PFR SPTS No. 16208

Outdoor lettuce virus disease project, year 2 report, 2018

Fletcher JD, Zhang Y, Kean AM, Davidson MM

April 2018

Confidential report for:
Vegetables New Zealand Incorporated

PUBLICATION DATA

Fletcher JD, Zhang Y, Kean AM, Davidson MM. April 2018. Outdoor lettuce virus disease project, year 2 report, 2018. A Plant & Food Research report prepared for: Vegetables New Zealand Incorporated. Milestone No. 75055. Contract No. 34998. Job code: P/313050/01. SPTS No. 16208.

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EXECUTIVE SUMMARY

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Over recent seasons, lettuce (*Lactuca sativa*) growers in the lower North Island, Nelson and Mid Canterbury have been concerned about poorly performing outdoor lettuce crops. In particular, those growing iceberg /crisp head lettuce types have noticed seasonal crop collapses with losses as high as 50%. As a result a 2-year research project was established to explore the problem. From our work in 2016–17 (Fletcher et al. 2017) we found *Lettuce necrotic yellows virus* (LNYV) and *Cucumber mosaic virus* (CMV) were strongly associated with necrosis symptoms of iceberg lettuce heads. Both viruses were found in *Sonchus* and *Lactuca* weed hosts near the monitored sites. Similarly *Nasanovia ribisnigri* was the most abundant aphid species found present on lettuce heads examined throughout the season at Marshland and Southbridge. Published literature and personal communications indicate there is strong evidence of *N. ribisnigri* transmitting *LNYV,* and some evidence of it transmitting CMV along with many of the other aphid species collected on lettuce heads. Along with this reservoirs of LNYV and CMV were commonly found in *Sonchus* and *Lactuca* weeds associated with both monitored sites. Various management and research recommendations were made from this report.

After consideration by Vegetables NZ the agreed objectives for 2017–18 were:

- Need to confirm that *N. ribisnigri* can transmit LNYV under New Zealand conditions. Laboratory experiments to confirm transmission would be undertaken using aphid colonies and virus isolates.
- Continue to monitor aphid populations and disease in Marshland and Sefton (following the closure of the Southbridge crop). Reference surveys at 'low disease' sites at Spotswood and Chertsey would also be completed. FruitFed Supplies would scout fields and collect wind trap contents and Plant and Food would continue to identify aphids and collate data.
- Regional surveys of Nelson and Horowhenua will be undertaken to confirm the incidence of LNYV and determine if measures taken by local growers, such as changes in chemical application, have been successful as has been reported anecdotally.
- Develop a more sensitive and faster diagnostic tool such as isothermal PCR amplification in order to improve field detection of LNYV in plants and aphid vectors.

Following on from 2017 we anticipated resolving the question of whether *N. ribisnigri* could transmit LNYV under New Zealand conditions. Unfortunately with the collapse of our original aphid colony and with the inability to replace it from a field source we were not able to progress this objective. We would hope further work in the future could be attempted to confirm *N. ribisnigri* importance as a virus vector.

The wet winter, warm spring and alternate hot and wet summer conspired to challenge lettuce production in Canterbury, Nelson and elsewhere. These factors also seriously affected other components of our project. It is fair to say, however, that these weather patterns did mean low levels of virus occurred in most crops throughout the season, which was certainly of some benefit for most growers.

The weather patterns seriously affected aphid vector activity as reflected in the low wind trap catches of virus vectoring aphids at Marshland and Sefton compared to the previous year. Similarly, field collections of aphids on lettuce plants this season were very low even where insecticides were scarcely used. Low vector numbers were reflected in the recorded incidences of LNYV and CMV, with a maximum of 10% in plantings at Marshland and 15% at Sefton (both harvested in late January and early February). Over the rest of the period virus incidence never exceeded 2%. Similar seasonal observations of low disease incidence were reported from Manawatu and Nelson.

Regional surveys of Canterbury, Manawatu and Nelson confirmed the presence of LNYV, CMV and sometimes *Beet western yellows virus* (BWYV) in local lettuce crops. A serious outbreak of necrotic disease occurred early in the season at Levin. Similarly, an early outbreak occurred at Chertsey but this was attributed to a missed insecticide drench application. Discussion with growers indicated that they were satisfied that insecticide drenches were largely effective at protecting seedlings and young plants from aphid establishment. However some growers felt they still needed an early follow-up spray to support the drench treatment. It was felt the effect of drench treatment may not be lasting as long as expected. It was suggested growers discuss with their seedling suppliers and confirm that chemicals were indeed being applied as directed by the manufacturer as a drench/soak and not just as a spray.

Additional work on *N. ribisnigri* survival and reproduction confirmed there was no evidence of a resistance-breaking strain of aphid in that tested population. An explanation of the initial infestation might lie with a mislabelled seed batch. It is worth noting that recently, resistance-breaking biotypes of *N. ribisnigri* (Nr:1) have been detected in Australia (Ausveg 2018). This does mean growers need to be on the alert for an incursion into New Zealand lettuce crops.

