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Tomato potato psyllid in Canterbury: Summary of trapping data and Liberibacter testing

Vereijssen J, Frampton R, Furlong J

May 2018















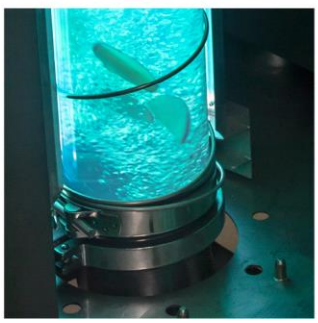


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CONTENTS

TECHNICAL ACRONYMS AND ABBREVIATIONS

CLso: *Candidatus* Liberibacter solanacearum, the bacterium vectored by the tomato potato psyllid and the putative causal agent of zebra chip disease in potato tubers

Ct: Cycle threshold in a qPCR, shows the number of cycles that it took to detect a real signal from the samples. It is a relative measure of the concentration of the target DNA in the PCR reaction. The lower the Ct value, the more CLso was detected in the psyllid. Ct values higher than 35 indicate that the qPCR method could not detect CLso.

CTAB: Cetyl trimethylammonium bromide, a detergent commonly used in DNA extraction methods

ITS: Internal transcribed spacer, region of DNA between the small ribosomal RNA subunit and the large ribosomal RNA subunit. Commonly used for identifying and classifying organisms. A qPCR assay has been developed for the TPP ITS region. This assay is used as a control to check the quality of the DNA extraction.

PCR: Polymerase chain reaction, is a molecular biological method for amplifying (creating multiple copies of) a short, well-defined part of a DNA strand, which is visualised as bands on a gel. This can be a single gene, or just a part of a gene.

TPP: Tomato potato psyllid, *Bactericera cockerelli*, a miniature cicada the size of a winged aphid, affecting solanaceous crops and a vector for CLso.

qPCR: A specialised technique that allows the results of a PCR reaction to be visualised in real time as the reaction progresses. It allows the measurement of minute amounts of DNA sequences (the amplified, well-defined part of a DNA strand – see PCR) in a sample; no gel is required for visualisation.

YST: Yellow sticky trap.

EXECUTIVE SUMMARY

Tomato potato psyllid in Canterbury: Summary of trapping data and Liberibacter testing

Vereijssen J, Frampton R, Furlong J
Plant & Food Research Lincoln

May 2018

Zebra chip disease of potatoes has been an increasing problem for growers in Canterbury over the past six years. The tomato potato psyllid (TPP) vectors the bacterium *Candidatus* Liberibacter solanacearum (CLso), the putative causal agent of zebra chip disease. A large-scale study was carried out in the 2017–18 season to explore the seasonality and directionality of TPP and CLso in potato crops in Canterbury. Yellow sticky traps were placed in fields and trapped insects counted by Fruitfed Supplies Ltd. staff. TPP were also removed from traps and tested by Hill Laboratories for the presence of CLso using composite sampling. The project was managed by the Foundation for Arable Research (FAR). This report summarises the trapping and CLso testing data provided to Plant & Food Research Ltd. (PFR) by FAR.

Yellow sticky traps caught TPP at all 10 potato crop locations in Canterbury. The seasonal peak of TPP catch was between mid-January and early February when totalled across all growers. Directionality (north, east, south, and west) differed among growers for first TPP caught and highest number of TPP caught. In general, very high numbers of TPP (more than 100 TPP per trap) were not observed.

CLso-positive TPP samples occurred throughout the trapping season, from December to March, but not consistently. The majority of composite TPP samples (81.6%) were negative for CLso, 13 (4.4%) were positive for CLso, and the remaining tests were reported as undetermined, likely due to insect misidentification. As expected, CLso haplotype A was found in CLso-positive samples.

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1 INTRODUCTION

Tomato potato psyllid (TPP), *Bactericera cockerelli* (Šulc) (Hemiptera; Triozidae), is a major pest for the New Zealand potato industry, especially for the seed and process/crisping potato growers. The psyllid is a vector of the bacterial plant pathogen *Candidatus* Liberibacter solanacearum (CLso) (haplotypes A and B), which is the putative causal agent of zebra chip disease in potato. To our knowledge, only CLso haplotype A is present in New Zealand.

In Canterbury and Hawke's Bay, research findings and observations have indicated that all TPP life-stages are present year-round (on non-crop hosts), adults are active year-round even in areas with no apparent host plants and in areas with snow, and CLso can be found in weed plants outside of the cropping season (Vereijssen & Scott 2013; Barnes et al. 2015; Vereijssen et al. 2015; Vereijssen et al. 2016), which is in line with findings overseas (Jensen et al. 2012, Horton et al. 2014; Murphy et al. 2013).

In some potato-growing areas in New Zealand TPP numbers on yellow sticky traps (YST) have generally been decreasing over the last six years, whereas TPP, and CLso, have been an increasing problem for potato growers in the Canterbury area over that period. Some areas in Canterbury seem to consistently have higher numbers of TPP or more zebra chip disease than others, while there are also reports of many TPP in a crop and hardly any zebra chip disease.

A small study was conducted in the 2016–17 season with growers in Pukekohe (Frampton et al. 2017), where all TPP from YST in potato crops were tested individually for the presence of CLso. The test results were used by growers to make TPP management decisions. This small study initiated the larger scale study in Canterbury described here.

This large-scale study was conducted in 10 selected potato crops in Canterbury. Firstly, to explore TPP seasonality using YST, with special attention paid to the direction at which TPP were caught. Additionally, presence of CLso in composite TPP samples caught on these traps was determined, to assess when positive TPP were coming into the crop or the area. Besides TPP, aphids and beneficial insects were also counted on each trap.

There are currently six described haplotypes (A-E and U) of CLso. The haplotypes are based on single nucleotide polymorphisms (SNPs) and insert-deletions (indels) in the bacterial rRNA operon. These differences correlate with different plant-insect associations and geographic distributions, however little is known about any biological differences between the CLso haplotypes (Nelson et al. 2011, Nelson et al. 2013, Teresani et al. 2014, Haapalainen et al. 2018). A recent publication demonstrated that symptom severity in tomato plants was more severe when infected with haplotype B compared to haplotype A (Mendoza-Herrera et al. 2018). Further research is required to determine if there are any differences in potato plants. New Zealand only has haplotype A and the introduction of further haplotypes could cause further damage to the potato as well as other horticultural industries.

