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Project number:	SFTF 170
Project leader:	Simon Segar (Harper Adams University), Glen Powell (NIAB EMR), Felicidad Fernández (NIAB EMR), Tom Pope (Harper Adams University).
Report:	Annual report, October 2020
Previous report:	n/a
Key staff:	Cindayniah Godfrey

Location of project: NIAB EMR

Industry Representative:	Loraine Boddington, National Association of Cider Makers, NACM Cider Makers Ltd., The Bounds, Much Marcle, Ledbury, Herefordshire, HR8 2NQ Charnee Butcher, Worldwide Fruit, Worldwide Fruit Ltd., Apple Way, Wardentree Lane, Spalding, Lincolnshire, PE11 3BB
Date project commenced:	23rd September 2019

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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.

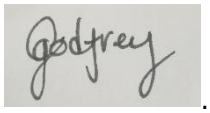
AUTHENTICATION

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.

Cindayniah Godfrey

PhD Student

NIAB EMR

Signature .....  ..... Date 30.10.20

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CONTENTS

GROWER SUMMARY

Headline

This project seeks to improve understanding of known woolly apple aphid resistance genes and to identify potential resistance genes from novel sources to include in a breeding programme. This project also aims to improve knowledge of woolly apple aphid lifecycle in the UK to better understand the pest.

Background

Woolly apple aphid (WAA; *Eriosoma lanigerum* Hausmann.) is an aphid originating from North America which has now spread across the world. In North America WAA has a sexual lifecycle, alternating between apple and American Elm (*Ulmus americana* L.) but in the rest of the world WAA has an asexual lifecycle feeding exclusively on apple. In the orchard WAA can overwinter on roots and low branches as early instar nymphs, which disperse to the rest of the plant in the spring, forming colonies which persist through the summer, especially on young growth and injury sites.

Woolly apple aphid saliva causes cells in the vascular tissue to rapidly divide, creating galls in plant tissue which reduce plant growth by disrupting water and carbohydrate flow, and through tissue disruption. The galls created by root-feeding WAA can affect above-ground growth, even in mature trees, and can have a knock-on effect on reducing fruit set. These galls often crack, especially after cold conditions, creating open wounds which are vulnerable to secondary infection, for example from European apple canker.

Chlorpyrifos and pimiricarb were used to control WAA until their withdrawal in 2016, although insecticides containing spirotetramat, for example Batavia, are still authorized for use. Spirotetramat is a systemic pesticide and can affect WAA feeding on the roots, even when applied to the above-ground parts of the plant. There are some natural enemies of WAA, such as the parasitoid wasp *Aphelinus mali* (Haldeman.) and several predators e.g. hoverfly larvae and earwigs. Unfortunately, natural enemies have not been recorded predating or parasitising the below-ground aphids. Resistant rootstocks offer an option to control these aphids, and ideally would be part of an Integrated Pest Management (IPM) strategy. Whilst resistant rootstocks can control below-ground WAA, other control methods, such as the use of *A. mali*, can be used to tackle WAA feeding above ground. There is then still the option to use conventional chemical pesticides where necessary for control.

Approximately ten *Malus* cultivars have been reported as showing WAA resistance and four distinct resistance-mediating genes have been identified. The gene *Er1* is derived from the

cultivar ‘Northern Spy’ and is the gene responsible for WAA resistance in the Malling-Merton (M.M.) rootstock series. This gene was the target of some work during the first year of the project.

Conventional plant breeding can take up to twenty-five years to bring a desirable trait to commercial introduction, especially for pest resistance. Marker-assisted selection (MAS) involves using the presence/absence of a marker linked to the target gene to identify whether the plant is resistant or susceptible, allowing quick and easy classification of any individual plant, making breeding programmes faster and more accurate.

Summary

In the first year of the project work focused on two main elements: mapping the resistance gene *Er1* and searching for potential novel sources of resistance within a range of crab apple species and domestic accessions. Both elements used similar methodology to achieve this year’s outcomes.

