Project title:	Understanding the scale and importance of *Raspberry leaf blotch virus* and its association with raspberry leaf and bud mite
Project number:	SF 148
Project leader:	Janet Allen, RSK ADAS Ltd Stuart MacFarlane, James Hutton Institute
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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

- Raspberry leaf blotch virus is transmitted and proliferated by the raspberry leaf and bud mite with Glen Ample and Octavia found to be the most susceptible varieties.

Background and expected deliverables

Crop damage to raspberry caused by raspberry leaf and bud mite (RLBM) feeding is an increasing problem around the UK. The damage, which was previously thought to be associated purely with the mite, is now also known to involve infection with *raspberry leaf blotch virus* (RLBV). It particularly affects Glen Ample but symptoms are increasingly being seen on other varieties (Figures 1 and 2). There is also a suggestion (preliminary results: J. Allen/S. MacFarlane) that the recently identified growth decline in certain varieties such as Octavia displaying poor lateral development, die back, blotchy leaves and malformed fruit (Figures 3 and 4), could be associated with this pest and/or virus. The association between the mite and RLBV has been proven. Increasingly however, damage symptoms are being observed without the mite being seen in the field.

This project aimed to carry out a UK-wide sampling of plantations and to conduct experiments to elucidate the links between the mites, the virus, plantation age, variety, yield loss and plant source, to inform strategies for control.

Symptoms

Figure 1. Minor leaf blotch symptom - primocane leaf

Figure 2. Moderate to severe infection to floricane lateral; blotches to primocane leaves

 

Figures 3 & 4. Severe infection - curled twisted chlorotic leaves and malformed fruit

Summary of the project and main conclusions

The project work was divided into six distinct objectives:

Objective 1 - To determine how widespread RLBV is in UK floricane and primocane raspberry plantations

Objective 2 - To confirm preliminary observations that RLBV is associated with plantation decline

Objective 3 - To identify the cropping situations where RLBV occurs

Objective 4 - To identify whether RLBV infection is associated with mite numbers infesting plants and/or the levels of damage caused to infected plants by the mites feeding

Objective 5 - To monitor how RLBV develops and spreads within a plantation

Objective 6 - To monitor varietal susceptibility to mite and RLBV

Objective 1 - To determine how widespread RLBV is in UK floricane and primocane raspberry plantations

In the first two years of the project, raspberry samples were collected from a range of raspberry plantations, spanning both new and old plantations and including 29 different varieties as well as different cropping systems. Of the 158 plantations sampled, 19% were positive for RLBV. This accounted for samples taken on 38% of the holdings tested and 38% of the varieties sampled. Of the varieties sampled, Glen Ample and Octavia were most commonly infected.

Mites were detected on 18% of samples, 82% of which were positive for RLBV. Both the mite and virus were detected in all of the key fruit growing regions of the UK on both small and large holdings and in both protected and containerised production. These results suggest that the mites and the virus are closely associated and widely distributed around the UK.

Objective 2 - To confirm preliminary observations that RLBV is associated with plantation decline

Where plantations were found to be positive for RLBV, the average proportion of the whole field showing RLBV symptoms was up to 50%. In some extreme cases this value was as high as 100% of the plantation showing symptoms, with several plantations being grubbed after they were surveyed.

Overall, very few asymptomatic plants were positive for the virus. In susceptible varieties, such as Glen Ample, there was clear association between characteristic symptoms of yellow leaf blotching and presence of the mite and or virus. Overall, however, 46% of samples showing typical yellowing symptoms tested negative for the virus, but in the newer plantations a greater proportion of ambiguous symptoms did yield positive virus results.

In this study the virus was rarely found in the absence of the mite, which is promising as there is a much greater potential to control the mite on farm than the virus. Careful monitoring and virus testing of plantations is important to identify the mite and virus early and to thereby avoid a build-up of the mite over time.

Objective 3 - To identify the cropping situations where RLBV occurs

On the sites surveyed in 2014 and 2015, the method of spawn management appeared to have some effect on levels of the mite and virus. Where mechanical methods were employed, such as strimming, greater levels of the mite and virus were apparent. This could be linked to seasonal carryover of mites. Mechanical methods tend to leave part of the spawn canopy behind and the presence and proximity of young primocane foliage to infested floricane leaves allows mites to migrate onto next year’s canes. There is also the risk of mites spreading through the debris created by mechanical spawn control.

Both RLBM and RLBV can be found on wild raspberry hosts. Presence of wild raspberry in close proximity to raspberry plantations seemed to increase the proportion of plantations affected. Mites were detected at harvest on some plantations that had not previously been identified as having mites at any earlier stage in the season. These particular plantations were noted as having wild raspberry on the site, which may act as a mite host. These results indicate the proximity of infested wild raspberry to commercial raspberry plantations is one pathway by which infection may be introduced to new plantations. Plantations of modern varieties, which were generally not found to be infected, were found to have the virus when in close proximity to an infected plantation.

