FINAL PROJECT REPORT

Project Title: Wooly Apple Aphid resistance in advanced rootstock selections

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Total Project Request: **Year 1:** $12,000 **Budget 1**

Organization Name: USDA ARS PGRU-FAZIO

Item	**2010**		
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies **	2,000		
Travel			
Miscellaneous			
Total	2,000		

Footnotes: ** DNA genotyping supplies.

Budget 2

Organization Name: WSU TFREC-BEERS

Item	**2010**		
Salaries			
Benefits			
Wages **	7,000		
Benefits	1,500		
Equipment			
Supplies***	1,500		
Travel			
Miscellaneous			
Total	10,000		

Footnotes: **Temporary labor help for greenhouse inoculations and data collection * Greenhouse supplies, fees.**

Background and Objectives: the woolly apple aphid (WAA), *Eriosoma lanigerum* has become a more severe pest in Washington apple production in the past few years. Milder winters have promoted overwintering survival on the aerial parts of the tree. In addition new plantings are rarely made on resistant rootstocks, and a very low percentage of the acreage is currently on resistant rootstocks. The transition from organophosphate insecticides to either insect growth regulators or neonicotinyl insecticides may also be contributing to higher pressure. It may be possible to control this pest significantly by utilizing genetically resistant rootstocks in new apple plantings. Resistant rootstocks would significantly reduce the ability of WAA to overwinter in the root zone and therefore decrease their survival and recurrence during the growing season. A previous greenhouse test of 8 clonally propagated rootstocks and 2 seedling rootstocks demonstrated that several of the new Geneva rootstocks to have virtual immunity to a Washington strain of woolly apple aphid due to the presence of the Robusta 5 derived *Er2* WAA resistance gene located on Linkage Group 17 of the apple genome. We have closely linked markers to this gene, however the ultimate test of the presence and efficaciousness of this gene can only be revealed by a good phenotypic replicated test. Although not part of this proposal we intend to clone the WAA resistance gene by probing a BAC library of G.41 possessing the Er2 gene. The proposed objectives were: 1. Test an array of Elite Geneva rootstocks and other commercially available rootstocks in a replicated greenhouse test for resistance to Wooly Apple Aphid (WAA) and disseminate knowledge to growers for planting recommendations.
2. Test the same array and as well as parents and other apple rootstocks for the presence of markers linked to wooly apple aphid the resistance genes.

Significant Findings
Objective 1: Nine rootstocks were categorized as resistant (3010, 3067, 4010, G.214, 4288, 4292, 4809, G.87, G.210), while five of the rootstocks were categorized as susceptible (2006, 3902, 4011, 4088, and M.9 Pajam). The final group (5890, 4814, 6969, 2406) were categorized as unsure; while the percentage of replicates rated as susceptible was low, there was enough evidence of woolly apple aphid establishment to take them out of the resistant group. These clones may exhibit an intermediate level of resistance to WAA.
Objective 2: Most published DNA markers linked to the different sources of wooly apple aphid resistance were difficult to work with in a high throughput marker assisted selection setting. Published markers linked to *Er2* were also difficult to ascertain and we had to rely on in-house developed markers to continue work on this resistance gene. Hence we developed additional markers like MalSSR2952 that were better suited at distinguishing the resistance in the progeny of Robusta 5 (the source of resistance).

Results and Discussion

OBJECTIVE 1

Despite repeated infestation, the first rating indicated only a moderate level of infestation in the susceptible 'M.9 Pajam' (ratings of 0-2). For this reason, the aphid populations were allowed to develop for an additional 6 weeks. At this time, most of the susceptible rootstocks were rated as 2 or 3.

For the purposes of summary, rootstocks rated as ≤1 were considered resistant, and those rated >1 were considered susceptible (Table1014.1). The percentage of reps falling into 'Resistant' or 'Susceptible' was calculated, and an overall rating assigned.

Nine of the rootstocks (3010, 3067, 4010, 4214, 4288, 4292, 4809, 5087, 6210) were considered 'Resistant' to woolly apple aphid, in that none aerial portions of the replicates had any rating >1, and only 1 replicate had a root system rated as susceptible (5087 was rated 1 and 3 for the top and roots, respectively). Given the consistency of the other replicates, this replicate may have been mislabeled.

Five of the rootstocks (2006, 3902, 4011, 4088, and M.9 Pajam) were categorized as susceptible, with 43 to 100% of either the tops or roots rated as susceptible. This group included the known susceptible 'M.9 Pajam'.