The second element of this year’s work was to take several crab apple species and accessions of domesticated apple and assess them for potential WAA resistance. Those which showed some resistance to WAA feeding will be used in the rest of the project to look at how resistance plants prevent aphid feeding, and the knock-on effects on growth and reproduction. A total of forty-one species of crabapple and domesticated apple accessions were phenotyped for WAA resistance. Of which, twelve were found to be WAA susceptible, eighteen resistant, and eleven as intermediate between resistant and susceptible.

To better understand how to control WAA in orchards it is important to understand its lifecycle in the UK and how it responds to feeding on different cultivars. To determine how the life cycles of populations of WAA around the world and within the UK differ, samples of WAA from different populations have been collected for genetic analysis. Sexually reproducing populations are expected to show higher genetic diversity than asexually reproducing populations, which may be a straightforward way of determining whether there is any sexual reproduction in the UK; it is currently thought that there is not.

Financial Benefits

This report summarises only the first year of a four-year project. As such there are no clear financial benefits yet outlined.

Action Points

There are no actions points from the first year of this project, as it is still in its early stages.

SCIENCE SECTION

Introduction

Four WAA resistance genes have been identified, all from different apple genotypes: *Er1-4*. The dominant major gene *Er1* is derived from Northern Spy (Knight *et al.*, 1962; King *et al.*, 1991) which is a parent of the commercially successful resistant rootstock MM106 (M.1 x Northern Spy). The second resistance gene *Er2* is derived from *Malus* x *Robusta* 5 (*M. baccata* x *M. prunifolia* Carr.; Robusta 5) (King *et al.*, 1991) and has been used as the source of WAA resistance in the Geneva rootstock series developed at Cornell University in the 1950s (Cummins and Aldwinckle, 1983). A better understanding of the four resistance genes already identified will help to understand how they can be incorporated into breeding programmes. Several wild species of apple (crab apples) are reported to show some resistance to WAA and may be sources of novel single resistance genes. The identification of novel resistance genes would increase opportunities for gene pyramiding and contribute to long-term rootstock breeding programmes.

Woolly apple aphid resistance is phloem-related: resistant varieties show thickened bundles of sclerenchyma around vascular tissue, mechanically preventing aphid feeding (Staniland, 1924). For *Er1-4* it has not yet been established how resistance affects aphids i.e., whether it prevents establishment or restricts feeding, causing colony collapse and death. This can be measured using parameters such as intrinsic rate of increase, mean relative growth rate, colony size, and wool production (Sandanayaka *et al.*, 2003).

Commercial use of crab apples

Many commercial apple varieties are self-incompatible (Broothaerts *et al.*, 2004), meaning that in a single-variety orchard an external pollen source is needed in order to guarantee pollination and fruit set, known as pollinisers. This is common practice in top fruit production, in apple orchards ornamental crab apple species are often used as pollinisers because they are easily distinguishable from the main crop (Kendall and Smith, 1975) and often have many flowers for pollinators (Church, Goodall & Williams 1974). Pollinisers need to have a similar flowering time to the crop and must be compatible to ensure successful pollination can occur (Sakurai *et al.*, 2000). It is crucial that pollinisers be close enough to the main crop to ensure pollen spread to all crop trees through pollinator activity (Free, 1962), resulting in pollinisers being planted regularly throughout the crop. These can, however, create reservoirs for pests and diseases if the pollinisers are susceptible. Resistant pollinisers may help to control pest build up within orchards.

This portion of the wider project set out to phenotype fifty-nine different accessions of *Malus* x *domestica* and some crab apple species to find potential sources of novel resistance genes. A range of levels of resistance is expected to be shown across the genotypes selected, allowing identification of accessions with potential for inclusion in a rootstock breeding programme.