The mites are unable to crawl far, so would have to be carried on the wind or by humans or animals to a new plantation. The infected raspberry plant may act as a host for the mite and virus, from where they can colonise healthy raspberry plantations or re-infect those that have been cleared of the mite.

Sites which used acaricides such as abamectin and/or releases of predatory mites appeared to have a lower level of RLBV and RLBM suggesting use of these agents could provide incidental control of the mite, and therefore reduce levels of the virus. However, it is likely that other factors are also important in determining the virus levels, such as variety, cropping system and proximity to wild raspberries.

Objective 4 - To identify whether RLBV infection is associated with mite numbers infesting plants and/or the levels of damage caused to infected plants by the mites feeding

The numbers of mites recorded on bud and leaf samples from the raspberries ranged from one or two mites to over 30. When tested, it was found that any level of mite infestation could lead to a positive result for RLBV.

Where mites were recorded on buds in the dormant season, they were recorded more frequently throughout the whole season on those plants. The presence of over wintering mites on the floricane did not necessarily lead to mites being present at bud burst. However, where there were high mite counts at dormancy, the floricane was infested at bud burst. This suggests that the mites are able to survive over winter and this would give the mites a base from which to build up the population in the following year.

The majority of mites were only first detected on the primocane at harvest and generally on plantations that had already had the mite during the season on the floricane. It is possible that during picking, the mites are knocked off and then spread from the floricane to the primocane, or when a worker moves from an infested plantation they may spread the mite to another plantation.

The initially small number of mites needed to cause infection and symptoms was confirmed in the transmission trials held at JHI, where mites and virus were transferred to healthy plants by clipping infested, symptomatic leaves to healthy plants. These plants were successfully infected with RLBV without high population bursts of mites being noted.

Objective 5 - To monitor how RLBV develops and spreads within a plantation

The results from both the field and at JHI indicate that the virus is very strongly linked to the presence of the mite. The virus was generally detected in the plants at the same sampling assessment that the mites were detected. On a few plantations there was a lag time in virus detection, from when the mites were detected. In these cases the virus was not present until the next sampling assessment. Mites tested during the dormant season were found to be positive for the virus even when the leaf bud they were on was negative for RLBV. This suggests that the mite introduces the virus to the part of the plant it is feeding on, but that it may take time for the virus to develop in the leaf.

The virus was not found on the primocane unless the mite was present, except in two plantations. Here the virus was found at bud burst. Both of these plantations had previously had mites present on the plant that were carrying the virus. The presence of the virus in the apparent absence of the mite may be due to: 1) a transient infective mite population, which was controlled or declined naturally, or 2) very low mite occurrence at sampling which was not possible to detect.

During the transmission experiments at JHI, RLBV was sporadically able to move across the graft junction into upper leaves but a productive infection was not maintained in the plants. Similarly, in the stem-taping experiment RLBV was initially able to move up to the top of the mite-inoculated plant but after two months virus could no longer be detected in these leaves.

When taken together with the previous grafting experiments in 2014 and 2015 it has been determined that RLBV only has a very limited ability to move via the vasculature from lower to upper leaves. However, in the absence of mites the virus infection does not persist. This suggests that repeated treatment to kill mites will prevent RLBV infection and disease symptoms from spreading through the plant. Whether the virus can spread within a single leaf after inoculation by one or a few mites, and in doing so cause disease symptoms, is not yet known.

Objective 6 - To monitor varietal susceptibility to mite and RLBV

The surveys and subsequent testing for RLBV performed on samples from the field, suggested that there was potentially some differential varietal susceptibility to the virus. Of the 29 varieties tested, Glen Ample, Octavia and older floricane varieties were the most commonly affected by the disease. However, more modern varieties including the coded variety CV-C and primocane varieties (previously considered less susceptible) were also found to be affected. The modern coded variety CV-A was the most sampled variety during the two year survey, with none of the samples testing positive for the virus.

Variety transmission tests at JHI confirm results from the field in Years 1 and 2 of this project, indicating that there is differential varietal susceptibility of raspberry cultivars to RBLV. Glen Ample has been shown repeatedly to be highly susceptible, showing the highest incidence of the virus on infected plants, with 100% of plants becoming infected. Under these experimental conditions, some varieties were noted as having extremely low incidence of the virus after being infected by mites, whilst others were more intermediate in their susceptibility. It is likely that the extreme susceptibility of Glen Ample to leaf blotch disease is a major factor in the emergence of this disease over the last ten years.

