Grower Summary

SF 148

Understanding the scale and importance of Raspberry leaf blotch virus and its association with raspberry leaf and bud mite

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GROWER SUMMARY

Headline

- The survey has confirmed a strong association between the *Raspberry leaf blotch virus* and the raspberry leaf and bud mite, with the virus rarely found in the absence of the mite.

Background and expected deliverables

Crop damage previously associated purely with the feeding of raspberry leaf and bud mite (RLBM), is an increasing problem around the UK and in some cases, is now also known to involve infection with a virus *Raspberry leaf blotch virus* (RLBV). It particularly affects Glen Ample but increasingly symptoms are being seen on other varieties (Figures 1 and 2). There is also a suggestion that the recent decline in certain varieties such as Octavia (poor lateral development, die back, blotchy leaves and malformed fruit, see Figures 3 and 4) could be associated with this pest and/or virus. The association between the mite and RLBV has been proven. However, increasingly, crop damage symptoms are being observed without the mite being detected in the field.

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

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

UK wide sampling and assessment

Following the sampling at 28 grower sites (95 raspberry plantations sampled) in 2014, a further 21 holdings (63 additional samples) were selected in 2015 to appraise the susceptibility to *Raspberry leaf blotch virus* (RLBV) and to infestation by raspberry leaf and bud mite (RLBM) of varieties and growing systems that were not included or were under represented in year one. At each site the grower was interviewed and up to five plantations which contained plants which were displaying the typical symptoms (symptomatic) of RLBV and or RLBM or not (asymptomatic) were selected for sampling. As in 2014, a wide range of floricane (summer) and primocane (autumn) fruiting varieties were sampled in order to try to establish why some plantations are affected by the mite and/or virus and why others are not. Within each plantation a single plant was selected and five of the newest fully emerged leaves from one of its current season primocane were collected and sent to JHI for molecular analysis by PCR (polymerase chain reaction). Detailed cropping information was collected for each site to support and inform the results, including: variety, planting date, planting material, spawn management, acaricide use, yield observed in 2014, presence of wild hosts of the mite and/or virus and level of symptoms displayed by the raspberry plants at the time of sampling.

Scale and severity of RLBM and RLBV

RLBV was confirmed in only two of the 63 plantations assessed in 2015 (Table 1). This was probably a result of sampling being skewed towards gaps in the 2014 data set, such as younger plantations with newer varieties and under-represented growing systems.

Across the whole of the dataset for 2014 and 2015, positive samples for RLBV were found in 19% of the plantations (30 out of 158) and 38% of the varieties sampled. Mites were detected on 18% of the samples, and of these, 82% were also positive for the virus. This suggests that the mites were a strong indicator of whether or not a sample would be positive for the virus.

Table 1. Association of *Raspberry leaf blotch virus* (RLBV) with presence of raspberry leaf and bud mite (RLBM) in UK Raspberry crops – 2015

Number of samples in each category			
RLBV - RLBM -	RLBV + RLBM -	RLBV - RLBM +	RLBV + RLBM +
61	1	0	1

Mite-virus interaction and links with plantation decline

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 plants in commercial plantations than the virus. Overall very few asymptomatic plants were positive for the virus. In susceptible varieties, such as Glen Ample, there was a strong correlation between characteristic symptoms of yellow leaf blotching and presence of the mite and virus. Overall, however, 46% of samples of foliage that were collected which were displaying yellowing symptoms were negative for the virus. In newer plantations a greater proportion of ambiguous symptoms displayed by the sampled foliage did however yield positive virus results. Therefore, careful monitoring and virus testing of plantations is important to identify the virus and mite early and to avoid build-up over time of one or both to a damaging level.

Table 2. Summary of some factors that appear to be associated with RLBV symptoms from examination of 158 raspberry plantations surveyed in 2014 and 2015

Factor	**Comment**	**High(er) Incidence***	**Low(er) Incidence***
Variety	11 out of 29 cv's positive	Glen Ample Octavia	Tulameen
Plantation age	Trend for incidence to be greater in older plantations	-	-
Region	Present in all regions	West Midlands	East Anglia South East
Planting material	Present in plantations grown from all types of planting material	Bare root short cane	Root Modules
Spawn management	Present whatever system used; worse where primocane removed mechanically	Mow or Strim	Hand or Shark
Wild raspberry	Greater chance of infected crop if wild raspberry is adjacent	Yes	No
Mite control	Less RLBV if a mite control strategy is in place (be it for RLBM or two spotted spider mite)	No mite control	Acaricide or predators used
Cropping system	Present in plantations grown utilising all types of crop management systems	Outdoor Soil	Tunnelled Containerised

*Only reported where sample size is greater than or equal to 10

Varietal susceptibility

To gain a more representative indication of the level of RLBM and RLBV in the UK raspberry industry, further sampling was conducted in 2015 to collect information from a larger number of plantations including those with no history of the pest or virus and located on sites in areas of the UK which, until recently, have not been used for raspberry production and have been planted with modern primocane fruiting raspberry varieties.

