large obstacle for many growers (Mazzola and Gu, 2007; Winkelmann *et al.*, 2018). Alternatively replanting an orchard with a rootstock different to the previous one can be effective in reducing ARD but the genetic resistance of the rotated rootstock and its genetic relationship to the previous rootstock are also important when deciding which rootstock to choose for rotation (Xu and Berrie, 2018; Deakin *et al.*, 2019; Shuttleworth, 2021).

In this study we present a continuation of the work from Deakin *et al* (2019) and report the results of rotating successive generation of rootstocks and different planting position. Here we report on (i) whether growth in the first 5 years after replant is greater in the alleyway then the corresponding tree station (hence ARD), and (ii) whether ARD severity was worse if the same or closely related rootstock genotypes were planted as those previously planted.

Materials and Methods

Orchard Design

The current study was conducted on a cider orchard in the West Midlands of England in Worcestershire (52.251020, -2.301711). Before grubbing in 2014, the orchard had been 'Katy' apples on MM.106 for blocks 1 and 2 and MM.111 for block 3. In the study both rootstock genotype and planting position (alleyway vs tree station) were investigated. The study consisted of eight rootstock genotypes paired in the previous tree station and the corresponding middle alleyway position approximately 2m away from the tree station. Three randomised blocks were used for the eight tree pairs. Each pair location within each block was randomly assigned one of the eight genotypes. The plot plan of orchard is shown in Figure 1. General characteristics and history of the orchard are described in detail in (Deakin *et al.*, 2019).

Rootstock and Scion Selection

Eight rootstocks were selected for the study based on their tolerance to ARD, vigour, and importance in the industry. The rootstocks used were M.9 (unknown pedigree), M.26 (M.16 × M.9), M.27 (M.13 × M.9), MM.106 (Northern Spy × M1), M.116 (MM.106 × M.27), G.11 (M.26 × M. robusta 5), G.41 (M.27 × M. robusta 5), and EM_SEL1 (M. robusta 5 × Ottawa 3) from the East Malling breeding programme. M.27, G.41, G.11, M.9, and EM_SEL1 are all dwarfing rootstocks. M.26 is a semi-dwarfing rootstock and M.116 and MM.106 are semi-vigorous rootstocks. M.27, G.41 and M.116 are reported to be tolerant to ARD. M.9 and M.26 are the most susceptible to ARD out of the eight genotypes. The root ball of each rootstock was washed before grafting to the cultivar 'Worcester Pearmain' in 2015. The trees were potted in a peat and sand mix and grown for 7 months. The land was subsoiled and rotavated prior to plating to prevent compaction. Most similar trees with matching rootstocks were paired for the tree station and corresponding alleyway and planted in the random position in each block on October 14th, 2015. Trees were managed conventionally during the trial period.

Growth Measurements and Statistical Analysis

Initial measurements of height (from ground level) and girth (5cm above the graft union) were taken for each tree. Each winter during dormancy (between January and March) from 2017-2021 trees were assessed for height and girth. Girth was measured as circumference of the tree 5cm above the graft union as the trunk may not be perfectly symmetrical. Trees were marked at the point they were measured for consistency in girth measurements. Height was measured from ground level to the end of the leader of the tree (not including any leaf height added to the branch at the leader's tip). Yield was calculated as the number of fruits per tree.

All statistical analysis were conducted in R V4.0.2 (R Core Development Team 2008). In a case where one of the tree pair had died (either alley or tree station) the corresponding healthy tree in the pair would be removed from statistical analysis. The rate of increase (R) of the height and girth measurements at for each year ($Year^x$) were analysed relative to the measurements the year they were planted ($Year^1$) and the rate of increase described by the following formula:

$$R = \frac{Year^x}{Year^1} \times 100$$

Mean height and girth height change was visualised using ggplot2 package v3.3.2 (Wickham, 2011). For yield the mean number of fruits was calculated and visualised in ggplot2. ANOVA was used to see significance of fruit differences between genotypes and between alley and tree station. Tukey HSD test was used to identify the identify of significance between mean fruit number for each genotype individual in the agricolae package v1.4.0 (de Mendiburu, 2020).

Results

After 5 years of growth a pattern began to emerge on which trees were performing better in the alley than the tree station and by our definition were affected by ARD. G.11 trees did not grow during the experiment, likely due to an issue in the grafting process or compatibility of the scion with G.11. G.11 trees were thus removed from any further analysis in this study. The rate of increase for girth highlighted three rootstock genotypes that performed better in the alleyway than the tree station: MM.106, M.116 and EM_SEL1 **(Figure 1)**. The remaining rootstocks performed similarly in the tree station as in the row. For M.116 the rate of increase in girth was clear as early as year 1. MM.106 began to show a clear difference in the rate of increase by year 2 and EM_SEL1 in year 3.