In the first year of this project two crosses expected to contain the resistance gene *Er1* were scored for WAA resistance following the methods given below. The results of this are not completed and so these data are not included in this report, but they will be used to improve genetic mapping of *Er1* which in the long term will aid Marker Assisted Selection.

Materials and methods

Plant material

Plant material was selected based on the following criteria: previously reported as having some WAA resistance or tolerance; having a flowering time which matches commercial cultivars; reported as having disease resistance. In some instances WAA resistance and resistance to pathogens such as fireblight have been found within a single accession (Miñarro & Dapena, 2009). Known susceptible accessions and sources of known resistance genes were included as positive and negative pseudo-controls respectively. A total of fifty-nine genotypes were selected, of which forty-one remained healthy enough for analysis, detailed in Table 1. The other eighteen died as a result of severe green apple aphid (*Aphis pomi*, de Geer) infestation which destroyed new season growth.

Graft wood was collected in late February. Fifteen accessions were provided by Frank P. Matthews nurseries (Tenbury Wells, Worcestershire) and the remaining twenty-six were collected on site at NIAB EMR (Table 1).

Table 1- List of apple accessions used and their origins

Genotype name	Type	Source
Polish 22	Rootstock	NIAB EMR
M.9	Rootstock	NIAB EMR
Mac 4	Rootstock	NIAB EMR
Malus fusca M	EM germplasm accession	NIAB EMR
Indian Magic	Commercial crab apple	F P Matthews
Alnarp 2	Rootstock	NIAB EMR
Malling Crab 'C'	EM germplasm accession	NIAB EMR
Malus florentina	EM germplasm accession	NIAB EMR
CG11	Rootstock	NIAB EMR
CG202	Rootstock	NIAB EMR
Malus koreana	EM germplasm accession	NIAB EMR
Malus × *robusta* 'Red Sentinel'	Commercial crab apple	F P Matthews
Malus praecox	EM germplasm accession	NIAB EMR
Malus pumilla 7728	EM germplasm accession	F P Matthews
White Angel	Commercial crab apple	F P Matthews
Gorgeous	Commercial crab apple	F P Matthews
White Star	Commercial crab apple	F P Matthews
Louisa	Commercial crab apple	F P Matthews
Mac 24	Rootstock	NIAB EMR
Malus robusta (EMLA)	EM germplasm accession	NIAB EMR
Malus hupehensis (EMLA)	EM germplasm accession	NIAB EMR
Northern Spy	Old scion variety	NIAB EMR
Scarlet Sentinel	Commercial crab apple	F P Matthews
Malus baccata	EM germplasm accession	NIAB EMR
Hashabi MH10.1	Rootstock	NIAB EMR
Malus kansuensis	Commercial crab apple	F P Matthews
Malus niedzwetzkyana	Commercial crab apple	F P Matthews
Novole	Rootstock	NIAB EMR
Malus baskatong	Commercial crab apple	F P Matthews
Malus x *magdeburgensis*	Commercial crab apple	F P Matthews
Malus coronaria 'Elk River'	Commercial crab apple	F P Matthews
Malus rubra 'Evelyn'	Commercial crab apple	NIAB EMR
Malus transitoria	EM germplasm accession	F P Matthews
Malus floribunda	EM germplasm accession	NIAB EMR
Malus floribunda J	EM germplasm accession	NIAB EMR
Malus halliana	EM germplasm accession	NIAB EMR
Mokum	Commercial crab apple	F P Matthews
Malus platycarpa (EMLA)	EM germplasm accession	NIAB EMR
Malus x *robusta* 5a	EM germplasm accession	NIAB EMR
Malus tschonoskii	EM germplasm accession	NIAB EMR
Malus x zumi 'calocarpa'	EM germplasm accession	NIAB EMR

All genotypes were grafted on to M.9 rootstocks in mid-March and left to grow under glasshouse conditions until the above-graft growth was at least 10cm in length. Three repeats of each genotype were used except for the following where there were six repeats: M.9, Northern Spy, *M. floribunda*. This is because these are expected to show clear resistance/susceptibility and will be used for further studies in the future. There would have been six repeats of some others including *M. robusta* 5a but they also died early in the experiment, as mentioned above.