Four of the rootstocks (5890, 4814, 6969, 2406) were categorized as 'Unsure'; while the percentage of replicates rated as susceptible was low, there was enough evidence of woolly apple aphid establishment to take them out of the 'Resistant' group. These clones may exhibit an intermediate level of resistance to WAA, or the infestation levels are the result of phenotypic variation.

Only two of the rootstocks in the 2010 evaluations were also in the 2009 evaluations (exp. 0912). One, '5087', was considered 'Resistant' in both evaluations. The other '4011', was considered 'Intermediate' in 2009, and susceptible in 2010. However, it was represented by only 4 replicates in 2009, and 8 in 2010, so the latter rating is felt to be more reliable.

Correlation of greenhouse experiments with molecular marker information

We have synthesized DNA markers listed on table 2 and assessed their presence/absence in an array of 96 apple rootstock DNAs that includes genotypes in Table 1 as well as commercial apple rootstocks and parents in the breeding program. We have discovered that several of the SCAR markers in Table 2 were very hard to amplify by PCR and therefore not suitable for large scale Marker Assisted Selection. A sample of one of the many DNA marker gel images generated during this study is in Figure 1.

Table 2. A list compiled by Bus et al. (2008) represents some of the markers that were used to tag resistance to WAA.

Marker name	**Marker type**	**Original RAPD/EST**	**WAA gene**	**Forward primer**	**Reverse primer**	**Product size (bp)**	**PCR conditions** [a]	**Linkage group**
NZsc_C20	SCAR	OPC20	*Er1*	TCTCTAACTCAATAACTCCCAAGAC	ACTTCGCCACCATTATCACTCCTGA	2,000	Td 70–60	8
NZsc_GS327	SCAR	GS327	*Er1*	GCCAAGCTTCAATGTCGGAGTAGAT	CAAGCTTCCCCTAAGGCTATTGCCA	1,600	Ta 60	8
NZsc_O05	SCAR	OPO05	*Er1*	CCCAGTCACTAACATAATTGGCACA	CCCAGTCACTGGCAAGAGAAATTAC	1,700	Ta 60	8
NZsn_O05	SNP	OPO05	*Er1*/*Er3*	AACGTCATGTCAATAT	CCCAGTCACTGGCAAGAGAAATTAC	880	Td 70–60	8
NZsc_E01	SCAR	OPE01	*Er3*	CCCAAGGTCCGAACACAAATGAGAG	CCCAAGGTCCAAAACTATCCCGAAG	1,350	Ta 60	8
NZsc_A01	SCAR	OPA01	*Er3*	CAGGCCCTTCAGCAAAGAG	CAGGCCCTTCACTACTAATA	1,250	Ta 60	8

				GTGTCT	AGAAC			
NZms_EB106753	SSR	EB106753	*Er1*/ *Er3*	TCTGAGGCTCCCAAGTCC	TAGGAGCAGAAGAGGTGACG	175	Td 65–60	8
NZms_EB145764	SSR	EB145764	*Er2*	TTCCAGCGATCCAAAACAAT	GCTCAGGAACACCTCGTTCT	198	Td 65–60	17

The SCAR and SNP primers were derived from RAPD markers (Operon Technologies, Alameda, CA), and the SSR primers were designed from ESTs of apple.

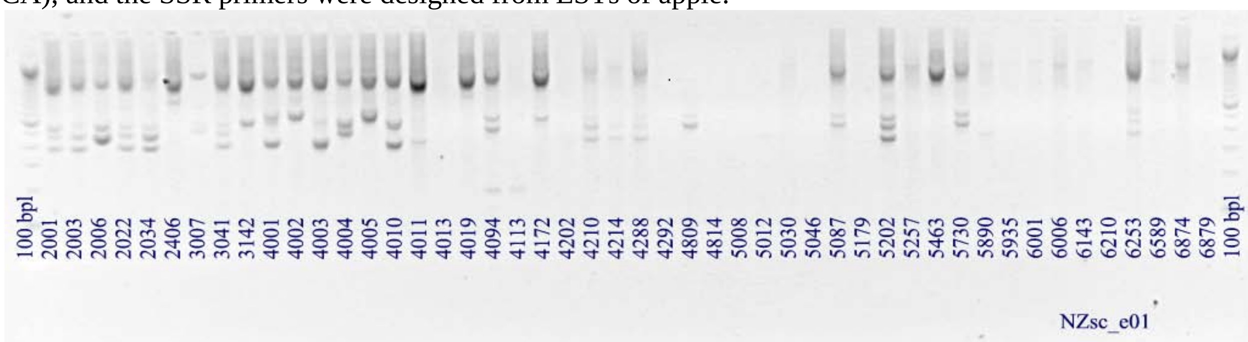

Figure 1. An example of a gel image of SCAR marker NZsc_E01 where the array of Geneva elites are tested for the presence of the resistance gene *Er3*.