The rate of increase in height was similar to the pattern seen for the girths. MM.106 and M.116 both had a higher rate of increase in height compared to the corresponding previous tree stations **(Figure 2)**. The higher rate of increase was clear as early as year 1 for both MM.106 and M.116 and remained higher throughout the trial. M.27 showed a slower rate of increase in the alley than in the tree station emerging in year 3 and persisting through to the end of the experiment. Rate of increase was similar for the other rootstocks genotypes. The effect on canopy vigour and health can be seen in **Figure 3**.

Fruit number was low for all genotypes on the orchard, ≥15 mean fruit per genotype across the trial in the final year of the study. There was more fruit on the trees planted in the alley compared to the trees in the previous tree station. P value for ANOVA was $p = 0.0984$ comparing fruit number between alley and tree station. Of the 7 genotypes, 6 had higher or equal fruit to the tree station trees, G.41 being the only tree with more mean fruit in the tree station **(Figure 4)**. ANOVA analysis of genotype showed significantly different mean fruit between groups ($p = 0.0188$). Tukey HSD test showed M.116 was significantly higher than M.27 and M.9 for mean fruit number.

Figures

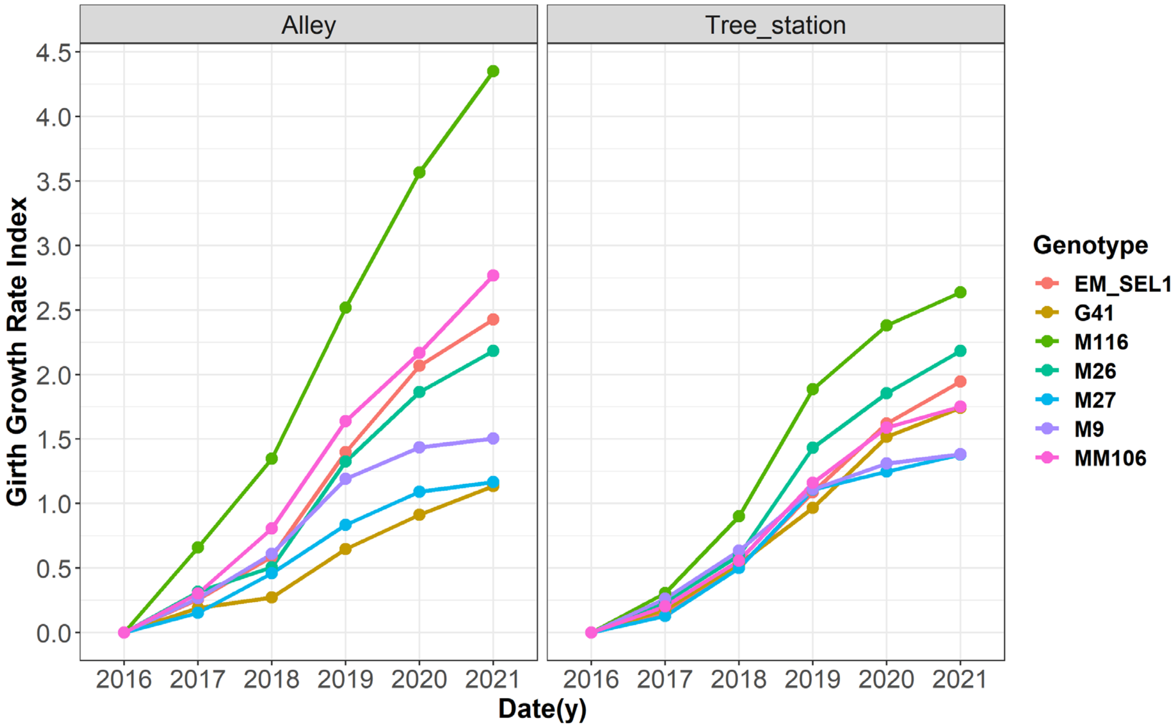

Figure 1: Mean girth growth rate index annually from 2016-2021. Each value was indexed based on their corresponding value in 2016. Colour of the line indicates rootstock genotype.