Aphids

Woolly apple aphids were collected from orchards at NIAB EMR from a wide range of apple genotypes, both commercial cultivars in the field and potted crab apples in glasshouse conditions. Aphids were collected by brushing aphids gently from where they were feeding into a small tub using a fine paintbrush, taking care to not damage the aphids' stylets.

Inoculation

Two inoculation sites were selected for each tree, spaced well apart. Grafting tape was used to secure a petiole to the main stem such that the space between was covered on all sides except above (see figure 1). A pea-sized amount of WAA, of mixed life stages, along with wax was placed into this space using a dry, fine paintbrush. The grafting tape kept the aphids in position, allowing colonies to feed and build.

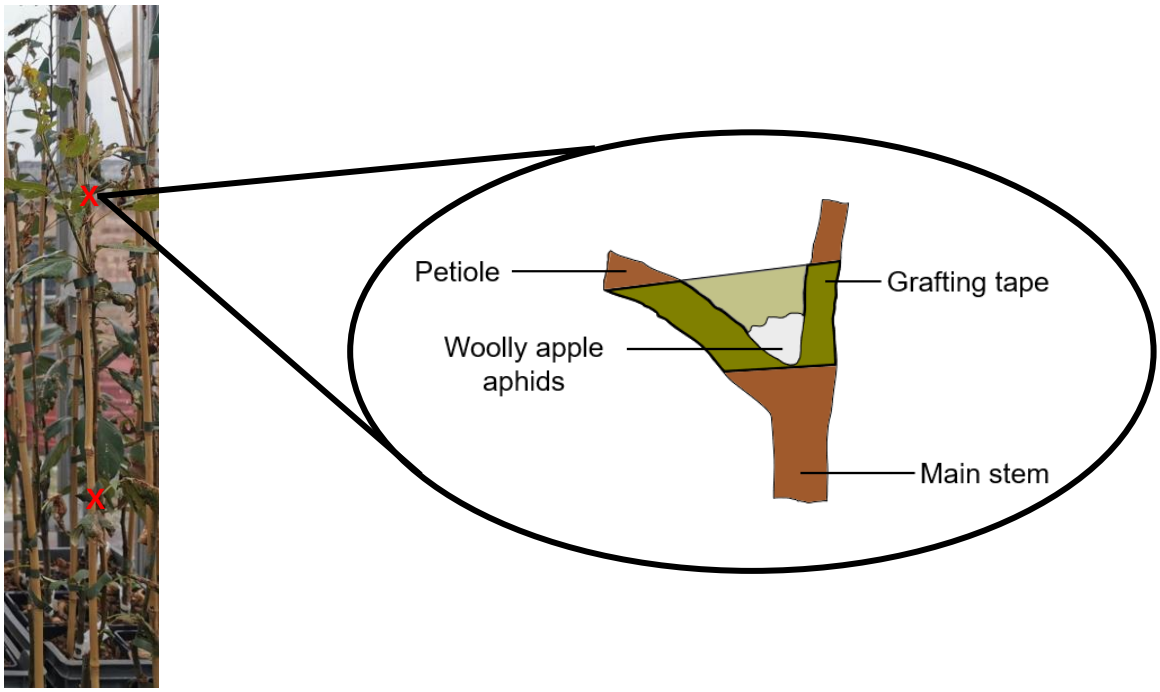

Figure 1- diagram of inoculation site showing grafting tape 'nest', WAA inoculum, and approximate inoculation sites marked on the tree.