The RT PCR test conditions were improved to give better resolution to this conventional assay. The development of a faster isothermal assay has been successfully initiated for LNYV but more work is need on this and on the CMV assay before it can be used for routine field application.

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1 INTRODUCTION

Over the past three seasons, lettuce (*Lactuca sativa*) growers in the lower North Island, Nelson and Mid Canterbury have been concerned about poorly performing outdoor lettuce crops. In particular, those growing iceberg/crisp head lettuce types have noticed seasonal crop collapses with losses as high as 50%.

From specimens submitted from the lower North Island and Canterbury during the 2015–17 period, Lettuce necrotic yellows virus (LNYV) was identified in most plants. LNYV is usually transmitted by the sow thistle aphid (*Hyperomyzus lactucae*). LNYV was not detected in Canterbury in the 2002–3 virus surveys (Fletcher et al. 2005); at this time Mirafiori lettuce big-vein virus (MLBVV) and Lettuce big-vein associated virus (LBVaV) were detected at 10–20%, and Turnip mosaic virus (TuMV) at 10%. In addition, in 2002 a serious new pest of lettuces arrived in the form of *Nasonovia ribisnigri*, the blackcurrant-lettuce aphid, which has been managed using insecticides and aphid-resistant cultivars.

A joint grower researcher meeting held on 17 August 2016 expressed concern that:

- A more virulent strain of LNYV virus has arrived from Australia (two virus strains exist)
- A new aphid that is able to transmit the virus has arrived in New Zealand
- Recent warm winters/summers in New Zealand have changed some dynamic with the behaviour of the sow thistle aphid
- Some interaction with other lettuce viruses and/or insects was occurring.

1.1 Year 1

In order to explore these possibilities, lettuce crops at two sites, Marshland and Southbridge in Mid Canterbury, were monitored weekly from November 2016 to mid-April 2017 for aphid vector activity over the spring, summer and autumn periods using field scouting and wind traps.

Scouts from FruitFed Supplies also inspected lettuce plants weekly for the presence of aphids and any virus-like symptoms. Mature adult aphid specimens were collected to confirm species identity. Collection tubes from the nearby wind trap were replaced weekly and collected specimens were conveyed to The New Zealand Institute for Plant and Food Research Limited (PFR), Lincoln for identification and counting. Three crop surveys were undertaken at the sites to determine the incidence of disease symptoms and identity of any associated viruses. Collected data were analysed and compared with existing recorded data held by PFR.

Our work found LNYV and *Cucumber mosaic virus* (CMV) were strongly associated with necrosis symptoms of iceberg lettuce heads. Both viruses were found in *Sonchus* and *Lactuca* weed hosts near the monitored sites. Similarly *N. ribisnigri* was the most abundant aphid species found present on lettuce heads examined throughout the season at Marshland and Southbridge. On weeds *N. ribisnigri, H. lactucae Aphis gossypii* and *M. euphorbiae* were detected on *Sonchus* and Lactuca weeds at both during November and December. Published literature and personal communications indicate there is strong evidence of *N. ribisnigri* transmitting LNYV, and some evidence of it transmitting CMV along with many of the other aphid species collected on lettuce heads. Along with this we found reservoirs of LNYV and CMV

were commonly found in *Sonchus* and *Lactuca* weeds associated with both monitored sites. From this work we recommended:

- Rapid removal/ploughing-under of harvested crops adjacent to growing crops.
- Zero tolerance of weed hosts of LNYV and CMV in and around lettuce plantings.
- An expansion of the area of suitable *Nasonovia*-resistant crisp head plantings over the critical infection periods.
- An examination of the efficacy of currently registered insecticides against *N. ribisnigri, Myzus persicae* and *H. lactucae.*
- Development of more sensitive and faster diagnostic tools such as isothermal amplification in order to improve field detection of LNYV and CMV in lettuce and to determine that *N. ribisnigri* is indeed another vector of LNYV and CMV.

1.2 Year 2

After a grower meeting in 2017, key points for further research in year 2 of the project were summarised as follows:

- We need to confirm that *N. ribisnigri* can transmit LNYV under New Zealand conditions. Laboratory experiments to confirm transmission would be undertaken using our aphid colonies and virus isolates.
- We should continue to monitor aphid populations and virus disease in lettuce crops in Marshland and, with the closure of the Southbridge site, move monitoring to Sefton, North Canterbury. Reference surveys at 'low disease' sites at Chertsey and Spotswood would also be completed. Fruit Fed Supplies would scout fields and collect wind trap samples and PFR continue to identify aphids and collate data.
- Regional surveys of Nelson and Horowhenua should be undertaken to confirm the incidence of LNYV and determine if measures taken by local growers, such as changes in chemical applications, have been successful as has been reported anecdotally.
- Continue to explore the stability of the Nr:0 resistance under New Zealand growing conditions.
- Undertake a laboratory leaf dip bio-assay to determine the continued efficacy against a field-collected population of *N. ribisnigri* of one established insecticide treatment and one new treatment.
- Develop a more sensitive and faster diagnostic tool such as isothermal PCR amplification in order to improve field detection of LNYV in plants and in aphid vectors.