In some tests, disease symptoms were observed and virus was detected by RT-PCR even though mites were not seen. It is possible that in these instances the mites were able to transmit the virus to the plant but then failed to become established themselves.

The variety experiments do not unequivocally show whether lack of susceptibility (failure to develop the disease) is due to effects on the mite or on the virus. To answer this question, in depth studies to look at the establishment and proliferation of mites on different varieties would be needed.

Main conclusions

Overall, RLBV and its associated vector are widespread across the country. However, at present one of the industry's primary varieties (referred to as CV-A in this report) appears to have tolerance to the mite and/or the virus, although direct challenge experiments using this variety were not carried out. This could change in the future, as the mite and virus are well established in wild raspberry populations in main cane fruit growing areas, for example in hedgerows. This residual population could easily allow the mite and virus to re-establish within plantations and cause severe damage very quickly. This is particularly a concern if a new variety adopted by the industry in the UK proved to be susceptible.

The current production practice of growing raspberries under polythene tunnels with plants and rows in close proximity to each other, with relatively high relative humidity and little wind movement, has created ideal conditions for RLBM. Although the use of acaricides and predatory mites seemed to confer some incidental control of the raspberry leaf and bud mite, there are currently no acaricides approved for providing control in outdoor crops. There is also a very limited choice of effective acaricides currently available. Abamectin (Dynamec) is the only option for use and this is only approved for use on protected crops or those in propagation. Predatory mites such as *Amblyseius andersoni* and *A. californicus* can provide some control but it is unlikely to completely clear an infestation. It is therefore difficult to eradicate the mite from infested propagation and commercial fruiting plantations.

Financial benefits

The total average cost of the disease to the UK raspberry industry can be calculated using the average figures for the industry from the DEFRA Horticulture Statistics. The current total raspberry area recorded in 2015 was 1,538 ha. Of this, the area occupied by the most susceptible varieties (such as Glen Ample and Octavia) is approximately 20 % (312 ha). The average raspberry yield across all varieties is 11.5 tonnes/ha and the average value across the year is £7,209. The total average value of this crop is therefore approximately £26m (312 x 11.5 x 7209 = £25,865,892). If we use the figures from this study that on average up to 50% of a plantation is subjected to the RLBV symptoms, this puts nearly £13m of crop at risk in the UK from the virus per annum. This is likely to be an underestimate of the cost of RLBV to the industry, as it is using averages across all varieties (for example Glen Ample and Octavia yields of 15 tonnes/ha are achievable when grown well under protection).

A number of key actions have been identified during this project, including growers being vigilant for symptoms, particularly in susceptible varieties. The project has also identified a benefit of being able to screen material at the breeding stage using a molecular test for RLBV. This ensures that highly susceptible varieties can be removed from breeding programmes.

Action points for growers

- Growers should be vigilant for symptoms and mites, although it can be hard to identify as occasionally symptoms are not linked to virus presence.
- If symptoms and mites are suspected, careful crop management is recommended to reduce the spread of mites. The use of acaricides and/or predatory mites will aid management of mites, but with no outdoor approval for acaricides such as abamectin, and limited choice for protected crops and propagation, complete control may be difficult to achieve.
- Future raspberry breeding should take the susceptibility of a new variety into account when selections are made. The susceptibility of a new variety can be determined relatively simply as demonstrated by the experiments performed by JHI as part of this project and the use of the molecular test for RLBV diagnosis will make the selection process faster and more robust.

SCIENCE SECTION

Introduction

Raspberry leaf and bud mite (RLBM), *Phyllocoptes gracilis* (Nalepa 1881) poses a considerable threat to the UK raspberry industry causing severe foliar damage, malformation of fruit and associated yield loss to the main UK raspberry cultivars (Alford, 1984; Mitchell, 2010). Considerable work has already been done to investigate chemical and biological control of the mite, but increasingly the leaf blotch and other symptoms are being observed in plantations in the absence of the mites (personal observation J. Allen/S. MacFarlane). Symptoms associated with RLBM feeding alone are similar to those seen in virus-type infections, however work done in the 1980s concluded that there was not a virus component to RLBM problem.

Using new techniques researchers at The James Hutton Institute demonstrated in 2012 that diseased plants do carry a newly described virus (named *Raspberry leaf blotch virus*; RLBV) (McGavin *et al.*, 2012). Virus testing has since been carried out in response to requests from individual growers and ADAS advisors on samples showing symptoms. All samples have been found to carry this virus, however, no systematic survey has yet been done to reveal how widespread this newly described virus is, whether it is always associated with mite feeding and whether it is also associated with chronic decline of raspberries. In addition, no experiments have yet been done to discover whether RLBM causes crop damage greater than normal feeding damage in the absence of the virus, or whether it is the virus that causes the majority of the disease symptoms. Figures 1 and 2 detail the sort of damage observed from this mite-virus interaction.