After inoculation trees were left in glasshouse conditions for two weeks to allow time for aphid populations to increase. Trees were watered daily and, although not kept under controlled temperature conditions, fans under manual control were used during periods of high temperature. After two weeks trees were scored for resistance/susceptibility to the criteria given in Table 2. Those trees which were scored as susceptible were discarded at this point. For those trees which scored as resistant the process was repeated to reduce the likelihood that a colony may not have developed for a reason other than host-plant resistance. Re-inoculation was carried out at different position on the tree and after an additional two weeks final scoring was carried out.

Table 2- criteria used to score individuals for WAA resistance.

Score	Description	Status
0	No colonies	Resistant
1	Single colony/two to three small colonies less than 1cm in diameter. Colony/colonies located around inoculation sites. These colonies do not persist beyond the end of the growing season.	
2	Two to three larger colonies greater than 1cm in diameter. Colonies located around inoculation sites. These colonies do not persist beyond the end of the growing season.	
3	Four or more small colonies less than 1 cm in diameter/two to three colonies greater than 1cm in diameter. Colonies spread over the plant away from inoculation sites. Colonies persist into winter.	Susceptible
4	Four or more large colonies greater than 1cm in diameter. Colonies may have begun to join up. Colonies well spread over the plant. Colonies persist into winter.	
5	Five or more large colonies greater than 1cm in diameter. Many small colonies. Often colonies have begun to join together. There are few parts of the tree without WAA colonies. Colonies persist into winter.	

Statistics

The final scores were analysed with a Chi-square test using R Studio with Rx64 version 3.6.1 (RStudio Team, 2020).

Results

The results of the aphid scoring work are summarised in Figure 2. Northern Spy and *Malus robusta*, known sources of WAA resistance genes *Er1* and *Er2* respectively scored as resistant to WAA, and M.9 which is known to be susceptible to WAA scored as such. Eighteen genotypes scored as completely resistant to WAA feeding but the majority showed variable levels of susceptibility. The differences in susceptibility between genotypes was found to be significant ($P<0.05$).