After consideration by Vegetables NZ the agreed objectives for 2017–18 were finalised as:

- Need to confirm that *N. ribisnigri* can transmit LNYV under New Zealand conditions. Laboratory experiments to confirm transmission would be undertaken using aphid colonies and virus isolates.
- Continue to monitor aphid populations and disease in Marshland and, with the closure of the Southbridge crop, move monitoring to Sefton, North Canterbury. Reference surveys at 'low disease' sites at Spotswood and Chertsey would also be completed.

FruitFed Supplies would scout fields and collect wind trap samples and PFR would continue to identify aphids and collate data.

- Regional surveys of Nelson and Horowhenua will be undertaken to confirm the incidence of LNYV and determine if measures taken by local growers, such as changes in chemical application have been successful as has been reported anecdotally.
- Develop a more sensitive and faster diagnostic tool such as isothermal PCR amplification in order to improve field detection of LNYV in plants and aphid vectors.

2 METHODS

2.1 Aphid and virus monitoring at Marshland and Sefton

From late November 2017 to late March 2018 two crops, one at Marshland and another at Sefton, were monitored weekly for likely aphid vectors. Crops were scouted for aphids on lettuce hosts; in each crop, 20 plants were examined and any aphid specimens were collected and taken to Lincoln for identification. The contents of a wind trap located near each crop were also collected and aphids were identified. It was mutually agreed with FruitFed this monitoring was to be performed by PFR staff owing to cost and distances required for FruitFed to undertake this work.

A visual estimate of virus incidence was determined at each visit by counting and visually estimating incidence in four groups of 50 plants showing leaf necrosis and yellowing symptoms, similar to that shown in Figure 1. Specimens exhibiting typical virus symptoms, similar to those shown in Figures 1 and 2, were also collected and tested for LNYV, CMV and BWYV to confirm virus identity. Virus assays were performed using double antibody sandwich (DAS) enzyme linked immunosorbent assay (ELISA) according to the manufacturer's instructions and Indirect (Ind) ELISA according to Fletcher (1993). A survey of *Sonchus* weeds was also undertaken at each site early in the season, *Sonchus* weeds found were taken back to the laboratory where aphids were removed and identified.

Figure 1. Typical *Lettuce necrotic yellows virus* (LNYV) symptoms in a lettuce crop at Marshland, January 2018.

Figure 2. Typical *Cucumber mosaic virus* (CMV) symptoms in a lettuce crop at Levin, March 2018.

2.2 Crop surveys

In addition to regular sampling of the monitored crops, surveys were undertaken early season at Spotswood North Canterbury, mid-season at Chertsey Mid Canterbury, and late season in Manawatu and Nelson. During these visits, crops were surveyed as described above for virus incidence and presence of aphid vectors. Selected plant specimens usually about 5 plants, exhibiting typical virus symptoms were also collected to determine if LNYV CMV and BWYV were present using ELISA Discussions were held with growers during these surveys concerning current practices for virus vector and disease management.

2.3 Virus transmission experiments

Virus transmission experiments were dependent on sustaining a viable colony of *N. ribisnigri.* While such a colony had been established in June it collapsed in November and no further *N. ribisnigr*i could be found in sufficient numbers to re-establish a colony. Consequently no transmission experiments could be attempted.

We do however report on earlier laboratory work to determine if aphids were overcoming the Nr:0 host plant resistance as suspected by a seed company representative.

2.4 Nasonovia ribisnigri survival experiment

In June a seed company detected heavy colonisation by *N. ribisnigri* of a new resistant lettuce variety grown in a trial in the lower North Island. There was concern that there may be a resistance-breaking population of *N. ribisnigri* present in this crop. Collections of this population of *N. ribisnigri* were made from infested lettuces and a colony established (with some difficulty) at Lincoln. To determine if evidence of such resistance breaking was present in this aphid population a simple challenge experiment was initiated. Seedling lettuce plants of 'Albanas' (resistant to Nr:0 populations of *N. ribisnigri*) and 'Winguard', a non-resistant cultivar, were obtained from Seedling Technologies of Kaiapoi and established in pots at Lincoln. Five *N. ribisnigri* nymphs were placed on 6 lettuce plants comprised of the two cultivars and placed in a Perspex colony cage (16h light 8h dark) on 3 August 2017 and monitored. A further 5 aphids were applied to all plants on 9 and 13 August 2017.

Counts of aphids present were recorded every 5 days over a 4 week period to measure the comparative survival and reproduction of *N. ribisnigri* on the two lettuce cultivars.

2.5 Improved virus assay

Dr Yubao Zhang, a visiting virologist from China, explored our current reverse transcriptase (RT) PCR assay for LNYV (Higgins et al. 2016) and worked to develop a new isothermal assay for both LNYV and CMV.

3 RESULTS

3.1 Aphid and virus monitoring at Marshland and Sefton

3.1.1 Aphid monitoring

Aphid species and numbers collected and identified from the wind traps from late November 2017 to late March 2018 are summarised in Figure 3.