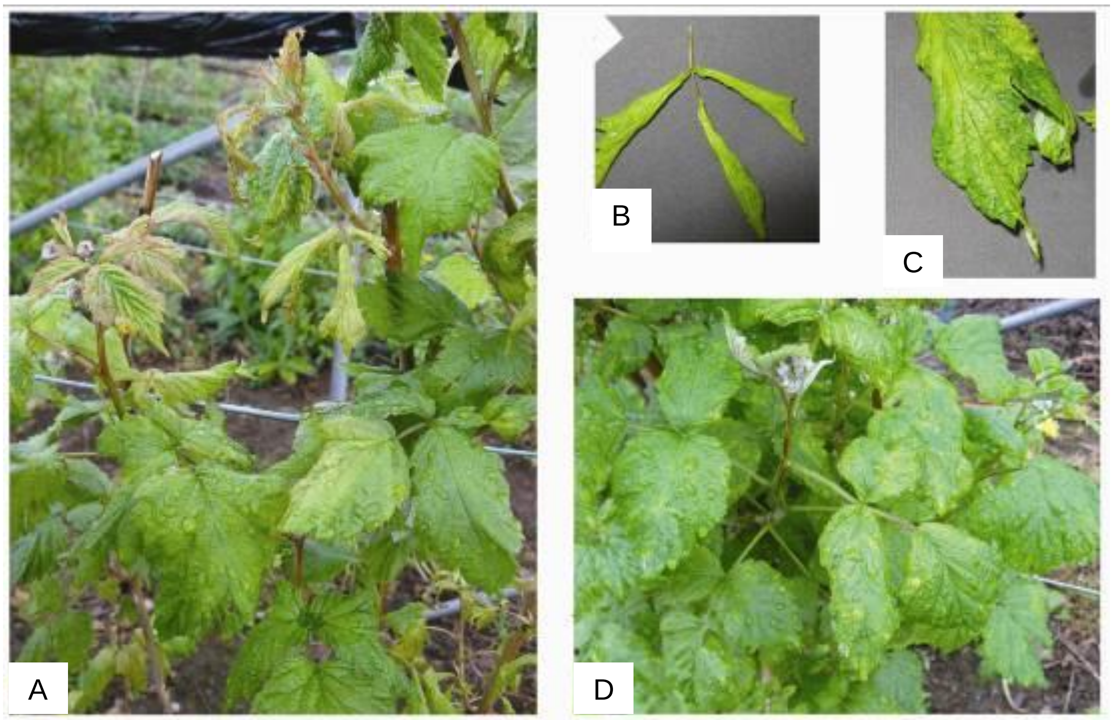

Figure 1. RLBV/RLBM symptoms on raspberry. A) Symptoms on floricane leaves, B) leaf rolling, C) mottled leaf blotch symptom, D) Symptoms on primocane leaves Source: JHI

Figure 2. Severe symptoms observed on Glen Ample in 2013, causing lateral die back and malformed fruit - confirmed RLBV, no mites observed.

The mite

Raspberry Leaf and Bud Mite (*Phyllocoptes gracilis* Nalepa; RLBM) is a microscopic mite of the family Eriophyidae (gall and rust mites) that colonises red raspberry and closely related *Rubus* species and hybrids. The mite has a very large geographical range, being found in most countries where raspberry is grown, particularly Europe and North America where they are known by the common name ‘Dry berry mites’ (Ellis *et al.*, 1991). In the UK they readily colonise raspberry in the wild and are frequently found on commercial stocks. The damage caused by these mites can range in severity depending on various factors. The two main factors appear to be 1) environment and 2) cultivar. The change in raspberry production in the UK from open-field production to semi-protected, tunnel grown crops that are well sheltered, warm and prone to dense humid canopies, along with the reliance of the industry on one or two highly susceptible cultivars (Glen Ample and Octavia), has led to a rapid increase in reports of damage attributed to these mites (Mitchell, 2010). The mite is able to overwinter on the leaf buds of raspberry plants and hibernates when the temperature drops below 11 °C. Like many other eriophyid mites, RLBM rely on wind movement to travel long distances and it is impossible to completely isolate plants to prevent spread. There is also the possibility that the mites may be transported and distributed to new plants on the hands or clothes of personnel, machinery or flying insects. Once infected, the population of mites within modern fruiting plantations can rapidly increase resulting in damage to fruiting cane leaves, primocane leaves and ultimately the ripe fruit, where quality is reduced due to misshapen and poorly formed drupelets. Little is known about when the virus is transmitted by the mites and to what plant tissues, buds or leaves, and whether the virus can move systemically through the plant.