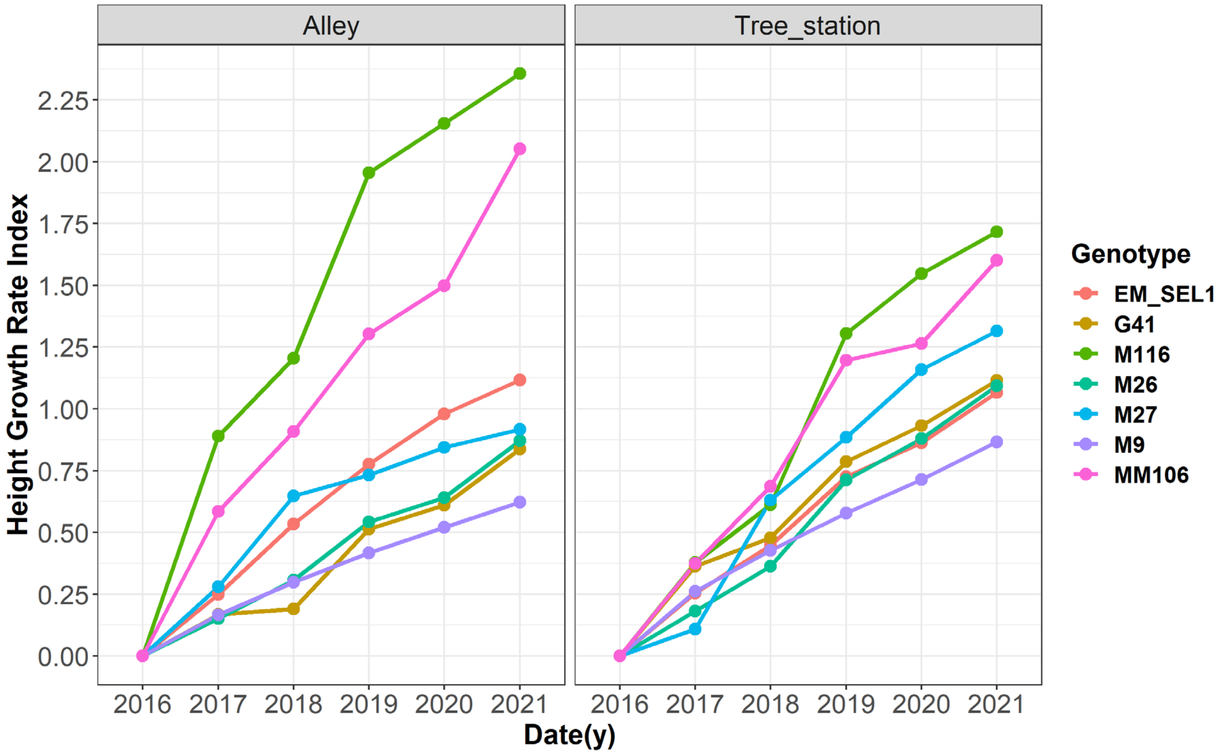

Figure 2: Mean height growth rate index annually from 2016-2021. Each value was indexed based on their corresponding value in 2016. Colour of the line indicates rootstock genotype.

Figure 3: ARD severity effect on MM.106 in the tree station (right) and the corresponding alleyway (left).