Over the past six years, PFR researchers (led by Dr Grant Smith and funded by the Plant Biosecurity Cooperative Research Centre) have developed new diagnostics for CLso based on *Liberibacter* genomes. One set of diagnostic assays enables the haplotype of CLso-positive samples to be determined. The haplotype A assay was used on some CLso positive TPP samples to confirm the haplotype present in New Zealand.

2 MATERIALS AND METHODS

2.1 Monitoring of TPP, aphids and beneficial insects and testing for CLso

Trapping of TPP was conducted by Fruitfed Supplies Ltd. and commenced on 6 December 2017 for McCain Foods Ltd. crops and 7 December 2017 for Talley's Group Limited crops. The last YST were placed on 28 March 2018. All dates are given as the date on which the YST was placed in the field. Ten crops spaced throughout Mid and South Canterbury were selected for this project (Appendix 1). Four YST were placed in a crop (one at each cardinal site) as part of the monitoring programme Fruitfed are providing to McCain and Talley's. Every week, YST were replaced at each crop, and the number of TPP, aphids and beneficial insects counted on each collected trap. Total numbers of each insect species per YST for each of the crops were reported weekly to PFR, through FAR.

After counting, selected TPP (Appendix 2) were taken off the YST (Appendix 3) by Fruitfed and sent to Hill Laboratories for CLso testing. The test results were reported weekly to PFR, through FAR.

PFR has developed a quantitative polymerase chain reaction (qPCR) diagnostic tool for the detection of CLso in insects (Beard et al. 2012). This tool is regularly used at PFR Lincoln. The test is not only sensitive (to three copies of the target DNA) but will also estimate the relative quantity of the bacterium contained in infective insects. An assay using the TPP internal transcribed spacer (ITS) region is used as a control to check that the extracted DNA is of high quality. The qPCR assays give cycle thresholds (Ct) as results. This number indicates when enough amplified DNA has been detected for the assay to be positive. The lower the Ct, the higher the amount of target DNA in the sample, and the higher the titre of CLso. The qPCR protocol as developed by Beard et al. (2012) was slightly revised by Hill Laboratories for this project, and where more than one TPP was collected from a YST, composite samples were tested (i.e. up to 10 TPP were collected in a tube, resulting in 1 test result). The results from the CLso and TPP qPCRs were assessed and the final test results were reported as described in Table 1.

Table 1. Quantitative PCR thresholds used to determine the *Candidiatus* Liberibacter solanacearum (CLso) status of tomato potato psyllid (TPP) samples tested by Hill Laboratories. Ct = cycle threshold.

Final test result	CLso test outcome	TPP ITS test outcome
Detected	Positive (Ct ≤ 35)	Positive (Ct ≤ 35)
Not detected	Negative (Ct > 35)	Positive (Ct ≤ 35)
Undetermined	Negative (Ct > 35)	Negative (Ct > 35)

2.2 Testing for *Candidatus* Liberibacter solanacearum haplotype A

A qPCR assay for CLso haplotype A has been developed at PFR through comparisons of CLso genomes. A genomic region that was unique to CLso haplotype A was chosen and screened against sequence databases as well as against multiple DNA panels in laboratory tests. This qPCR assay was used to test 10 TPP samples that were positive for CLso. The assay has a sensitivity similar to the assay developed by Beard et al. (2012).

3 RESULTS

3.1 Monitoring of TPP, aphids and beneficial insects and testing for CLso

Fruitfed and Hill Laboratories data received through FAR were summarised and explored. There were missing data from some growers or specific YST at various times during the trapping period (Table 2).

Table 2. Missing data over the trapping period for each grower or for a specific yellow sticky trap (YST). (N = trap placed at north side of crop, E = trap placed at east side of crop, S = trap placed at south side of crop, W = trap placed at west side of crop, TPP = tomato potato psyllid, CLso = *Candidatus* Liberibacter solanacearum).

Sample date as on Fruitfed form	Week	Grower no.	Trap	Comments
6/12/2017	1	5	W	Predator counts missing
6/12/2017	1	8		missing data - no table
7/12/2017	1	10		missing data - no table
6–7/12/2017	1	2, 5, 6, 8, 10		CLso tests do not match TPP counts
13/12/2017	2	3	S	Aphid counts missing
13/12/2017	2	3	N	CLso test, no TPP on trap
13/12/2017	2	6	N	CLso test, no TPP on trap
13/12/2017	2	6	E	TPP on trap, no CLso test
13/12/2017	2	9	E	TPP counts missing
13/12/2017	2	9	N	TPP counts missing
19/12/2017	3	2	N	TPP on trap, no CLso test
19/12/2017	3	2	W	CLso test, no TPP on trap
19/12/2017	3	4	S	CLso test, no TPP on trap
19/12/2017	3	9	E	TPP counts missing
19/12/2017	3	9	N	TPP counts missing
20/12/2017	3	2	N	not tested for CLso, insect was damaged
29/12/2017	4	8	W	not tested for CLso, insect was damaged
29/12/2017	4	9		missing data - no table
5/01/2018	5	5	W	Predator counts missing
5/01/2018	5	7	W	TPP on trap, no CLso test
5/01/2018	5	8	N	TPP on trap, no CLso test
11/01/2018	6	5	N	Aphid counts missing
16/01/2018	7	3	E	TPP on trap, no CLso test
16/01/2018	7	5	E	TPP on trap, no CLso test
25/01/2018	8	5	N	Predator counts missing
25/01/2018	8	8	N	Aphid counts missing
31/01/2018	9	4	S	TPP counts missing

Sample date as on Fruitfed form	Week	Grower no.	Trap	Comments
31/01/2018	9	9		TPP counts missing
8/02/2018	10	3	W	not tested for CLso, insect was damaged
8/02/2018	10	9	S	not tested for CLso, insect was damaged
8/02/2018	10	2	E	not tested for CLso, insect was damaged
14/02/2018	11	10		missing data - no table
21/02/2018	12	10		missing data - no table
28/02/2018	13	4	W	not tested for CLso, insect was damaged
28/02/2018	13	10		missing data - no table
7/03/2018	14	10		missing data - no table
14/03/2018	15	5	N	not tested for CLso, insect was damaged
14/03/2018	15	8	W	TPP counts missing
14/03/2018	15	10		missing data - no table
21/03/2018	16	1	W	TPP counts missing
21/03/2018	16	4	N	TPP on trap, no CLso test
21/03/2018	16	10		Missing data - No table

In general, very high numbers of TPP (e.g. more than 100 TPP on a trap) were not observed in the selected crops. Across all growers, the peak of TPP caught was between mid-January and early February, with most of the TPP caught in the month of January compared to other months (Figure 1).