The virus

JHI studies have shown that the virus is present in the mite, can be transmitted by the mite and, importantly, has been detected in almost every sample of symptomatic raspberry that has been tested to date (samples obtained from southern England, Scotland and mainland Europe). The virus belongs to a group of similar viruses that are each transmitted by an eriophyid mite. In at least one example, a virus of legumes, it appears that the mite cannot multiply efficiently unless it carries the virus, and plants that are resistant to the virus are very poor hosts for the mite. These studies have not been done for the raspberry mite (RLBM) and virus (RLBV).

In an earlier study (1970s), different raspberry cultivars were examined for their ability to support mite populations, Malling Promise was found to be a much poorer host for the mite than Malling Jewel. A previous AHDB project (SF 81) reported that Glen Ample and Malling Landmark were highly susceptible and supported high numbers of mites, whereas Glen Magna was found to have significantly fewer mites and this was linked to leaf hair density. During 2011, virus-infected samples that were collected from England and Scotland have primarily been of Glen Ample, but also include Malling Jewel and Glen Rosa often in close proximity to other varieties not showing symptoms. These results suggest that there are differences in cultivar reaction to this disease, although it is not known whether these cultivars resist the mite, the newly identified virus or both.

It is also not clear whether the disease symptoms seen in plants infected by RLBV are caused by the virus alone or result from a combination of virus effects and mite effects, although it has been shown that the bright yellow sectors of symptomatic raspberry leaves do carry higher levels of virus than the adjacent green sectors of the same leaves (S. MacFarlane, unpublished results). One method, in principle, to separate the virus from the mite vector is to transfer the virus from an infected plant to a healthy plant by grafting, which should bypass the need to have mites to carry out the virus transmission.

Knowledge requirements

Attempts to control the problem by spraying plants with acaricides to kill the mites have not been particularly successful, with the development of strong symptoms even in plantations where few mites can be detected. If it can be shown that the virus is the major cause of the observable symptoms then a strategy to identify and incorporate virus-resistance into new cultivars might be appropriate. Currently, however, our knowledge of the interaction between mite, virus and raspberry plant is very poor. For example, we do not know;

- Whether the virus can be present in the plant in the complete absence of mites
- Whether spread of the virus in the plant requires movement of the mite from leaf to leaf (i.e. is the virus systemic or localised in the absence of the mite)
- Whether the virus can cause disease symptoms in the absence of mites
- Whether the mites cause the symptoms in the absence of the virus

Aims and objectives

The project aim is to understand how widespread *Raspberry leaf blotch virus* (RLBV) is in the UK and to better understand the association between the virus and mite infestations.
The specific aims in year three (2016) were to:

- Monitor how RLBV develops and spreads within raspberry plants and whether the presence of mites is necessary for virus persistence
- Propagate, inoculate and assess historical varieties from the JHI germplasm collection with reported tolerance/resistance to leaf and bud mite disease.

Materials and methods

The testing of plant material for the Raspberry leaf blotch virus (RLBV) and the raspberry leaf and bud mite interactions took place at research facilities at the James Hutton Institute, Invergowrie, Dundee DD2 5DA. Two strands of experiments were conducted, one investigating the spread of the virus within the plant, as well as from plant to plant. The other was to identify and confirm differential varietal susceptibility to the virus.

Virus identification

In all experiments the identification of the presence of the virus was performed using reverse transcription polymerase chain reaction (RT-PCR). For this leaf samples were stored at - 80 °C prior to extraction and the leaf was processed to isolate total plant RNA. The RLBV sequences present in the RNA sample were amplified, 2 µl of RNA was mixed with 46 µl RNase-free water, 1 µl each of RLBV primers 1095 and 1087 (derived from RLBV RNA3 encoding the capsid protein) and combined with an Illustra Ready-To-Go RT-PCR bead (GE Healthcare). Presence or absence of the virus was recorded.

Role of mites in RLBM transmission and persistence

A simple experiment was performed to examine transmission from plant to plant and assess the longevity of the infection of a raspberry plant when mites are actively removed.

RLBV and mites were transferred from an infected plant to four healthy Glen Ample plants by attaching an infected, mite infested leaf to a lower leaf of each test plant. The virus infection was confirmed using RT-PCR.

The plants were treated after one month with acaricide to kill the mites and were re-potted into insecticide-containing compost. The stem immediately below the top-most leaf was wrapped with plastic tape to provide a physical barrier to any surviving mites and to identify which leaves were from new growth. The plants were visually examined for the mites and RT-PCR tested twice more at monthly intervals for the presence of RLBV.