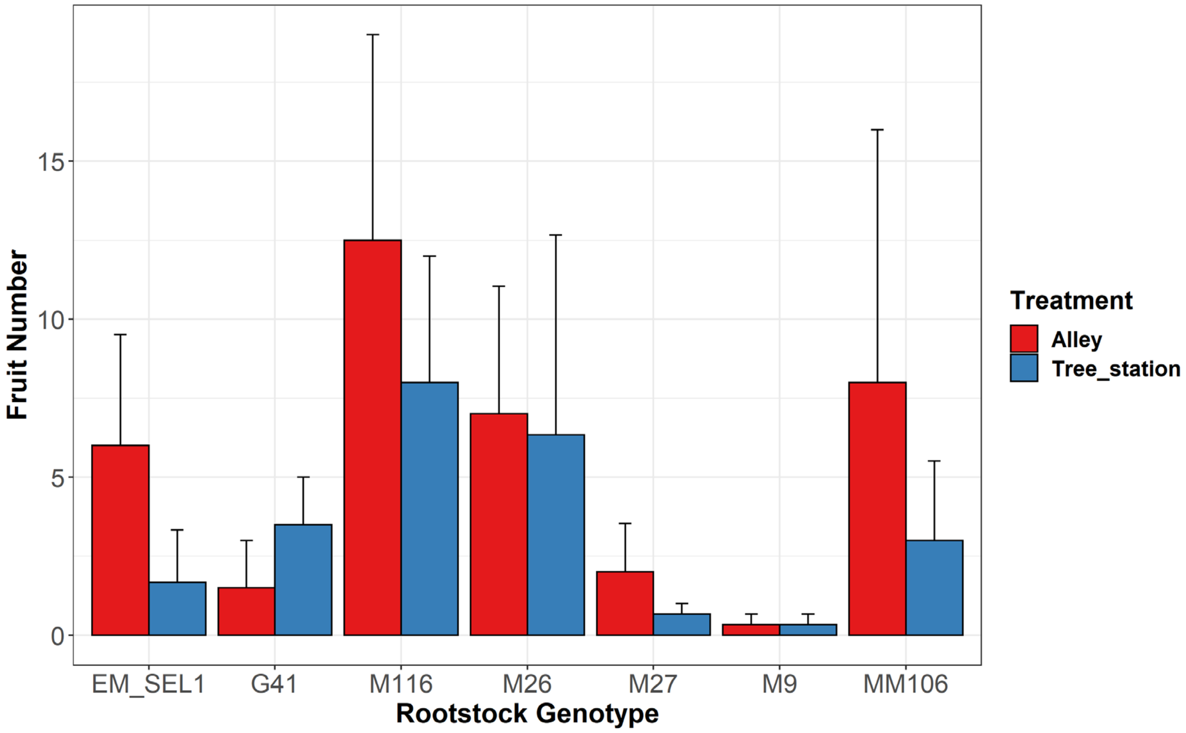

Figure 4: Mean number of fruits for each rootstock genotype planted in the Alley (Red) and in the previous Tree Station (Blue).

Discussion

This report presents a portion of the data for a larger experiment on the impact of rotating rootstock genotypes and planting the alleyways to alleviate ARD symptoms. This work shows the impact of rotating rootstocks and the alleyway effect on both tree establishment and yield. Interpretation of the data should focus on the growth parameters of the tree as the yield data may not be as significant due to the low number of fruits. To confirm all fruit was being counted accurately (not being picked before analysis) the orchard was visited in July, but as numbers were still low it may be that Worcester Pearmain produces fruit slower in the early years after planting.

Greater growth in the alleyway than in the tree station, the more severe that rootstock is affected by ARD. The results showed M.116 was the most severely affected by ARD on this site despite being previously described as tolerant to ARD. MM.106 and the coded EM_SEL1 were both also affected by ARD over the trial period. As MM.106 was one of the previously planted rootstocks on the site and M.116 is derived from a cross including MM.106, we have confirmed the hypothesis that rootstock rotation away from the previous planted genotype is effective at alleviating ARD. Tree vigour (data not shown) was also reduced when comparing the MM.106 and M.116 trees in the previous tree station with their corresponding pair in the alleyway.

Despite the low fruit number and the caution that must be taken when interpreting results described above, fruit number was higher for all rootstock genotypes apart from one in the alley. The increased fruit could be due to larger more vigorous trees but could also be interpreted as more nutritious soils allowing energy flow into fruit production. M.116 and MM.106 had the largest disparity between the number of fruits in the alley and in the tree station, again highlighting the most severe ARD in those genotypes. The more vigorous trees did have more fruit than the smaller dwarfing trees which is to be expected due to the size of the tree. This report has focused on just one aspect of the experiment whilst I concurrently conduct the following tests:

- Metabarcoding of ITS and 16S regions to compare microbial populations in soils between alley and tree station as well as between rootstock genotypes.
- Soil functional assessment to see differences in soil carbon utilisation and bacterial enzyme activity between planting positions and rootstock genotypes.

Conclusions

- Closer related rootstocks to previously planted rootstocks will have more severe ARD than those more distantly related.
- Planting in the alleyway next to the tree station can alleviate ARD symptoms.
- ARD onset can occur as early as Year 1 or Year 2 after planting.
- Detrimental growth and yield due to ARD are still prevalent 5 years after planting even in vigorous rootstocks.
- Further work on the microbiome differences between alleyway and row may determine some of the causes of ARD in the UK.

Knowledge and Technology Transfer

National Fruit Show 2018/2019

Fruit Focus 2018/2019

AHDB Tree Fruit Day 2019/2020/2021

AHDB soft fruit day 2019/2020/2021

NACM Parliament Cider Tasting 2020

NACM orchard visit 2019

AHDB Industry visit Dundee 2019

Worshipful Company of Gardeners presentation 2019

University of Nottingham DTP presentation 2019

University of Reading undergraduate presentation 2019

International canker workshop New Zealand 2020

AHDB Crops PhD Student Conference Best Poster Winner – 2021

Glossary

ARD – Apple Replant Disease

ANOVA – Analysis of Variance

HSD – Honestly Significantly Different

PGPR – Plant growth promoting rhizobacteria

AMF – Arbuscular Mycorrhizal Fungi

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