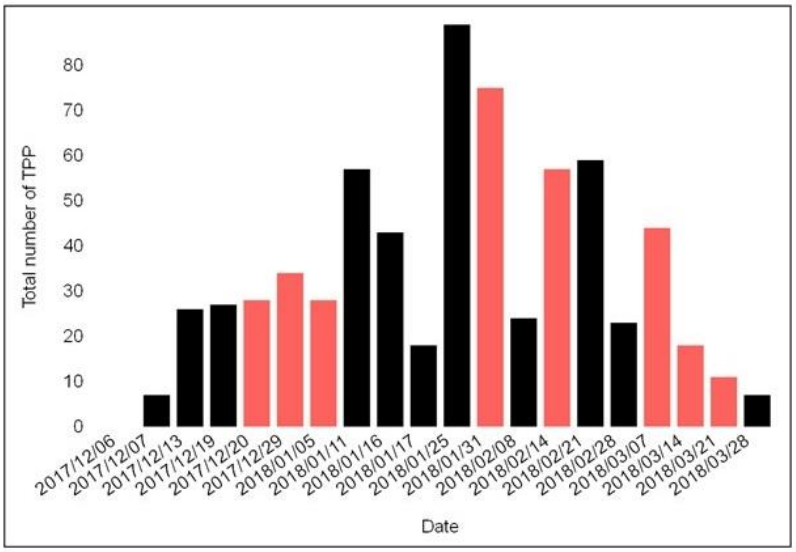


Figure 1. Total number of tomato potato psyllid (TPP) caught on all four yellow sticky traps (YST) in 10 potato crops in Canterbury in the 2017–18 growing season. Red bars indicate one or more TPP samples tested positive for *Candidatus* Liberibacter solanacearum. Black bars indicate no TPP samples tested positive from the YST for that date.

Over the whole season and across all growers, more TPP were caught on the east trap (226 TPP), with the other three traps catching similar numbers (N = 154; S = 159 and W = 136 TPP) (Figure 2). Two CLso-positive TPP samples were reported from the start of December, however, the YST count data for that week were missing. These results are not used in the following figures or grower results but were used in the haplotype testing. On 20 December the first confirmed positive TPP sample was found at Grower 1. Grower 1 and 4 had more positive TPP samples than the other growers. Across all growers, the south trap had noticeably more positive samples (5) than the north (1), east (3) and west (2) traps.

Table 3. Dates tomato potato psyllid (TPP) samples (containing one or more TPP) from yellow sticky trap tested positive for *Candidatus* Liberibacter solanacearum per grower.

Date CLso-positive TPP was detected	Grower no.
*6 December 2017	8
*6 December 2017	5
20 December 2017	1
29 December 2017	1
29 December 2017	4
29 December 2017	10
5 January 2018	6
5 January 2018	8
31 January 2018	4
14 February 2018	8
7 March 2018	7
14 March 2018	7
21 March 2018	7

*TPP counts from week 1 (6-7 December 2017) do not align with CLso test results. These two samples are not included in the following figures or grower results, but were used in the haplotype tests.
NT: not tested.

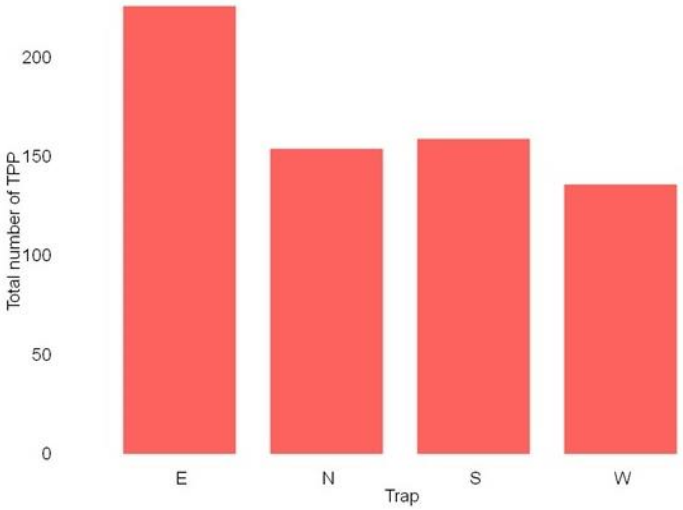


Figure 2. Total number of tomato potato psyllid (TPP) caught on all four yellow sticky traps in 10 potato crops in Canterbury in the 2017–18 growing season. Red bars indicate one or more TPP samples tested positive for *Candidatus* Liberibacter solanacearum.