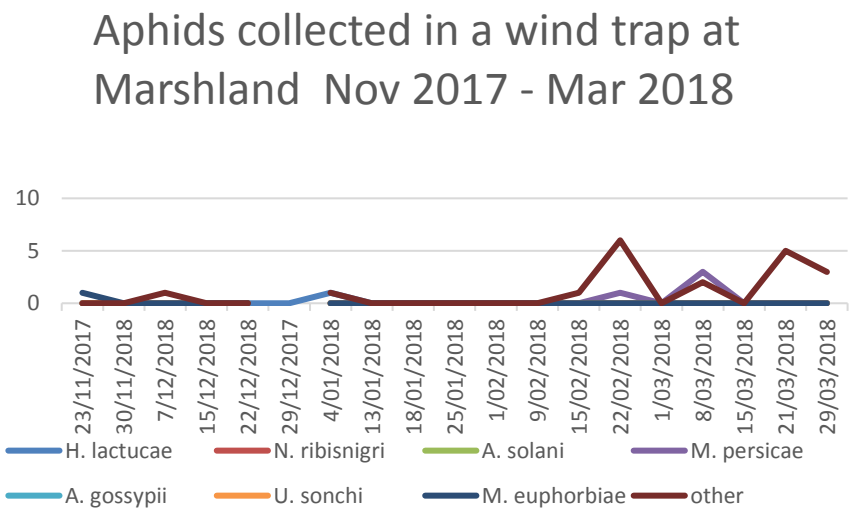

Figure 3A

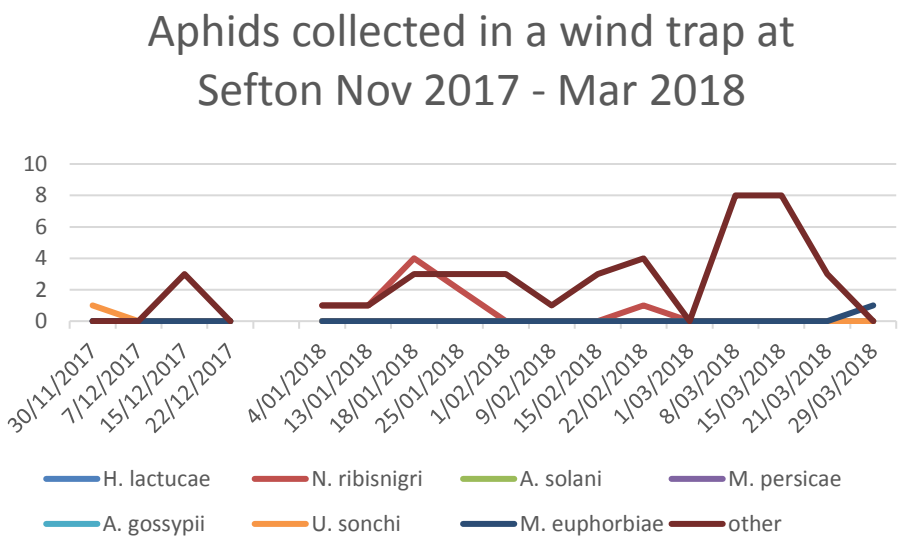

Figure 3B

Figure 3. Aphids collected in wind traps located in two lettuce crops at (A) Marshland and (B) Sefton, Mid Canterbury, November 2017–March 2018. *Hyperomyzus lactucae, Nasonovia ribisnigri, Aulacorthum solani, Aphis gossypii, Uroleucon sonchi, Macrosiphum euphorbiae, Myzus persicae and other aphid species.*

Over the trapping period, numbers of trapped aphid vectors of lettuce viruses were very low (Figure 4). At Marshland maximum per visit of three *M. persicae,* one *H. lactucae* and no *N. ribisnigri* were trapped over the period. At Sefton a single *M. euphorbiae* and 4 *N. ribisnigri* were trapped. 'Other' non virus vectoring aphids dominated trap catches over the trapping period at both sites. At both sites apart from seedling drenches with insecticide no other insecticides were applied as they were not really necessary.