With regards to the resistance gene Er2 derived from Robusta 5 we tested several markers located on chromosome 17 of apple that were reported to be linked. The markers and genomic location in parenthesis were: NZms_EB145764 (1,585Kb), MR1133 (3,576Kb), MalSSR2952 (5,014Kb), CH05g03 (5,324Kb), GD153 (9,138Kb), and GD96 (11,820Kb). We found inconsistencies according to the genetic map by Bus et al. (2008) which placed Er2 between markers GD96 and GD153 (Figure 2) next to NZms_EB145764. This report led us to believe that we could use GD96 and GD153 as predictors of the presence of the resistance gene.

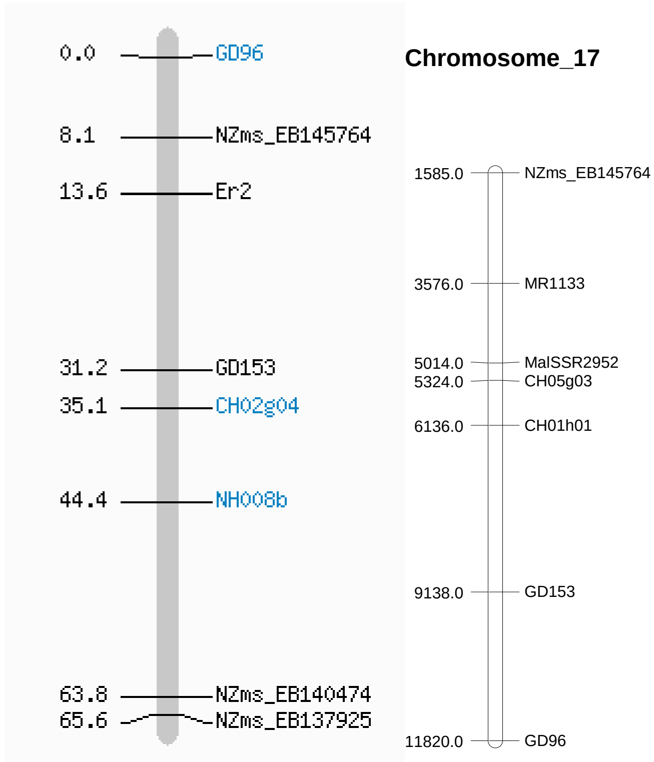

Figure 2. Putative map of the location of the *Er2* gene by Bus et al. (2008). Physical map of chromosome 17 with correct marker locations in Kilobases.

As it turns out the predictive power of GD96 and GD153 was not good and had to synthesize the NZms_EB145764 marker to detect *Er2*. While the new marker had good predictive power for most Robusta 5 ((R5) progeny in crosses with Ottawa 3 it was not able to detect the Robusta 5 resistance allele in another family whose progeny is G.41 and G.202 which have been experimentally classified as immune to the wooly apple aphid. Further study of this marker in a segregating population indicated that it was not behaving in a true codominant manner like most SSR markers do, rather one of the alleles was null. This hinders the utilization of such marker for large scale marker assisted selection. Alternative markers are being sought for easy visualization/selection on gels. With regards to the rootstocks classified as U (unsure) in the greenhouse phenotypic experiments summarized on table one, G.969 (6969), G.890 (5890) should be resistant according to the molecular marker information. Rootstock CG.4814 does not possess any markers for resistance, however this rootstock may be a recombinant showing no markers bust still possessing the gene(s) for resistance. Rootstock CG.2406 is has a very different genetic background than the others tested here, because it is the only one with *Malus micromalus* in its pedigree. This rootstock did not seem to possess any marker allele related to the R5 resistance, however in the test for the marker NZsc_GS327 linked to the Northern Spy (NS) resistance gene on Chromosome 8 we detected a marker with similar molecular weight indicating the possibility that a similar gene might be acting, in addition we detected a unique allele for the NZmsEB106753 marker also linked to the NS resistance. Susceptible rootstocks CG.2006, CG.3902, CG.4011, CG.4088, did not possess markers for any of the detectable resistance genes. More work is needed to study the inheritance and presence of aphid resistance in potential parent material for further crosses. In the meantime the possibility of combining resistance of the Er1 gene on chromosome 8 and Er2 gene on chromosome 17 may be real on third generation crosses made recently.