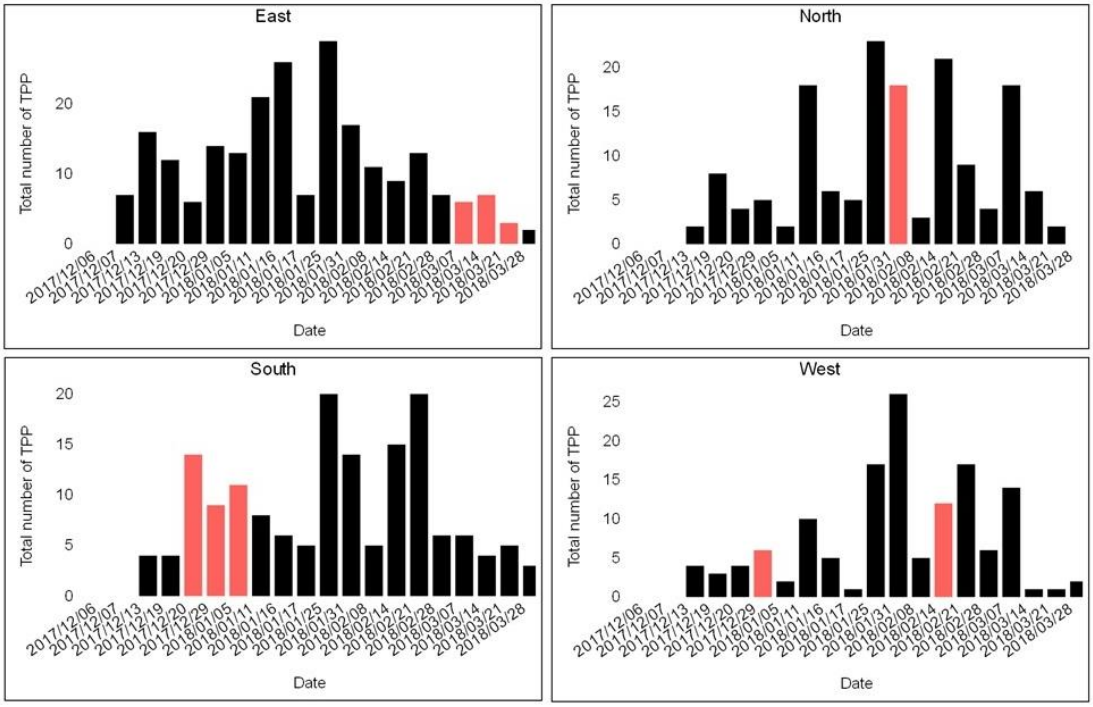


Figure 3. Total number of tomato potato psyllid (TPP) caught on all four yellow sticky traps (YST) in 10 potato crops in Canterbury in the 2017-18 growing season for each of the traps (east, north, south, west). Red bars indicate one or more TPP samples tested positive for *Candidatus* Liberibacter solanacearum. Black bars indicate no TPP samples tested positive from the YST on that date.

Over the season a total of 293 CLso tests were carried out by Hill Laboratories (Table 4). The majority of tests (81.6%) were negative for CLso and 13 (4.4%) TPP samples were positive for CLso, of these four were single TPP, seven were composite samples and two were of unknown TPP number. The remaining 41 tests were reported as undetermined; in these tests the TPP ITS reaction was negative as was the CLso test.

Table 4. Summary of quantitative PCR tests for *Candidatus* Liberibacter solanacearum carried out by Hill Laboratories.

Test outcome	Number of tests	Single TPP samples	Composite TPP samples	Unknown sample size
CLso detected	13	4	7	2
CLso not detected	239	98	128	13
Result undetermined	41	25	13	3
Total CLso tests	293	127	148	18

Besides TPP, aphids and beneficial insects (primarily lacewings, hoverflies, and ladybirds) were also counted on the YST. Aphids were present throughout the trapping season, but more abundant at the start of the season until approximately mid-December (Figure 4), with a slight increase in March. Across all growers, the number of aphids caught was similar for all traps (Figure 5). Beneficial insects were present during the trapping season, with lower numbers for January (Figure 4). Across all growers, the number of beneficial insects caught was similar for

all traps (Figure 6). Patterns differed per grower, probably depending on crop management, crop location, alternative host plants and surrounding crops, and local weather (Appendix 4).

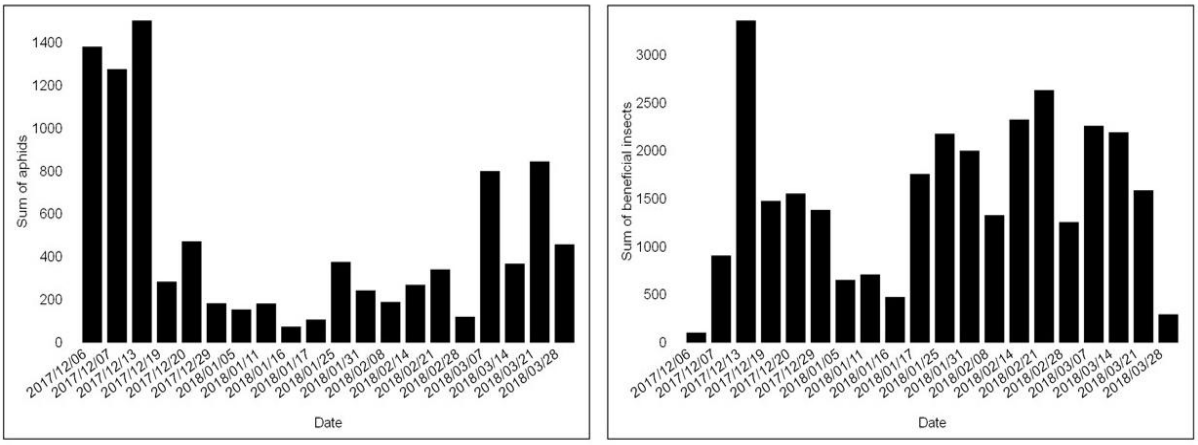


Figure 4. Sum of aphids (left) and beneficial insects (right) caught on four yellow sticky traps in 10 crops in Canterbury over the trapping season.