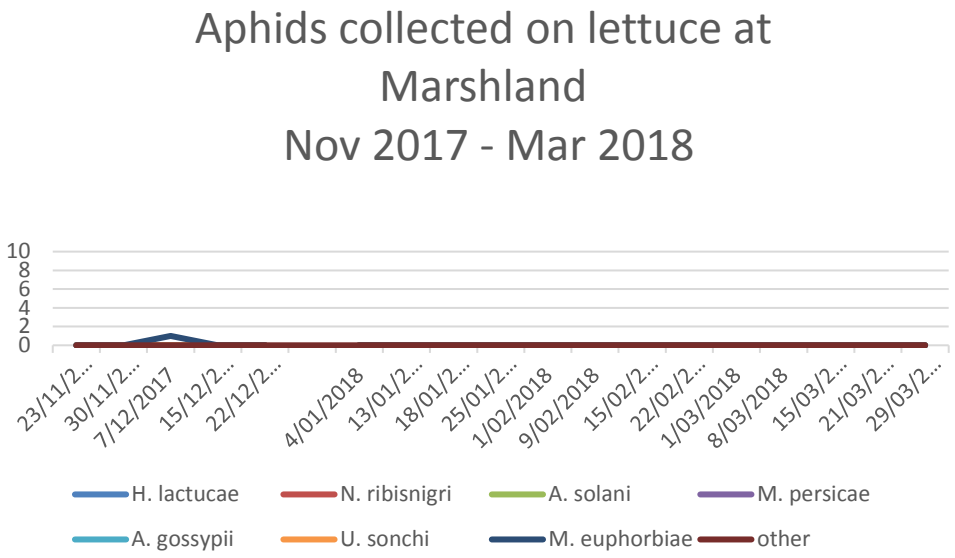

Figure 4A

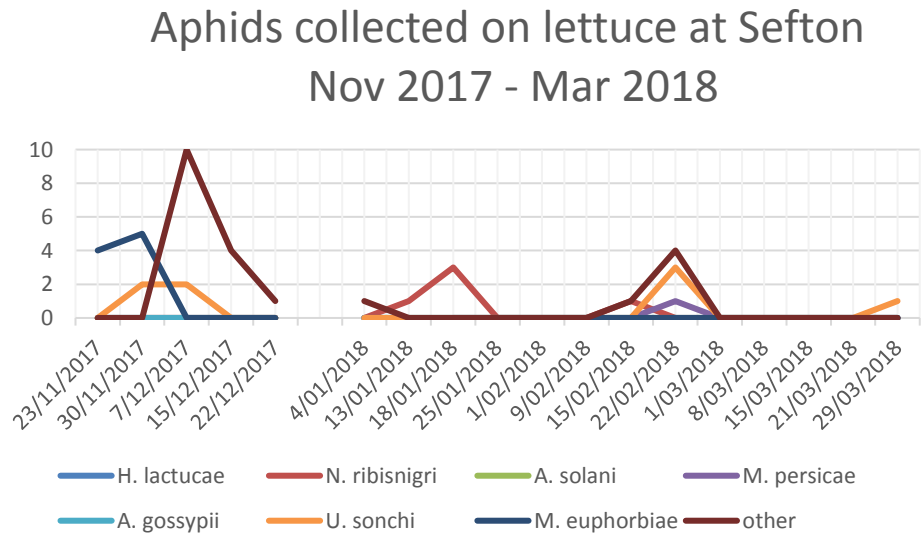

Figure 4B

Figure 4. Aphid species and numbers collected and identified from individual lettuces sampled and examined over the period November 2017 to March 2018 at (A) Marshland and (B) Sefton, Mid-Canterbury. *Hyperomyzus lactucae, Nasonovia ribisnigri, Aulacorthum solani, Aphis gossypii, Uroleucon sonchi, Macrosiphum euphorbiae, Myzus persicae.*

As reflected in the wind trap figures, aphid numbers recorded from scouted lettuces were also very low this season. We noted that *H. lactucae* was not detected on lettuces examined during November at either site. In fact only a single aphid (*M. euphorbiae*) was detected at Marshland all season. *N. ribisnigri* was detected only at Sefton at low levels in January and February. Again 'other' aphid species also prevailed on plants at Sefton. We detected no plants infested with *N. ribisnigri* during this season's monitoring. On weeds, only *H. lactucae* and *M. euphorbiae* were detected on *Sonchus* spp. during November and December.

3.1.2 Virus monitoring

Lettuce crops were monitored weekly for symptoms of virus infection. Results of visual virus estimation are presented in Figure 5. Virus ELISA assays of plants sampled over the season to verify visual symptoms detected LNYV in 66% of plants exhibiting typical symptoms that were collected at Marshland and 78% at Sefton, CMV (Figure 5) in 22% collected at Marshland and none at Sefton, and *Beet western yellows virus* (BWYV) in 5% collected at Marshland and 14% at Sefton. On *Sonchus* weeds surveyed at Marshland we detected LNYV in 5/5 *Sonchus oleraceus,* 2/2 *S. asper* and 1/1 *Lactuca serriola.* There had been a considerable effort to remove all weeds at Marshland which probably contributed to the season's lower overall virus incidence at that site. At Sefton surveys of the same weed species failed to detect any virus present in any plants.