Glen Ample and some of the older floricane varieties, as expected, were the varieties most commonly affected by the condition, but more modern varieties including coded CV-C and some primocane varieties (previously considered less susceptible) were also found to be affected particularly when growing in close proximity to infected plantations. Coded variety CV-A was the most sampled variety in the two years, however none of the samples were positive for the virus. Nevertheless a wide cohort of varieties can be infested with both the mite and virus.

Trends in cropping situations where RLBV occurs

Some interesting observations have been made from this survey that indicate that how crops are managed may affect this condition. How spawn is managed appears to have some effect on levels of the mite and virus with physical methods (as opposed to chemical) seemingly showing greater levels of the mite and virus. Potentially this could be related to seasonal carryover of mites, as the presence and proximity of young primocane foliage to infested floricane leaves allows mites to migrate onto next year's canes. Earlier, rapid and complete removal of the first or second flush of primocane could hinder this migration and or systemic movement of the virus.

Sites which used acaricides, such as abamectin (e.g. Dynamec), 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 were also important in determining the virus levels, such as variety, cropping system and proximity to wild raspberries.

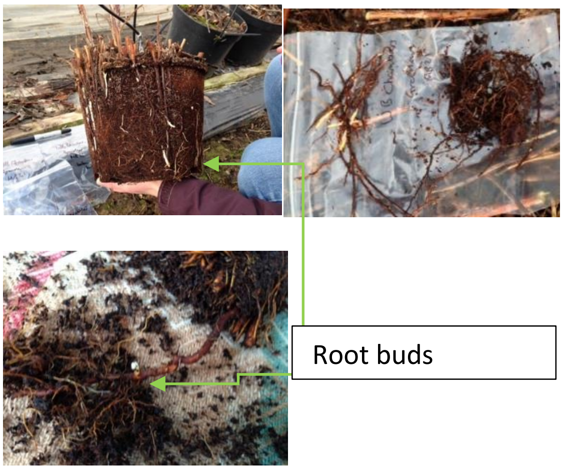
Presence of wild raspberry in close proximity to raspberry plantations seemed to increase the proportion of plantations affected. Both RLBM and RLBV can be found on wild raspberry hosts; these results indicate that proximity of infested wild raspberry to commercial raspberry plantations is one pathway by which infection may be introduced to new plantations. During the survey, plantations of modern varieties 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 to the new plantation. The infected raspberry may act as a host for the mite and virus, from where they can colonise healthy raspberry cultivations or re-infect those that have been cleared of the mite.

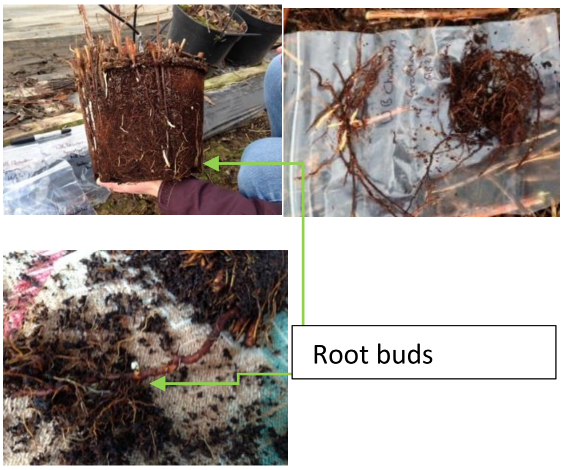
Traditional bare root (short and long cane) planting material showed a greater incidence of RLBV infection. However, this could be an artefact of variety, plantation age or growing system. Tunnel protection and in substrate, e.g. pot grown production, seemed to decrease the instances of mites and virus infection. This result needs to be interpreted with caution, however, as it may be as a result of other factors, such as location on new sites without a history of raspberry or hybrid berry i.e. Tayberry production, proximity of wild raspberry and other infected plantations. It may also be related to the age of plantation, as a high proportion of pot grown raspberries only have a three year commercial cropping life, decreasing the chance of the virus building up in the plantation.

In-depth mite-virus assessment

In 2015, ADAS consultants re-visited 20 of the plantations identified in 2014 as having *Raspberry leaf blotch virus*, raspberry leaf and bud mites or both virus and mites. At each site a subset of plants were assessed for mites and virus throughout the year. Both primocane and floricane raspberry types were sampled in the detailed sampling. Three plants were selected within a row of each plantation, the middle plant of the three was marked with tape and sampled five times during the year. The type of sample and timing of the collection from the sample plants is shown in Table 3. All samples were sent to JHI for mite and PCR analysis.

Table 3. Detailed sampling throughout 2015.