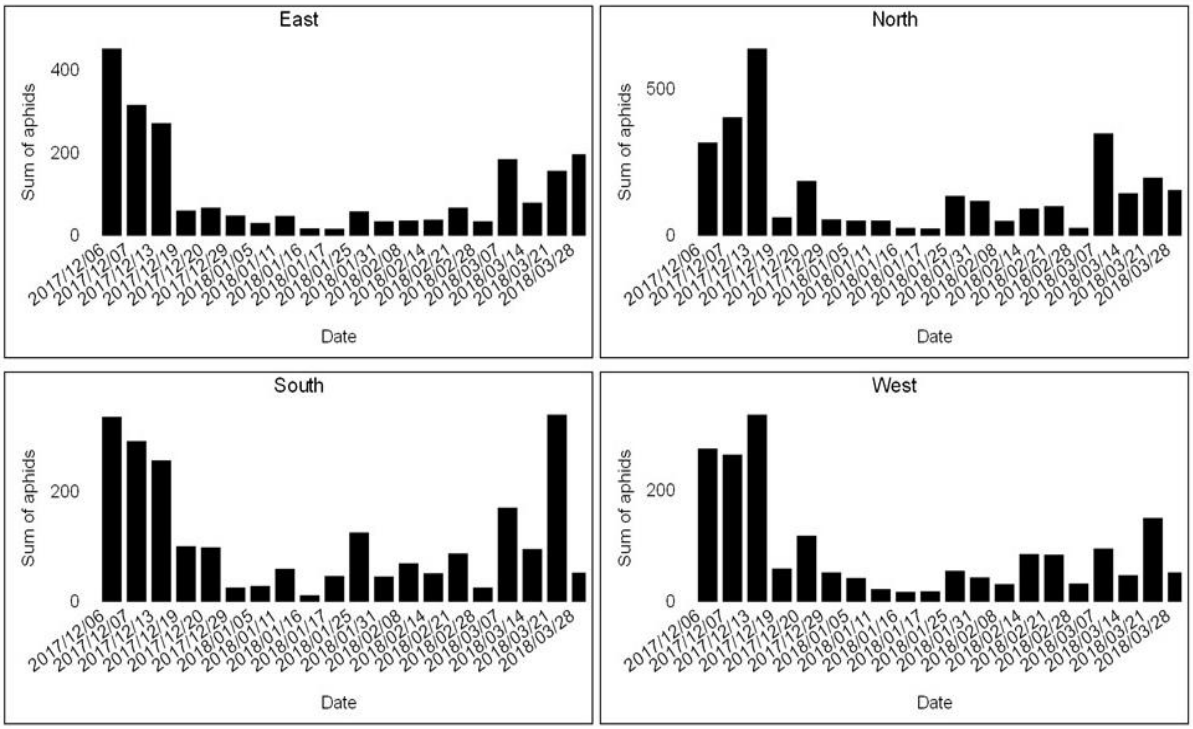


Figure 5. Sum of aphids caught on yellow sticky traps placed on the north, east, south, and west sides of 10 crops in Canterbury over the trapping season.

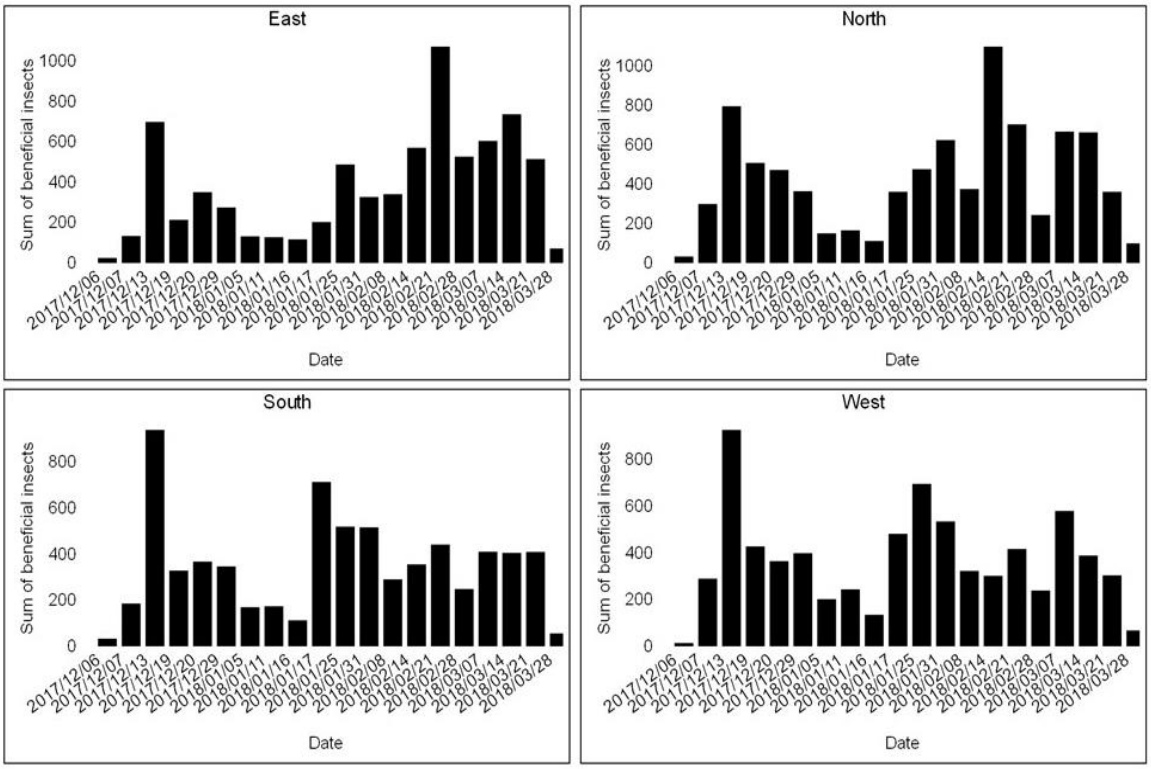


Figure 6. Sum of beneficial insects caught on yellow sticky traps placed on the north, east, south, and west sides of 10 crops in Canterbury over the trapping season.

For Grower 1, the first TPP were trapped on 20 December 2017 and the composite psyllid sample from the south trap tested positive for CLso on that date (Appendix 4). In the following week there was another positive sample, but no more positive TPP were collected from the traps for the remainder of the season. More TPP were caught on the south trap than the other traps, but overall numbers were low. Aphids were mainly trapped early in the season (December) and late March. Beneficial insects were trapped throughout the season, with higher numbers in December. For both aphids and beneficial insects, there were different patterns between the four traps.

For Grower 2 the first TPP was caught on 13 December 2017 (Appendix 4). None of the TPP samples tested over the trapping period were positive for CLso. The east trap caught more TPP than the other traps, but overall numbers were low. The number of aphids caught on the trap was highest in December and late March. The number of beneficial insects caught was highest mid-December.

For Grower 3, seven TPP were caught in the first week of trapping (Appendix 4). None of the TPP samples tested over the trapping period were positive for CLso. The east trap caught more TPP than the other traps. The number of aphids caught on the trap was highest in December and late March. The number of beneficial insects caught was high through December and January.