Virus transmission within the plant

Grafting was employed in 2016 to elaborate further on observations in 2015 that RLBV was unable to move from lower to upper leaves of raspberry plants. In 2015 two other viruses *Black raspberry necrosis virus* (BRNV) or *Raspberry vein chlorosis virus* (RVCV) were first moved by grafting from plants of other varieties onto Glen Ample. These viruses are mobile within raspberry plants and were used to test that virus transmission could be achieved across the graft.

The graft was achieved by using the bottle graft method, where a soft tissue stem of approximately 15-20 cm (scion) was cut from the infected plant (NB older, harder stems cannot be used and so these experiments can only be done during part of the growing year). All the leaves apart from those at the tip were removed and a 1 cm upward incision was made a quarter of the way from the top of the stem. A complementary incision was made into the stem of the healthy (recipient) plant and the two stems were joined together using a tongue and groove connection. The join was held together with a strip of soft, air-tight (Nesco) film and the base of the scion section was placed into a tube of water until the graft union successfully formed.

Once a functional graft junction formed (approximately within one month), the bottle was removed. The top two or three nodes of the recipient plant stem (above the graft junction) were removed and, after a further six weeks or so, the new leaves that emerged from this resected stem were tested for the presence of virus. After this, the grafted plants were managed in the normal way with the removal of older stems over the winter period, and new growth occurring in the next year was also tested for the presence of the viruses.

In 2016 the plants infected with BRNV or RVCV were also infected with RBLV by leaf clipping from an infested source plant. The plants were confirmed to have both virus combinations (BRNV + RLBV or RVCV + RLBV) by RT-PCR and mites were removed by acaricide treatment. Scions from these dual infected plants were grafted onto healthy Glen Ample plants, which were treated further and the plants were examined visually to confirm the absence of mites.

The initial growth formed on these plants just above the graft junction was tested for the presence of the viruses by RT-PCR after one month. The new growth was removed and new leaves allowed to emerge. These new leaves were then tested with RT-PCR to identify whether these viruses were able to establish a long-lasting infection.

Testing varietal susceptibility to RLBV

Over two years, 2015 and 2016, experiments were performed to find whether there is a difference in susceptibility of different raspberry varieties and breeding selections to leaf blotch disease. In this period 101 plants were tested from 15 different varieties or selections from the JHI germplasm collection.

In each experiment at least four healthy plants of a particular variety/selection were grown in pots in a plastic tunnel. A leaf from a Glen Ample plant carrying mites and showing symptoms of leaf blotch disease was attached using a paper clip to a single leaf of each healthy, recipient plant. The plants were monitored visually for the development of symptoms and leaves collected at three time points for analysis. The leaves were examined using a binocular microscope to identify the presence of mites and samples from these leaves were tested by RT-PCR for the presence of RLBV.

Results

Role of mites in RLBM transmission and persistence

When the plants were tested one month after the initial transfer of virus and mites to the healthy Glen Ample plants, three out of four plants contained detectable RLBV in the new growth, with one showing some symptoms.

After one month, during which a repeated acaricide treatment was done to ensure removal of the mites, no RLBV was found in the top-most leaves of any of these plants, which had initially been infected with the virus.

Virus transmission within the plant

When the initial growth that had formed above the graft junction was tested by RT-PCR, after one month, RLBV and BRNV was detected in 2 of 4 plants. At this time RVCV was not detected in any of the plants.

When the regrowth was removed and new leaves allowed to emerge, none of the plants (either stem or emerging leaves) were found to contain RLBV. However, both of the other viruses, BRNV or RVCV, were found in one of each set of grafted plants. This result shows that a functional graft junction was formed in the plants and that in some of these plants the viruses BRNV and RVCV were able to move through it and establish a long-lasting infection, even though RLBV was not able to do so.

Testing varietal susceptibility to RLBV

The results from the varietal susceptibility testing can be found in **Table 1**. There was variable susceptibility to RLBV seen across all of the varieties/selections used in this trial.

Out of the 15 varieties/selections tested only three of them were found to have not been infected with RLBM following inoculation with raspberry leaf and bud mites. These were September, Latham and Selection G2. A further variety (Autumn Bliss) had very low incidence of infection with only a single plant out of 15 (representing 7% of plants) returning a positive sample.

The results confirmed previous observations that Glen Ample is especially susceptible to mite establishment, symptom production and virus accumulation. Schoenmann and Selection P4 were similarly highly susceptible.

In addition to these cultivars, intermediate susceptibility was found for Malling Promise, Glen Moy and several of the selections.