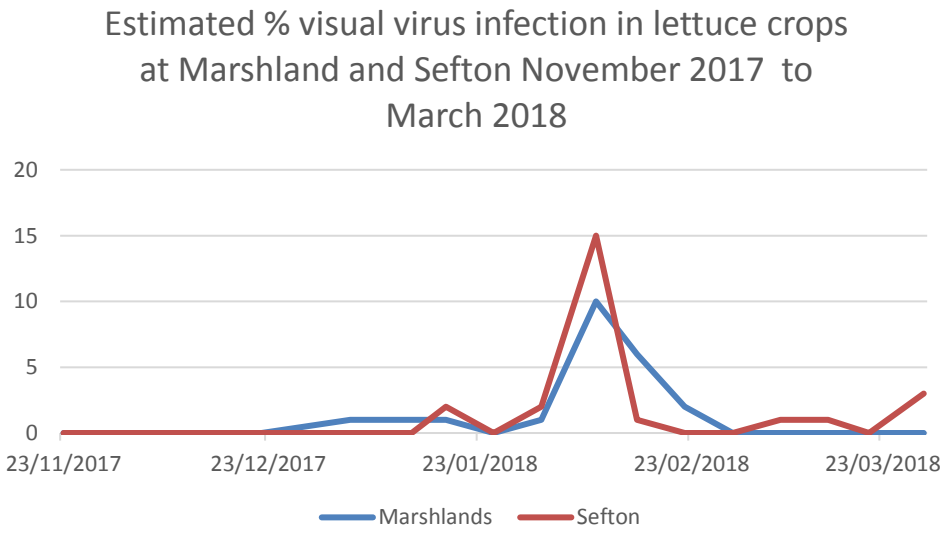

Figure 5. Estimated percentage (%) visual virus infection in monitored lettuce crops at Marshland and Sefton Mid Canterbury November 2017 to March 2018.

3.2 Crop surveys

3.2.1 Canterbury

A survey of a new lettuce cropping site at Spotswood in North Canterbury in late November did not detect any early season virus. A weed survey did however detect LNYV present in single plants of *S. oleraceous* and *S. arvensis.* Discussion with the grower late in the season indicated virus infection had not been a concern this season.

Another crop at Marshland was visited in early December and in March where LNYV was detected by viral assay in three sampled specimens but visual incidence was below 1%. In March, visual incidence was estimated at 1% and the grower indicated virus had not been a serious problem during the season. Seedling treatment with Durivo (thiamethoxam and chlorantraniliprole) (to be applied as a seedling drench 24 h before planting), had been instituted this season. In March a visual incidence of 1–1.5% virus yellowing and necrosis symptoms was observed. Collected specimens were tested and confirmed 3/3 with LNYV.

Crops at Chertsey were visited in late January where it was reported that there had been an early season prevalence of *H. lactucae* in plantings not treated with Confidor (imidacloprid) insecticide (which was to be watered evenly over seedlings 24 h before planting). In this case application is usually made by spray to run-off over the glasshouse raised seedlings. Routine spraying continued every 7–14 days throughout the season if scouting detected aphid presence. Collected symptomatic lettuce specimens were tested by viral assay and confirmed 4/5 with LNYV. High aphid pressure was experienced in late February and March but was insufficient to require further sprays. Some virus was observed on visual inspection but at less than 1% incidence.

In mid-March crops were visited at Palmerston North and in the Manawatu. The season had been one of climatic extremes with stormy wet weather and periods of high temperature. The Palmerston North grower reported no virus problems in recent years. He confirmed that seedlings were treated before planting with Confidor insecticide at the nursery. During winter non-Nasanovia resistant lettuce cultivars were successfully grown. Around Levin, a major grower had experienced a 10–20% virus incidence over December–January. Some of this might be attributed to poor efficacy of the seedling insecticide treatment. Currently Durivo is applied at the nursery before shipping to the property. Highest virus incidences seemed to occur close to urban areas. A zero aphid tolerance policy applies and crops are scouted regularly and if necessary sprayed every 7–14 days. Visual incidence appeared to be 1% LNYV with 2–3% CMV. Of the symptomatic specimens collected, 4/5 had LNYV and 4/5 CMV. Three were mixed virus infections. Two specimens exhibited CMV-like symptoms (plant stunting and yellowing with little or no necrosis), the others typical LNYV. At a nearby property the grower indicated they had had no disease problem in lettuce apart from Sclerotinia, however, they do apply insecticide as a precaution.

In late March crops were visited in the Richmond plains growing area of Nelson. The season had been one of climatic extremes with a wet spring followed by a long period of drought into January moving into cyclonic weather in January–February. Crop losses were experienced because of these conditions. On the first property few virus problems had been experienced this season; incidence in the visited crops was less than 0.5%. Control was attributed to Confidor drench and follow-up insecticides. At the second property an incidence of 1–1.5% virus was observed in a crop under harvest. Seedlings were produced on the property and were treated with Confidor or Durivo. Aphids and virus had not been a serious problem this season in the fields although there was concern about full control of aphids in the glasshouse where seedling infection might become a concern. In the last property virus had not been a serious problem this season but had caused problems in the past. Seedlings are treated with Durivo and shipped to Nelson for planting. Close scouting is practised and chemical applied if necessary every 7–14 days.

3.3 Virus transmission experiments

As explained above no transmission experiments were undertaken as a colony could not be established.

3.4 *Nasonovia ribisnigri* survival experiment

Results of the aphid survival assay experiment are summarised in Figure 6.

Over the 4 weeks of monitoring, reproduction of *N. ribisnigri* continued on the three plants of the non-aphid resistant variety 'Winguard' to maximum populations of between 43 to 52 aphids/plant. In contrast reproduction on Nr resistant variety 'Albanas' only reached populations of between 1 and 4 aphids/plant. This result did not provide evidence of any breakdown of resistance in this population of *N. ribisnigri* to N:O conferred plant resistance.