For Grower 4, the first TPP were caught on 13 December 2017 (Appendix 4). There were two dates TPP samples tested positive for CLso, 29 December 2017 and 31 January 2018, on the west and north trap respectively. The east and west traps trapped the highest number of TPP over the season, with the south trap trapping the least. Most aphids were caught on the first

week of trapping. Beneficial insects trapped showed a different pattern from most other growers, where high numbers were caught regularly throughout the trapping season.

For Grower 5, the first TPP were caught on 13 December 2017 (Appendix 4). None of the TPP samples tested over the trapping period were positive for CLso. The east trap caught most TPP over the trapping season. The number of aphids caught on the traps was highest in December. Most beneficial insects were trapped from mid-February till late March, a different pattern to most other growers.

For Grower 6, the first TPP were caught on 13 December 2017 (Appendix 4). The only TPP sample that tested positive for CLso was collected from the south trap placed on 5 January 2018. The north trap trapped the largest number of TPP over the season. Most aphids were caught on the first week of trapping, with very low numbers compared to other crops for the remainder of the trapping season. Beneficial insects trapped showed a different pattern to most other growers, where high numbers were caught regularly throughout the trapping season.

For Grower 7, the first TPP were caught on 13 December 2017 (Appendix 4). For three weeks in a row, 7, 14 and 31 March, TPP samples tested positive for CLso, all from the east trap. This pattern is different to the other growers, where positive TPP samples were found early in the season. The east, north, and south traps caught most TPP, with marked lower numbers for the west trap. The number of aphids caught on the traps was highest in December. The number of beneficial insects were relatively high throughout the trapping season, with a peak in mid-December.

For Grower 8, the first TPP were caught on 20 December (Appendix 4). TPP samples tested positive for CLso on 5 January and 14 February 2018, on the south and west trap respectively. The east, south and west traps caught most TPP. The number of aphids caught on the traps was highest in December. The numbers of beneficial insects was relatively high throughout the trapping season, with a peak in December and late January.

For Grower 9, the first TPP were caught on 13 December 2017 (Appendix 4). None of the TPP samples tested over the trapping period were positive for CLso, just one of four growers where this was the case. Most TPP were caught on the north trap. Number of aphids caught on the trap was highest in mid-December and March. Late January there was a peak of beneficial insects caught.

For Grower 10, the first TPP were caught on 13 December 2017 (Appendix 4). The only TPP sample that tested positive for CLso was caught on a trap placed 29 December 2017 (south trap). The highest number of TPP were caught on the east and north trap, with the south and west trap catching marked lower numbers. The number of aphids caught on the trap was highest in mid-December. The number of beneficial insects was relatively high throughout the trapping season, with marked lower numbers trapped late December to early January.

3.2 Testing for *Candidatus* Liberibacter solanacearum haplotype A

A total of 13 TPP samples were found to be positive for CLso based on the testing carried out by Hill Laboratories. Ten of these samples were further tested by PFR in order to determine the CLso haplotype. The March samples were not tested. All 10 TPP samples were positive for haplotype A.

4 DISCUSSION

TPP were caught on YSTs in potato crops at all 10 growers' sites. Trap catches are influenced by canopy density, plant foliage quality, and temperature which determine adults' tendency to fly, and wind can discourage flight, reducing trap catches. Also, trap catches may temporarily drop after a pesticide application. On the other hand, adult numbers may temporarily increase on traps after foliage disturbances, such as irrigation or applying an insecticide. Therefore, a sudden increase in TPP caught in a YST does not always mean that an outbreak will occur. Farmers will have to use data from traps in combination with other observations to make crop management decisions. Also, low numbers on traps do not necessarily mean low numbers of TPP in the crop. Traps are catching only the adults, not the juvenile, non-flying, stages of TPP. YST monitoring should therefore always be followed by a visual inspection of the crop.

Directionality (north, east, south, and west) of trapped numbers differed among growers for first TPP caught and highest number of TPP caught. The direction associated with a trap's placement is not explicitly indicative of the direction from which insects are entering a crop. Both the front and back side of a YST provide catch data; total catch from one trap is summed from both sides of a trap. Insects on the outward-facing side of a trap may be more likely to have come from outside the crop, but also insects from within the crop could be present. The inward-facing (towards the crop) side of the trap presents itself to insects within the crop and those flying over from alternative directions. Host plants bordering a crop may also further confound the estimation of movement directionality with traps. Insects could build populations on host plants and inflate trap catch data as they enter a crop from whichever direction is bordered by the host plant.

Six growers had TPP samples that tested positive for CLso. This does not mean that there were no CLso-positive TPP in the potato crops of the growers without positive samples, as the four sticky traps sample only a small area of the crop.

The majority of CLso-positive samples were found quite early in the growing season (December–January), except for Grower 7 (three positive samples in March) and Grower 8 (a mid-February sample). This is in contrast to the small study in Pukekohe (Frampton et al. 2017), where most CLso-positive TPP were found from February to early March 2017, with only two CLso-positive TPP found late December 2016. It must be noted however that in the Pukekohe study, all TPP trapped on a YST were assessed individually for CLso, whereas in this study, individual TPP or composite samples of up to 10 TPP were tested.

The accuracy of composite testing has not been assessed and as colony TPP were used for the initial assessment of the method, there is a risk of false negative results. The colony TPP reared by PFR have a high titre of CLso (and hence a low Ct when tested used qPCR). Field-collected TPP, from crops and other host plants, generally have much lower titres of CLso, leading to higher Ct values. Combining multiple TPP from YST may dilute the CLso DNA and further increase the Ct, potentially leading to a false negative result. Essentially, it is plausible that one or more field-collected CLso-positive TPP adults could elude detection in a composite test.

There were 41 ‘undetermined’ CLso tests (14% of total) from Hill Laboratories. The TPP ITS test for these samples was negative. This could be caused by the DNA extraction not working or by misidentification of the insects on the YST. This issue has not been observed with the Pukekohe testing also carried out this season by Hill Laboratories. It was an issue for the Pukekohe tests in the 2016–17 season but was resolved after further training in TPP identification (Frampton et al. 2017). It is likely that insect misidentification was the cause of these test results.