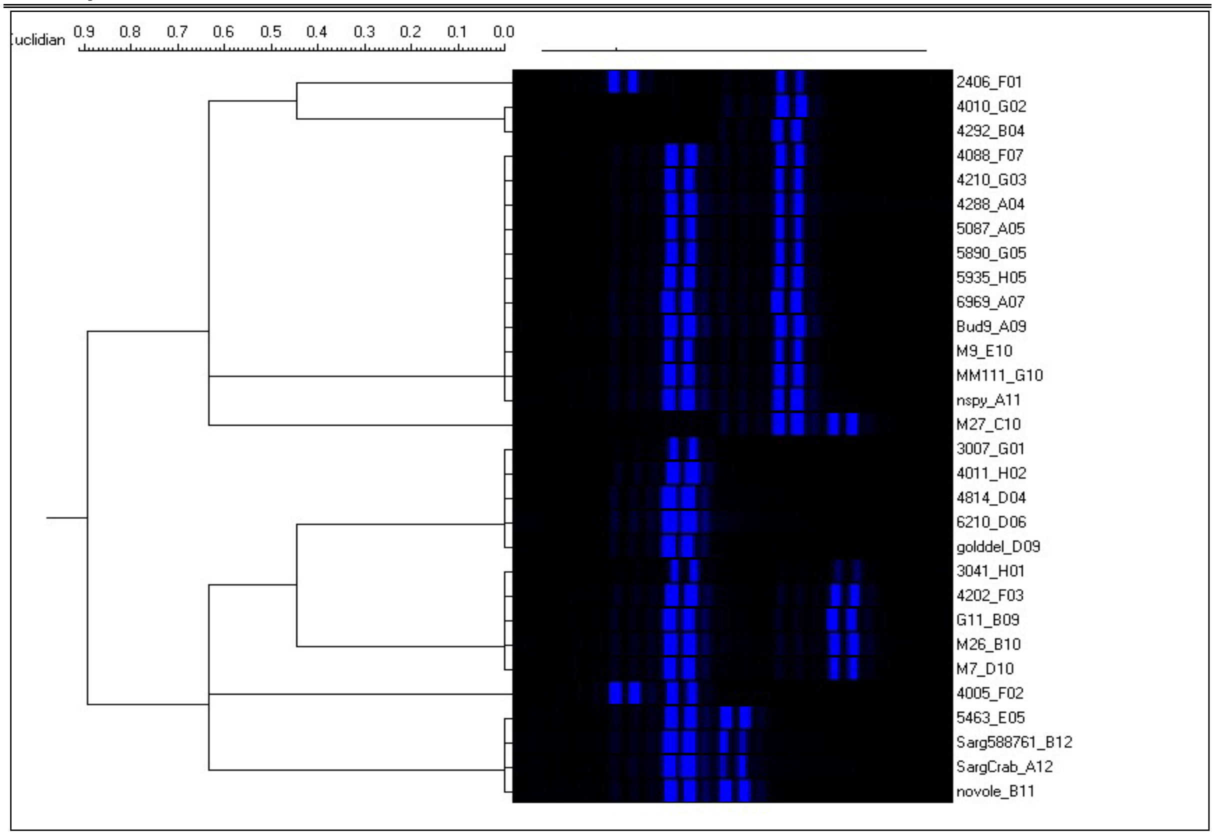

Figure 3. Marker NZmsEB106753 supposedly diagnostic for the Er1/Er3 resistance on Chromosome 8, does not show differences between resistant Northern Spy (nspy) and susceptible B.9 and M.9 rootstocks – hence it not useful as a diagnostic tool.

Table 1. Rootstock rating, 27 August 2010.