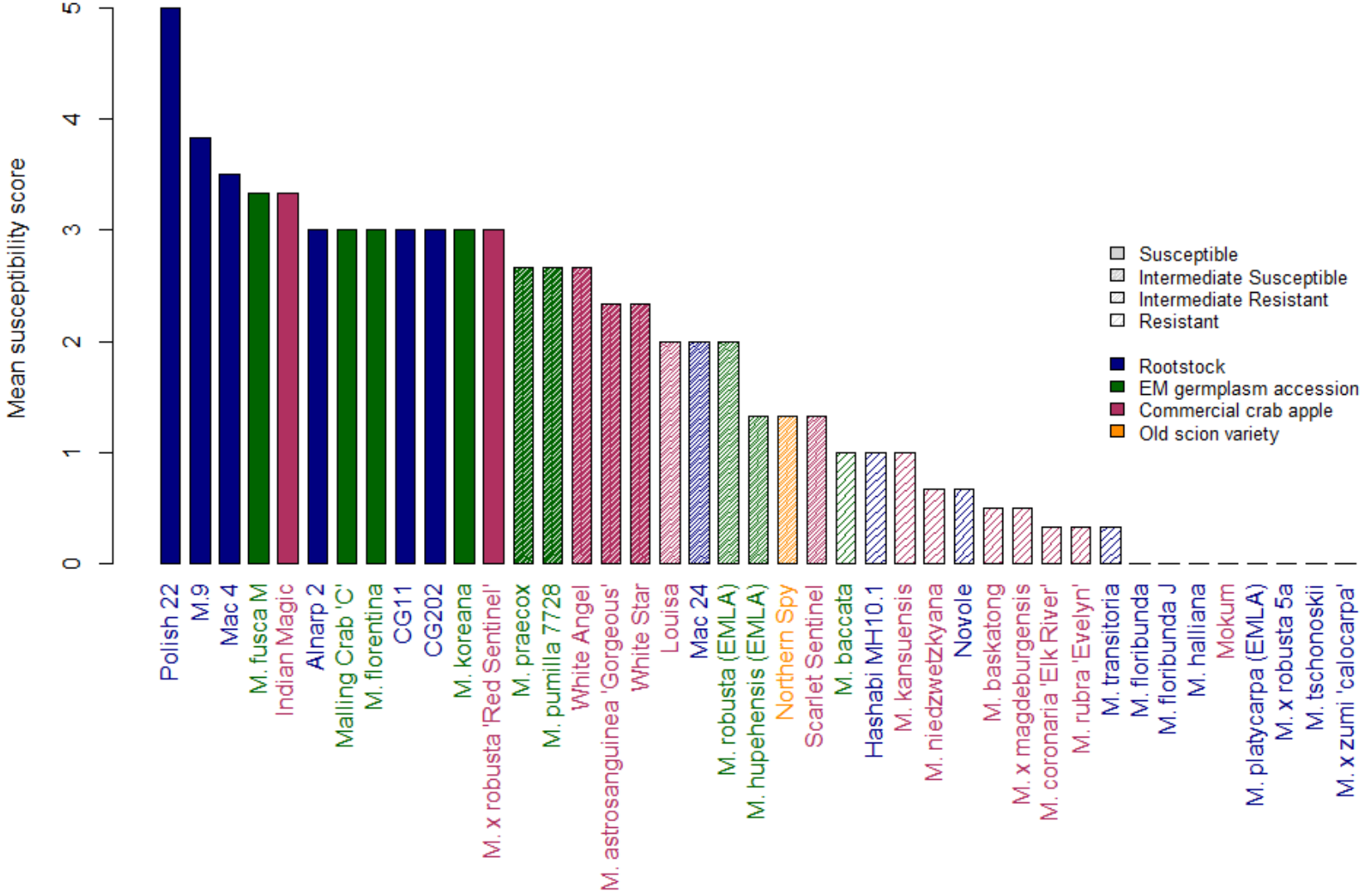

Figure 2- graph showing the mean susceptibility scores for woolly apple aphid feeding on different apple genotypes.

Discussion

The results presented here identified eight crab apple accessions as being resistant to WAA, five of which have not previously been investigated. In addition, twenty-one accessions may harbour WAA resistance or tolerance and are good candidates for further study.

Genotypes used

This work included a much wider range of genotypes than commercial breeding programmes normally would, where there is more emphasis on inheritance of a single resistance gene and scoring tends to focus on a binary resistant/susceptible classification. A similar methodology and scoring criteria were used when mapping *Er1-3*, although using galls produced over several months as a measure of infestation, along with colony growth, as here (Bus *et al.*, 2008). Genotypes with an intermediate resistance score may arise from wild types not showing binomial segregation of resistance genes seen in resistant cultivars. Variable resistance may occur if the resistance gene is part of a QTL or under different combinations of host and aphid genotype (Kanvil *et al.*, 2014). Although here some WAA were found feeding on resistant accessions, namely Northern Spy and *M. robusta* EMLA, it is unlikely that these aphids are of a resistance breaking biotype but rather that environmental conditions have affected WAA performance and/or expression of resistance genes (Bus *et al.*, 2008). There is limited anecdotal evidence for resistance-breaking WAA in the UK but samples of these aphids were taken for genetic analysis. It is likely that these aphids will die out before winter, leaving the plants clean, but a third scoring event will help to identify whether this is the case.

Both accessions of *M. floribunda* used here showed complete resistance to WAA. This is in agreement with some previous work, such as that of Sandanayaka *et al.* (2005) who found reduced WAA settlement on *M. floribunda*, compared to commercial varieties. These authors also found that WAA survival on *M. floribunda* was greatly reduced. *Malus floribunda* 821 is already used in the EM breeding programme (Fernández Fernández, 2020, pers. comm.) and accessions of *M. floribunda* could in future be used to identify and map the novel resistance gene carried by *M. floribunda*. The self-incompatability locus of *M. floribunda* 821 is known (Verdoot *et al.*, 1998) which, if compatible with the crop variety, would make it an ideal candidate for a resistant polliniser. Of the other six genotypes which scored as completely resistant only one, *M. robusta* 5a, has previously been studied for WAA resistance.