Table 1. Incidence of mites, symptoms and virus in raspberry plants exposed to mite and virus infected leaves.

Cultivar	Mites seen	Symptoms seen	Virus detected by RT-PCR
Glen Ample	17/19	19/19	19/19
Schoenmann	4/4	4/4	4/4
Autumn Bliss	0/15	0/15	1/15
Malling Promise	3/13	3/13	6/13
September	0/5	0/5	0/5
Latham	0/5	0/5	0/5
Glen Moy	1/5	2/5	3/5
Selection C5	2/5	4/5	5/5
Selection K1	0/5	1/5	2/5
Selection P1	0/5	0/5	2/5
Selection P2	0/5	1/5	2/5
Selection P3	1/5	2/5	2/5
Selection P4	3/5	4/5	5/5
Selection G2	0/5	0/5	0/5
Selection D2	0/5	1/5	2/5

During this series of experiments accurate counts of mite numbers were not done, however, there was not any obvious “burst” of mite reproduction during the short time when plants were under test. This reinforces the notion that the production of disease symptoms requires only very low mite numbers to be present.

In some tests disease symptoms were observed and virus was detected by RT-PCR even though mites were not seen. It is possible that in these instances the mites were able to transmit the virus to the plant but then failed to become established themselves.

Discussion

The results from the varietal susceptibility tests confirm results seen in years 1 and 2 of this project that there is some differential varietal susceptibility. As with the samples from plantations, Glen Ample had the highest incidence of virus transmission, with 100 % of the plants becoming infected with RLBV once the mites and virus were introduced. Some cultivars showed intermediate susceptibility to the virus, whilst others remained healthy and uninfected. Autumn Bliss showed no incidence of infection from samples in the field and in the transmission tests showed a similar lack of susceptibility, with only a single plant out of 15 becoming infected.

In some of the varietal susceptibility tests disease symptoms were observed and virus was detected by RT-PCR even though mites were not seen. It is possible that in these instances the mites were able to transmit the virus to the plant but then failed to become established themselves. However, for this to be confirmed, more experiments should be done in which varying numbers of mites are transferred onto raspberry plants for defined periods before being actively removed.

These experiments do not unequivocally show whether lack of susceptibility (failure to develop the disease) is due to effects on the mite or on the virus. To answer this question, in depth studies to look at the establishment and proliferation of mites on different varieties are needed.

The virus transmission tests indicated that RLBV was sporadically able to move across the graft junction into upper leaves but a productive infection was not maintained. Similarly, in the stem-taping experiment RLBV was initially able to move up to the top of the mite-inoculated plant but after two months virus could no longer be detected in these leaves. When taken together with the previous grafting experiments we conclude that RLBV has a very limited ability to move via the vasculature from lower to upper leaves and that, in the absence of mites the systemic virus infection does not persist. This suggests that repeated treatment to kill mites will prevent RLBV infection and disease symptoms from spreading through the plant. Whether the virus can spread within a single leaf after inoculation by one or a few mites, and in doing so cause disease symptoms, is not yet known.

In addition, the extreme susceptibility of Glen Ample to leaf blotch disease is likely to be a major factor in the emergence of this disease over the last ten years. Lack of susceptibility ("resistance") is present in some varieties, and can and should be introduced into new cultivars during the breeding process – use of the molecular test for RLBV diagnosis will make this selection process faster and more robust.

Conclusions

Spread of RLBV in UK floricane and primocane raspberry plantations

- Of the 158 plantations sampled in 2014 and 2015, 19% were positive for RLBV, this accounted for samples taken on 38% of the holdings tested and 38% of the varieties sampled.
- Of the varieties sampled, Glen Ample and Octavia were most commonly infected.
- Mites were detected on 18% of samples, 82% of which were positive for RLBV.

- Both the mite and virus were detected in all of the key fruit growing regions of the UK on both small and large holdings utilising both protected and containerised production.
- These results suggest that the mites and the virus are closely associated and widely distributed around the UK.

RLBV and associated plantation decline

- Where plantations were found to be positive for RLBV, the average proportion of the whole field showing RLBV symptoms was up to 50%. In some extreme cases this value was as high as 100% of the plantation showing symptoms, and several plantations were grubbed after they were surveyed.
- Overall, very few asymptomatic plants tested positive for the virus. In susceptible varieties, such as Glen Ample, there was good agreement between characteristic symptoms of yellow leaf blotching and presence of the mite and or virus.
- The virus was rarely found in the absence of the mite, which is promising as there is a much greater potential to control the mite on farm than the virus.
- Careful monitoring and virus testing of plantations is important to identify the mite and virus early and to thereby avoid a build-up of the mite over time.