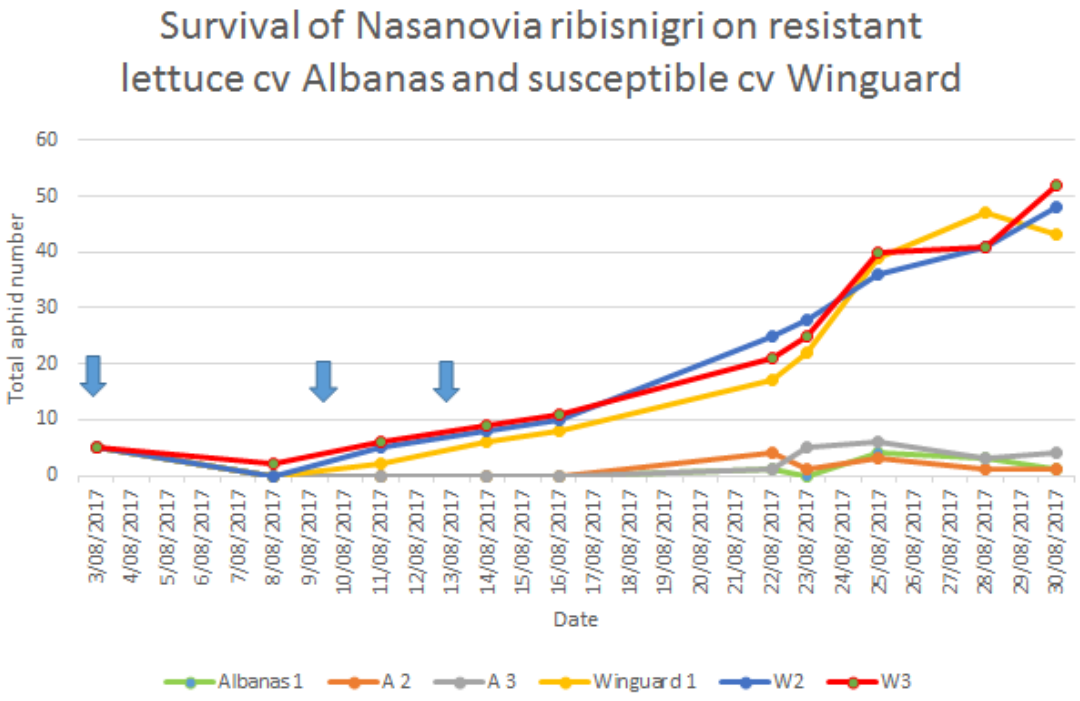

Figure 6. Measurement of the survival and reproduction of *Nasanovia ribisnigri* on resistant lettuce cultivar 'Albanas' and susceptible cultivar 'Winguard' over a four week period. Five aphids were applied to each plant on three dates over the period (indicated by arrows).

3.5 Improved virus assay

RT PCR

The RT PCR conditions for LNYV (Higgins et al. 2016) were modified to improve resolution and the conditions were modified as follows:

Viral RNA was obtained from lettuce plant specimens using a Sigma Spectrum™ total RNA extraction kit following the manufacturer's instructions. Internal controls used were BCNG 3 and BCNG4 internal primers.

Temperature	Time	Cycle/s
50°C	30 min	1x
94°C	2 min	1x
94°C	15 sec	35x
55°C	30 sec	35x
68°C	1 min	35x
68°C	10 min	1x

Total time 2h 14 min

RT-Isothermal detection development

Isothermal detection primers for LNYV and CMV were designed and tested and conditions refined.

Reactions were carried out at 60–65°C for 60 min followed by incubation at 80°C for 10 min. Amplification products were visualised on a 2% agarose gel. See Appendix 1.

4 DISCUSSION

Following on from work last year we anticipated resolving the question of whether *N. ribisnigri* could transmit LNYV under New Zealand conditions. Unfortunately with the collapse of our original aphid colony and with the inability to replace it from a field source we were not able to progress this objective. We would hope further work in the future should be attempted to confirm *N. ribisnigri* importance as a virus vector.

The wet winter, warm spring and alternate hot and wet summer conspired to challenge lettuce production in Canterbury, Nelson and elsewhere. The monitored site at Marshland was in fact flooded at one point during the season (Figure 7).These factors also seriously affected other components of our project. It is fair to say, however, that these weather patterns did mean low levels of virus occurred in most crops throughout the season, which was certainly a benefit for most growers.

The weather patterns seriously affected aphid vector activity as reflected in the low trap catches of aphid virus vectors at Marshland and Sefton compared to last year. Similarly, field catches of aphids on lettuce plants this season were very low even where insecticides were scarcely used. Low vector numbers were reflected in the recorded incidences of LNYV and CMV, with a maximum of 10% in plantings at Marshland and 15% at Sefton (both harvested in late January and early February). Only at Sefton did increased virus incidence appear to coincide with previous *N. ribisnigri* activity in the crop and with wind trap catches. Over the rest of the period virus incidence never exceeded 2%. Similar seasonal observations of low disease incidence were reported from Manawatu and Nelson.