Type of raspberry	Timing	Sample
All raspberry types i.e. flori & primocane fruiting cultivars	End of winter, before bud break	• Root hairs and root buds  • Dormant buds 
Floricane – Summer fruiting varieties	Floricane at bud break or soon after	• First emerged leaves at base of the lateral bud, taken from middle of the floricane • Newest emerged primocane leaves
	Flowering	• Floricane leaves from middle of fruiting lateral • Newest fully emerged primocane leaves
	Harvest	• Floricane leaves from end of fruiting lateral • Newest fully emerged primocane leaves
	Post-harvest	• Newest fully emerged primocane leaves

Type of raspberry	Timing	Sample
All raspberry types i.e. flori & primocane fruiting cultivars	End of winter, before bud break	• Root hairs and root buds  • Dormant buds 
Primocane – Autumn fruiting varieties	First visit only – at bud break	• Where floricane crop being produced first emerged leaves at base of the lateral bud, taken from middle of the floricane
	At bud break or soon afterwards when first primocane emerging	• Newest fully emerged primocane
	Flowering	• Newest fully emerged primocane
	Harvest	• Newest fully emerged primocane
	Post-harvest	• Newest fully emerged primocane

Movement of mites during the season

Mites were found to overwinter in the leaf buds on 25% of the plantations assessed for the detailed sampling. Mite numbers were variable on the same plant, with some buds having no mites, whilst others contained up to 30 mites. Mites were recorded more frequently throughout the whole season on all of the plants that had mites recorded in floricane buds in the dormant season. Over wintering mites did not necessarily lead to mites being present at bud burst. However, where there was a high mite count at dormancy (10 or more mites per bud), the floricane was infested at bud burst.

Mites were found on the floricane of some infested plants from bud burst. These were plants that had high numbers of mites present during the dormant season. The earliest time mites were seen on the primocane was at plant flowering, but this was only noted at one site. The majority of mites were present at harvest on the primocane. This was true for plants that had not previously been identified as having mites earlier on in the season.

Movement of virus during the season

With the exception of the dormant season, the virus was generally detected in the plants at the same time that the mites were detected. On a few plantations there was a lag time in virus detection, where the mites were detected, but the virus was not present until the next sampling assessment.

The virus was not found on the primocane unless the mite was present, except for two plantations where the virus was found at bud burst. Both of these plantations had previously had mites present on the plants that were carrying the virus. This may be due to: 1) A systemic virus infection present following a historic mite infestation. 2) There was a transient infective mite population, which was controlled or declined naturally, or 3) mite occurrence was very low at sampling and were not possible to detect.

The virus was detected in the root hairs and root buds in some plantations. The mites were not found in the root buds, suggesting that the virus has some ability to move within the plant and lay dormant. Where the mite was not detected on the plantation throughout the season, no further virus infection was found. On the four samples where mites were present in leaf buds during dormancy, the mites tested positive for the virus and all of the plants had the virus at some point in the season.

Virus transmission trials

It is not clear whether the disease symptoms seen in plants infected by RLBV are caused by the virus alone moving within the plant systemically or results from movement of mite carrying the virus to different parts of the affected plant. 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 but the presence of virus in root samples suggests there can be movement of the virus without mite feeding.

One method used in research studies to separate the virus from the mite vector is to transfer the virus from an infected plant to another healthy plant by grafting, which should bypass the need to have mites to carry out the virus transmission. Grafting is a long-established method for virus-testing and is known to achieve the transfer of the great majority of viruses from plant to plant. Grafting experiments were initiated in 2013 to investigate whether the virus is moving systemically within raspberry in the absence of the mite. To do this a section of an infected raspberry plant is grafted to a growing healthy raspberry plant using the bottle graft method.

Grafting in raspberry is a seasonal activity, requiring the advanced production of recipient rooted plants and the sourcing of RLBV and mite-infested source material (usually from local affected plantations). As it is known that viruses in combination can sometimes cause an increase in virus content and symptom, severity tests were carried out that attempted to mix RLBV with other viruses. So far, three separate sets of grafting experiments have been done.

In none of these experiments was RLBV found to be able to move across the graft junction from the source plant to the recipient plant. This was regardless of whether the source plants had RLBV only or RLBV with a second virus, or whether the recipient plants initially were virus-free or carried another virus different from RLBV.

In one experiment, plants carrying both RLBV and Black Raspberry Necrosis Virus (BRNV) were used as source plants in a graft test. Five of the seven recipient plants were found to have become infected with BRNV via the graft but none of the seven plants tested positive for RLBV.

This last experiment shows that, even when the graft is proved to be functional (by the transfer of BRNV), RLBV seems not to be transferred by this means. This is strong evidence that RLBV does not move by itself through the vascular system of raspberry plants, and gives support to the notion that spread of the virus in raspberry is likely to require the assistance of the mite. This does not explain the presence of the virus in the roots of the plants, so further investigation is required to understand the movement and longevity of the virus within the plant.

Financial benefits

- This research will lead to increased knowledge of the scale, severity and main causes of this disorder, which is becoming an increasing problem for many commercially grown varieties, and one which, increasingly does not appear to be effectively controlled through conventional strategies.
- It also offers the potential to reduce crop damage through development of a rational control strategy based on this better understanding of the mite-virus interaction.

Action points for growers

- Careful monitoring and virus testing of young plantations is important to identify the virus and mite early and to avoid build-up over time of one or both to a damaging level.

- Highly susceptible varieties, such as Glen Ample, could be used as indicator plants in new plantations.