The missing data listed in Table 2 indicate some problems with information flow between the different parties involved in the YST counting, TPP floating, and CLso testing. The TPP counts and CLso test data from week 1 do not align but this issue appears to have been resolved in subsequent weeks. This level of inaccuracy could impact the quality of the data and steps should be taken to improve these systems in the future.

5 REFERENCES

Barnes AM, Taylor NM, Vereijssen J 2015. Non-crop host plants: prime real estate for the tomato potato psyllid in New Zealand? New Zealand Plant Protection 68: 441.

Beard S, Pitman A, Kraberger S, Scott I 2012. SYBR Green real-time quantitative PCR for the specific detection and quantification of '*Candidatus* Liberibacter solanacearum' in field samples from New Zealand. European Journal of Plant Pathology 136: 203-215.

Frampton R, Kalamorz F, Drayton G, Sachtleben T, Scott I, Dohmen-Vereijssen J 2017. HortiCentre yellow sticky trap testing. A Plant & Food Research report prepared for FAR and ProGrow. Milestone No. 70935. Contract No. 33817. SPTS No. 14687.

Haapalainen M, Wang J, Latvala S, Lehtonen MT, Pirhonen M, Nissinen AI 2018. Genetic variation of '*Candidatus* Liberibacter solanacearum' haplotype C and identification of a novel haplotype from *Trioza urticae* and stinging nettle. Phytopathology in press.

Horton DR, Munyaneza JE, Swisher KD, Echegaray E, Murphy AF, Rondon SI, Sengoda VG, Neven LG, Jensen AS 2014. What is the source of potato psyllids colonizing Washington, Oregon, and Idaho potato fields? Potato Progress 14: 1-6.

Jensen AS, Rondon SI, Murphy AF, Echegaray E 2012. Overwintering of the potato psyllid in the Northwest on *Solanum dulcamara*. In: Workneh F, Rashed A, Rush CM eds. Proceedings of the 12th annual zebra chip reporting session, 30 October - 2 November 2012, San Antonio, TX, USA. Pp. 63-64.

Mendoza-Herrera A, Levy J, Harrison K, Yao J, Ibanez F, Tamborindeguy C, 2018. Infection by '*Candidatus* Liberibacter solanacearum' haplotypes A and B in *Solanum lycopersicum* 'Moneymaker'. Plant Disease in press.

Murphy AF, Rondon SI, Jensen AS 2013. First report of potato psyllids, *Bactericera cockerelli*, overwintering in the Pacific Northwest. American Journal of Potato Research 90: 294-296.

Nelson, WR, Fisher, TJ, Munyaneza, JE 2011. Haplotypes of "*Candidatus* Liberibacter solanacearum" suggest long-standing separation. European Journal of Plant Pathology 130:5-12.

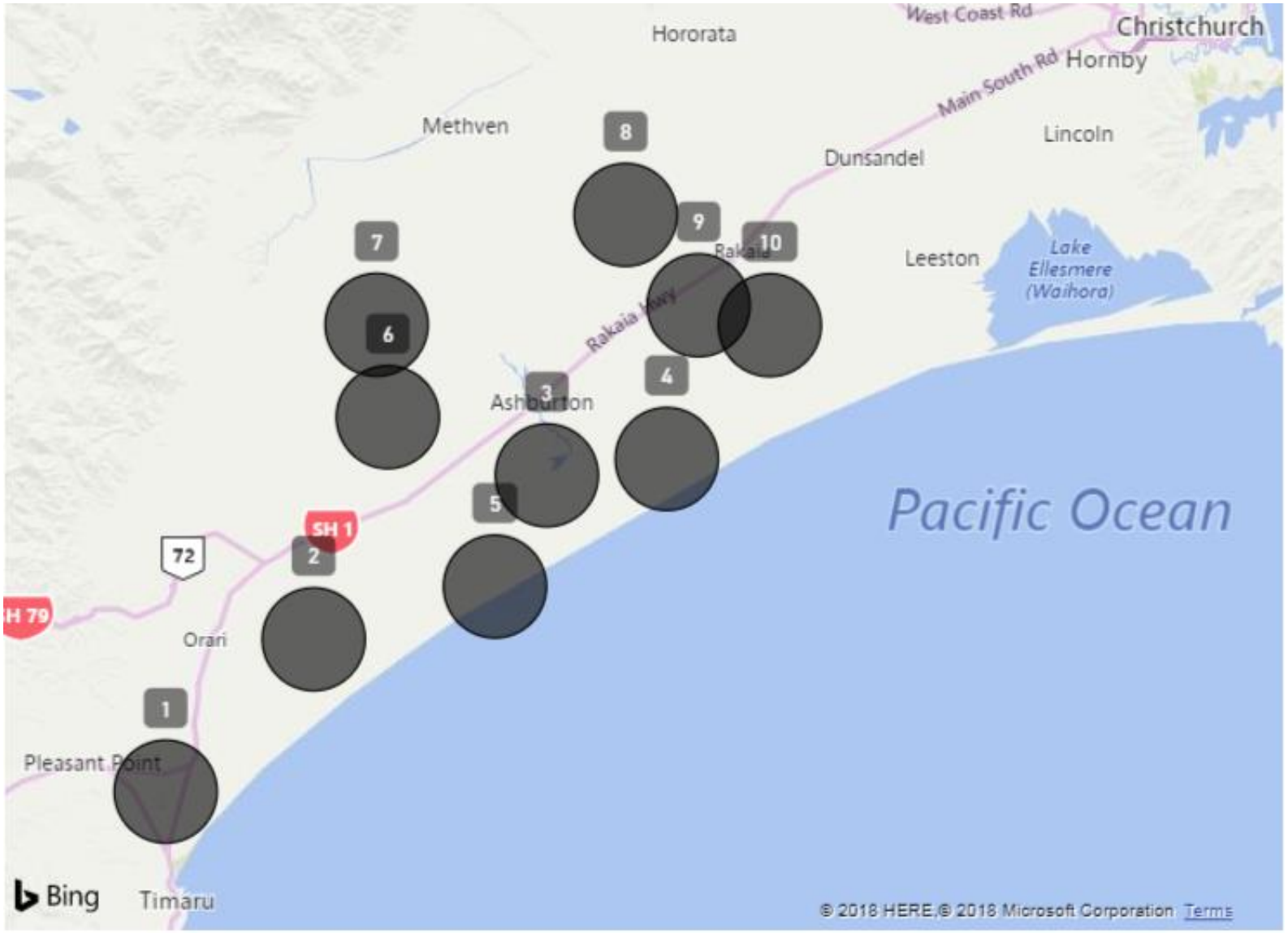
Nelson, WR, Sengoda, VG, Alfaro-Fernandez, AO, Font, MI, Crosslin, JM, Munyaneza, JE 2013. A new haplotype of "*Candidatus* Liberibacter solanacearum" identified in the Mediterranean region. European Journal of Plant Pathology 135:633-639.Teresani, GR, Bertolini, E, Alfaro-Fernandez, A, Martinez, C, Tanaka, FAO, Kitajima, EW, Rosello, M, Sanjuan, S, Ferrandiz, JC, Lopez, MM, Cambra, M, Font, MI 2014. Association of '*Candidatus* Liberibacter solanacearum' with a vegetative disorder of celery in Spain and development of a real-time PCR method for its detection. Phytopathology 104:804-811.