Regional surveys confirmed the presence of LNYV, CMV and sometimes BWYV in local lettuce crops. A serious outbreak of necrotic disease occurred early in the season at Levin. Similarly, an early outbreak occurred at Chertsey but this was attributed to a missed insecticide drench application. Discussion with growers indicated that they were satisfied the Durivo (Insecticide Resistance Action Committee (IRAC) Group 4A Neonicotinoids & Group 28 Diamides) treatment was largely effective at protecting seedlings and young plants from aphid establishment. Confidor (Insecticide Resistance Action Committee (IRAC) Group 4A Neonicotinoids) was still found to be effective by some growers. In discussion, some growers felt they still needed a follow-up spray to support the drench treatment. It was felt the treatment effect may not be lasting as long as expected. It was suggested growers discuss with their seedling suppliers and confirm that chemicals were indeed being applied as directed by the manufacturer as a drench/soak and not just as a light spray. The practice in early 2000's on farms at Pukekohe and elsewhere was to immerse lettuce seedling trays in a solution of Confidor. This practice may have provided a better drenching effect than application by a watering can or a spray.

The additional work on *N. ribisnigri* survival and reproduction confirmed there was no sign of a resistance-breaking strain (Nr:1) of aphid in that tested population. An explanation of the initial infestation might lie with a mislabelled seed batch. It is worth noting that recently, resistance-breaking biotypes of *N. ribisnigri* have been detected in Australia (Ausveg 2018). This does mean growers need to be on the alert for an incursion into New Zealand lettuce crops. A recent fact sheet has been produced by Rijk Zwaan Australia (Rijk Zwaan Australia 2018) which may be useful to NewZealand growers. Further strategies for the management of resistance breaking *N. ribisnigri* biotypes are also outlined in Arend (2003).

The RT PCR test conditions were improved to give better resolution to this conventional assay. The development of a faster isothermal assay has been successfully initiated for LNYV but more work is need on both this and on the CMV assay before it can be used for routine field application.

Figure 7. Extreme weather conditions flooded and destroyed part of the monitored site at Marshland in mid-January 2018.

5 ACKNOWLEDGEMENTS

We sincerely thank the following colleagues and contributors: Mark & Ryan Kiesanowski, Marshland and Cam Booker, Sefton for use of their properties to monitor aphids and virus disease. In particular we thank Roger Banfield, Terranova Seed for helpful discussions and the submission of this proposal to Vegetables New Zealand. We thank growers in Canterbury, Manawatu and Nelson and in particular their helpful field staff for allowing property access for field surveying, sampling and helpful discussions. We also thank Mark O'Connor for his helpful coordination and discussions. We especially thank Stuart Paul at FruitFed Supplies for coordinating and hosting grower meetings throughout the project. We thank Seedling Transplants, Kaiapoi for helpful discussions and supplying seedling lettuces and Dr Zeger van Herwijnen of Rijk Zwaan Breeding B.V., the Netherlands for very helpful discussions on lettuce/*Nasonovia* host responses. Thank you to Pam Fletcher for her helpful field assistance. We finally thank John Seymour and the research committee of Vegetables New Zealand for their funding support of this project.

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APPENDIX 1

RT-Isothermal detection for LNYV/CMV in Lettuce

	Reaction Mixture	uL
1	Template (RNA)	1
2	Thermpopol buffer (10×)	2.5
3	Betaine (5M)	4
4	dNTP (10mM)	3.5
5	MgSO4 (100uM)	1.5
6	B3 Primer (10uM)	0.5
7	F3 Primer (10uM)	0.5
8	FIP Primer (100uM)	0.4
9	BIP Primer (100uM)	0.4
10	FL Primer (100uM)	0.25
11	BL Primer (100uM)	0.25
12	Bst pol (8u/uL)	1
12	Thermo reverse transcriptase (1.5u/uL)	0.5
13	RNA-free Water	8.7
Total		25

The reaction was carried out at 60–65°C for 60 min followed by incubation at 80°C for 10 min.

Amplification products were made visible on a 2% agarose gel.

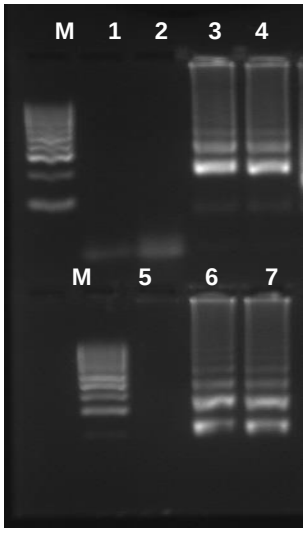

Figure 8. RT-LAMP for detecting Lettuce necrotic yellows virus (LNYV). Lane M, 100-bp DNA marker; lane 1, 2, 5 negative control. Lane 3,4,6,7, lettuce samples.

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