	Tops					Roots			
Rootstock	Mean rating	n reps susceptible (TOP)	n reps	% Susceptible (TOPS)	Rating	Mean rating	n reps susceptibl e (ROOTS)	n reps	% Susceptible (ROOTS)
3010	0.63	0	12	0.0	R	0.42	0	12	0.0
3067	0.46	0	12	0.0	R	0.21	0	12	0.0
4010	0.08	0	13	0.0	R	0.00	0	13	0.0
4214	0.40	0	15	0.0	R	0.10	0	15	0.0
4288	0.13	0	8	0.0	R	0.00	0	8	0.0
4292	0.04	0	12	0.0	R	0.00	0	12	0.0
4809	0.00	0	12	0.0	R	0.13	0	12	0.0
5087	0.17	0	12	0.0	R	0.33	1	12	8.3
6210	0.11	0	9	0.0	R	0.00	0	9	0.0
5890	0.30	1	10	10.0	U	0.45	1	10	10.0
4814	0.38	2	13	15.4	U	0.15	0	13	0.0
6969	0.77	2	13	15.4	U	0.88	2	13	15.4
2406	0.40	1	5	20.0	U	0.00	0	4	0.0
2006	1.14	3	7	42.9	S	1.31	4	8	50.0
3902	1.46	6	12	50.0	S	2.50	12	12	100.0
4011	2.10	8	10	80.0	S	1.90	7	10	70.0
4088	1.89	8	9	88.9	S	1.83	5	9	55.6
M.9 Pajam	2.18	13	14	92.9	S	1.46	8	14	57.1

R=Resistant; S=Susceptible; U=Unsure

References

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Previous work:

Resistance of Rootstock Selections to a Washington Strain of Woolly Apple Aphid

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Keywords: woolly apple aphid, *Eriosoma lanigerum,* host plant resistance, Robusta 5 gene

Abstract: Ten rootstock selections were tested for their ability to host woolly apple aphid aerial colonies. Differences among the various rootstocks were apparent within a few weeks of artificial infestation. After 4 wk, the susceptible rootstocks (including M.9, M.26, Bud 9, Bud 118, and seedlings from New York and Washington) were heavily infested. On MM.111 (whose resistance is derived from 'Northern Spy'), colonies established successfully, but were small and poorly developed. The majority of the replicates of the Geneva 'Robusta 5' derived resistant rootstocks (G.202, G41, and 4210) were free from infestation; but some replicates had a few very small colonies.

Host plant resistance is a little used tactic in tree fruit pest management, and the long-known resistance of certain rootstocks to woolly apple aphid is one of the few examples. This resistance is based on a naturally occurring resistance in the apple cultivar 'Northern Spy'. The characteristic was incorporated into the Malling-Merton 100 series of rootstocks in the 1920s, and these stocks were widely planted for this reason. Two phenomena occurred to effectively curtail their use. First, new rootstocks with more favorable characteristics in terms of precocity, productivity and size control were introduced. These included the Malling series (of which M.9 and M.26 have been widely planted in Washington), and the Budagovsky series. Both these series are susceptible to woolly apple aphid. The second phenomenon was that biotypes capable of overcoming the 'Northern Spy' based resistance were discovered in three areas of the world (Gilliomee 1968, Sen Gupta and Miles 1975, Klimstra and Rock 1985).

More recently, a new line of woolly apple aphid-resistant rootstocks have been introduced from the apple rootstock breeding program at Cornell's Geneva Experiment Station (Robinson et al. 2003). This resistance is based on a *Malus × robusta* selection known as 'Robusta 5'. This parent also confers a degree of fireblight resistance, and has been widely used in the Geneva program.

The objectives of this test were twofold: 1) to determine if a Washington strain of woolly apple aphid had overcome the 'Northern Spy'-based resistance, and 2) to confirm the 'Robusta 5' based resistance in our area.

Materials and Methods

Apple rootstock liners, from ¼ to⅜ inch diameter, were planted in a soil mixture of equal parts peat, perlite, and vermiculite on 21 April. Ten rootstock types were used: The Geneva line 4210, Geneva 41, Geneva 202, Bud 9, Bud 118, M.9, M.26, MM.111, seedlings from Washington

(Willow Drive Nursery), and seedlings from New York. Ten of each type were planted. Trees were infested about one month after planting, when new shoot growth was approximately 6 cm.