Cropping situations where RLBV occurs

- Mechanical methods of spawn management, such as strimming, seemed to show greater levels of the mite and virus.
- Both RLBM and RLBV are found on wild raspberry hosts. Presence of wild raspberry in close proximity to raspberry plantations seemed to increase the proportion of plantations affected. The wild raspberry may act as a mite host.
- The proximity of infested wild raspberry to commercial raspberry plantations is one pathway by which infection may be introduced to new plantations.
- Sites which used acaricides and/or releases of predatory mites appeared to have a lower level of RLBV and RLBM suggesting use of these agents could provide incidental control of the mite and, therefore, reduce levels of the virus.
- However, there are currently no acaricides approved for providing control in outdoor crops, and there is a restricted choice of effective acaricides i.e. only abamectin (Dynamec) for use on protected crops or those in propagation. Predatory mites such as *Amblyseius andersoni* and *A. californicus* can provide some control but it is unlikely to completely clear an infestation. It is therefore difficult to clean up infested propagation and commercial fruiting plantations of the mite.

RLBV infection association with mite numbers and movement

- When tested, it was found that any level of mite infestation could lead to a positive result for RLBV.
- Where mites were recorded on buds in the dormant season, they were recorded more frequently throughout the whole season on those plants.
- High mite counts at dormancy tended to lead to infestation of the floricane at bud burst. This suggests that the mites are able to survive over winter and would give the mites a base from which to build up the population in the following year.
- The majority of mites were only first detected on the primocane at harvest and generally on plantations that had already had the mite on the floricane during the season. It is possible that during picking the mites are knocked off and then spread from the floricane to the primocane, or when a worker moves from an infested plantation they may spread the mite to another plantation.
- Small numbers of mites needed to cause infection and symptoms was also noted in the transmission trials held at JHI, where plants were successfully infected with RLBV without high population bursts of mites being noted.

Development and spread of RLBV in plants and plantation

- The results indicate that the virus is very strongly linked to the presence of the mite. The virus was detected in the plants at the same sampling assessment that the mites were detected.
- On a few plantations there was a lag time in virus detection, from when the mites were detected. In these cases the virus was not present until the next sampling assessment.
- Mites tested during the dormant season were found to be positive for the virus even when the leaf bud they were on was negative for RLBV. Suggesting that the mite introduces the virus to the part of the plant it is feeding on, but that it may take time for the virus to develop in the leaf.
- The virus was not found on the primocane unless the mite was present, except in two plantations. Here the virus was found at bud burst. Both of these plantations had previously had mites present on the plant that were carrying the virus.
- RLBV does have a very limited ability to move via the vasculature from lower to upper leaves, however, in the absence of mites the virus infection does not persist.
- Repeated treatment to kill mites may prevent RLBV infection and disease symptoms from spreading through the plant. Whether the virus can spread within a single leaf after inoculation by one or a few mites, and in doing so cause disease symptoms, is not yet known.

- The mite and virus are well established in wild raspberry populations in main cane fruit growing areas, for example in hedgerows, and could easily come back and severely damage plantations very quickly. This is particularly a concern if a new cultivar adopted by the industry in the UK proved to be susceptible.

Varietal susceptibility to mite and RLBV

- Of the 29 varieties tested Glen Ample, Octavia and older floricane varieties were the most commonly affected by the disease.
- Modern varieties including CV-C and primocane varieties (previously considered less susceptible) were also found to be affected. The modern variety CV-A was the most sampled variety during the two year survey, with none of the samples testing positive for the virus.
- Variety transmission tests at JHI confirm results from the field in Years 1 & 2 of this project, indicating that there is differential varietal susceptibility of raspberry cultivars to RBLV.
- Glen Ample has been shown repeatedly to be highly susceptible, showing the highest incidence of the virus on infected plants, with 100% of plants becoming infected.
- Under these experimental conditions, some cultivars were noted as having extremely low incidence of the virus after being infected by mites, whilst others were more intermediate in their susceptibility.
- It is likely that the extreme susceptibility of Glen Ample to leaf blotch disease is a major factor in the emergence of this disease over the last ten years.
- The variety experiments do not unequivocally show whether lack of susceptibility (failure to develop the disease) is due to effects on the mite or on the virus. To answer this question, in depth studies to look at the establishment and proliferation of mites on different varieties are needed.
- It is very important that future raspberry breeding programmes take the susceptibility of a new cultivar into account when selections occur. The susceptibility of a new cultivar can be determined relatively simply as demonstrated by the experiments performed by JHI as part of this project.

Knowledge and Technology Transfer

Presentation given at the AHDB/EMR Soft Fruit Day (25th November 2015) summarising results

Article in AHDB Grower magazine April 2016 (pg 24)

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