Limitations of inoculation and scoring

This work shows the full range of susceptibility completed to the scoring criteria given in table 1. Although these criteria and the inoculation protocol are defined there is still a degree of

subjectivity: the number of aphids used to inoculate is hard to standardise, the temperature conditions across the study period varied considerably, as did the time period between first inoculation and final scoring. Although the time between inoculation and scoring was standardised to two weeks, the time between first scoring and re-inoculation could not be standardised because of a lack of aphid inoculum available. Individuals which were immediately re-inoculated would receive their final score after four weeks of aphid growth whereas those with a larger time interval would be expected to have more aphid growth. Whilst standardising this time period is not always possible, for this reason, including the time period or the re-inoculation pre score as a confounding factor may help to determine the extent to which the time between inoculation events influences the ultimate resistant/susceptible score given.

This last point may explain the genotypes indicated in figure 2 as being intermediate i.e. falling between resistant and susceptible. Standardisation of the time for full completion of the work would help to eliminate some variation but this was not possible because of a shortage of WAA for inoculation. A third inoculation event at the end of the season may help to clarify some intermediate genotypes.

Natural enemy control

Woolly apple aphid has a number a natural enemies, most notably the parasitoid wasp *Aphelinus mali* (Haldeman) which can very effectively control WAA in commercial conditions (Cohen *et al.*, 1996). Aphid population control by *A. mali* was not controlled for and at times numbers in the glasshouse were very high which may have led to populations being smaller than usual and potential over-representation of resistant genotypes in the data. Temporal separation of the two inoculations should reduce the likelihood of this because they will occur at different points in the seasonal lifecycle of *A. mali*. It is possible to control *A. mali* with selective insecticides as carried out by Bus *et al.* (2008) in a similar study, which could be challenging but may be an option if *A. mali* becomes a limiting factor.

Monitoring *A. mali* numbers and parasitism levels will allow this to be included as a variable. *Aphelinus mali* has been shown to be attracted to yellow sticky traps, stapled vertically to the tree trunk (Beers, 2012), giving an option for easily monitoring parasitoid numbers in a glasshouse environment. The developmental times for *A. mali* across a range of six temperatures from 13°C to 30°C have been published (Asante and Danthanarayana, 1992) which will allow rate of parasitism to be calculated and included as a factor in WAA survival.

Conclusions

Eight varieties were found to be completely resistant to WAA feeding and, therefore, warrant further investigation, of which five have not been studied before. Most genotypes however showed some susceptibility, suggesting variation in resistance. As this work was the first completed experiment of the project it acted as a useful metric for determining whether the techniques used here will be feasible going forward. There is good potential for inclusion of some of these genotypes in a resistance breeding programme both in the short term, for those for which research has already been carried out such as *M. floribunda* accessions, and in the longer term for less-well categorised accessions.

Despite the limitations to this study discussed above, this work has identified a number of potential targets for future study. The range of susceptibilities shown here gives a good platform to investigate the effects of resistance on WAA survival.

Knowledge and Technology Transfer

- 30.10.19 – presentation to CTP students and industry representatives.
- 14.11.19 – poster at the Berry Garden Growers' conference.
- 28.11.19 – presentation at Harper Adams PGR colloquium.
- 29.01.20 – presentation at AHDB Crops PhD student conference.
- 27.02.20 – poster and brief presentation at AHDB Tree Fruit Day.
- 01.04.20 – presentation to PhD students at NIAB EMR.
- 08.04.20 – presentation to Genetics, Genomics and Breeding department at NIAB EMR.
- 04.08.20 - presentation to CTP students and industry representatives.

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