Parentage of rootstocks

Rootstock	Parentage
G.41	M.27 × Robusta 5
G.202	M.27 × Robusta 5
4210	O.3 × Robusta 5
MM.111	N. Spy × Merton 793
M.9	Juane de Metz clones
M.26	M.16 × M.9
B.9	M.8 × Red Standard
B.118	Moscow pear × M.9 or M.8

Insects were collected from Mountain View Orchard, East Wenatchee. Stem sections 4-6 cm long, each with 50-200 aphids, were placed at the base of each tree on 19 May (Plate 0625.1). First instars were seen on the trees the next day. Fresh stem sections were collected on 22 May and placed on any trees that appeared to have a low number of first instars. This included all of Geneva 41, Geneva 202, 4210, and about half of the other trees. Trees were arranged on a greenhouse bench in a randomized complete block design (10 types × 10 reps) (Plate 0625.2).

Aphids had matured by 8 June and had begun to produce new first instars. Aphid densities on aerial parts of the tree were evaluated on 16 June. Two types of evaluations were performed: 1) a numerical rating system and 2) digital estimation.

Rating system: Tree were rated on scale of 0 to 4, where 0=no colonies; 1=few, small colonies; 2=few, normal colonies; 3 = heavily infested; 4 = very heavily infested with a large volume of waxy filaments.

Root evaluation. Evaluations of the root systems were performed on 14 July. Root systems were rated as infested or not infested, and with or without galls. Results are given as a percentage of the replicates which were positive for either infestation or galls.

Results and Discussion

All of the Geneva rootstocks (G.41, G.202 and 4210) were virtually immune to woolly apple aphid (Table 0625.1, Fig. 0625.1; Plate 0625.3). Only a few of the replicates had any colonies established, and those consisted of only a few aphids. By comparison, at the same point in time, the Malling, Budagovsky, and seedling rootstocks were highly infested (Plates 0625.4. 7). The MM.111 was intermediate between the two extremes (compare Plates 0625.5 and 6), however, colonies were able to establish on most replicates, but grew very slowly by comparison, never reaching the level of infestation of the susceptible rootstocks. Apparently, this is typical of the MM rootstocks, in that the resistance was never complete, but rather expressed as a marked degree of antixenosis. Based on this, there is no evidence that the Washington strain of woolly apple aphid has changed its ability to infest this resistant series over time.

Because the rootstocks in the trial were either clonally propagated or seedlings, their phenotypic expression of resistance was throughout the tree (roots and shoots). Not surprisingly, then the degree of infestation and galling on the roots mirrored that of the shoots. Neither the Geneva rootstocks nor MM.111 had any root infestation or evidence of galls by the end of the test (56 d after initial inoculation). The susceptible rootstocks had 60-100% of the replicates with root infestation, and 10-70% had evidence of gall formation. Given the high pressure of this test, the percentages of both categories would have been higher given sufficient time. However, the trees were nearly dead at the time of root evaluation, so continuing it was not feasible.

Literature Cited

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EXECUTIVE SUMMARY

We have successfully classified rootstocks G.41, G214, G.210, G.87, G.202 as resistant to the wooly apple aphid by direct plant assays and by molecular methods. We have confirmed the susceptibility of G.935, CG.4011, CG.4088, CG.2006, CG.3902 and controls M.9, M.26, B.9 by direct plant assays and by molecular methods. Rootstocks G.890 and G.969 although exhibited the correct configuration of molecular markers for resistance were not able to be classified – the test was inconclusive in the current greenhouse test because insufficient data. Rootstock G.4814 did not possess the molecular markers for resistance and its greenhouse test was also inconclusive. Rootstock CG.2406 was also classified as inconclusive in the greenhouse test and because of a very different pedigree was not able to be classified correctly using molecular markers possessing new, unique marker types; however if classified as resistant in future studies this rootstock may be used as a novel source of resistance for future crosses aimed at combining genes for durable resistance. The ‘Northern Spy’ resistance to wooly apple aphid in the MM series of rootstocks was of an incomplete type when compared to the ‘Robusta 5’ resistance, which in the case of G.41 and G.202 and other resistant Geneva rootstocks was described as virtual immunity. This experiment has been very useful for the classification of new rootstocks. The planting rootstocks possessing a high level of resistance to the wooly apple aphid may provide the benefit of eliminating areal colonies altogether as aphids may not be able to overwinter in the root zone. This feature may be very important in organic production. Added benefits from this study are the possibility of identifying the genes/products that provide a natural barrier to wooly apple aphids – synthesizing these products and using them as more eco-friendly treatments on susceptible orchard canopies.