Vereijssen J, Agnew NM, Barnes A-M, Butler RC, Finlay KJ, Logan DP, Powell K, Puketapu AJ, Sandanayaka M, Thompson S, Valenzuela I, Yen AL 2016. Understanding the role of alternative host plants in tomato potato psyllid and Liberibacter life cycle and ecology. A Plant & Food report prepared for Plant Biosecurity Cooperative Research Centre. SPTS No. SPTS 13841.

Vereijssen J, Taylor NM, Barnes AM, Thompson SE, Logan DP, Butler RC, Yen AL, Finlay KJ 2015. First report of '*Candidatus* Liberibacter solanacearum' in Jerusalem cherry (*Solanum pseudocapsicum*) and thorn-apple (*Datura stramonium*) in New Zealand. New Disease Reports 32.

Vereijssen J, Scott IAW 2013. Psyllid can overwinter on non-crop host plants. NZGrower 68: 14–15.

APPENDIX 1. LOCATIONS OF POTATO CROPS THAT WERE MONITORED FOR TOMATO POTATO PSYLLID USING YELLOW STICKY TRAPS



APPENDIX 2. PROTOCOL FOR SELECTING TOMATO POTATO PSYLLIDS ON A TRAP

FOUNDATION FOR ARABLE RESEARCH
EXPERIMENT PROTOCOL

Summarising TPP trapping data and CLso Assay results to produce weekly updates for growers

Objectives:

The aim of the project is to provide growers weekly updates on TPP numbers and if psyllids are CLso positive ('hot')

Project plan:

Traps and Data Collection

10 fields throughout Canterbury currently being monitored with yellow sticky traps for the presence of TPP (tomato potato psyllid) will be selected to monitor in this project.

4 sticky traps per site will have every psyllid tested as composite samples of 10

TPP monitoring data from Fruitfed Supplies (number of TPP, number of native psyllids, number of beneficial insects) will be sent to PFR

Fruitfed will also extract TPP composite samples from 4 sticky traps at each site and send to Hills labs

qPCR assay data of a composite sample will be tested by Hills Labs and sent to PFR

Sampling will continue at each site until decided by FAR

PFR will amalgamate and summaries the data. Hotspots, trends, unexpected results ect will also be reported

A final report from PFR will conclude the project

TPP data and Extracting Psyllids

Fruitfed staff will weekly gather data on number of TPP, number of native psyllids, number of beneficial insects) as per usual trapping information. This data will then be sent to Jessica Dohmen-Vereijssen (Jessica.Dohmen-Vereijssen@plantandfood.co.nz) on a weekly basis.

Fruitfed staff will be trained in extraction. Protocols on number of psyllids to extract from each trap and where on the trap they will be extracted from will be supplied to the Fruitfed Staff member doing the extraction and trap collection for our sites.

- 10 sites throughout Canterbury
- Sites are current McCain and Talleys monitoring sites
- Map of sites given to both Fruitfed and PFR.

All psyllids will be extracted from each trap (x4) at each site. Each trap will be labelled with a site name and trap direction (eg North, South)

Psyllids will be removed in composite samples of 10 and sent to Hills Labs weekly in microfuge tubes (composite sample of 10 in each tube). All samples being sent will be labelled with the site number, the date, sticky trap location.

If there are more than 10 psyllids from that trap eg 18 then that trap will be sent in two samples (North 1 and North 2)

Sending Samples to Hills

Fruitfed staff will be trained in extraction. Protocols on number of psyllids to extract from each trap and where on the trap they will be extracted from will be supplied to Fruitfed Staff

After extraction samples will be sent to Hills Labs weekly

1 person from Fruitfed will be responsible for traps and sending samples to Hills

A submission form will be filled out and sent with samples (FAR to supply)

Microfuge tubes will be supplied to Fruitfed by FAR

Psyllids will be removed in composite samples of 10 and sent to Hills Labs weekly in microfuge tubes (composite sample of 10 in each tube). All samples will be labelled with the site number, the date, sticky trap ID (eg Fruitfed label 4 sticky traps North, South, East, West).

All psyllids will be sampled in composite samples

Sample numbers is unknown and will be determined by psyllid numbers but this will be at least 4 weekly and increase as numbers increase (40 psyllids per trap will be 4 samples from that trap)

Each site of 4 microfuge tubes (1 per sticky trap) will then be placed in a plastic zip lock bag and labelled again with site number and date

All samples will then be bubble wrapped and couriered to Hills Labs, Hamilton. Address supplied on the submission form.

APPENDIX 3. EXTRACTING ('FLOATING') TOMATO POTATO PSYLLID OFF YELLOW STICKY TRAPS

By Gabby Drayton, Plant & Food Research Lincoln

1) Ensure you have identified the tomato potato psyllid (TPP) on the yellow sticky trap (YST).

2) Put on gloves (new pair of gloves for each YST).

3) Using scissors, carefully cut out sections of YST containing TPP. Leave a 1–3 cm margin between TPP and the cutting point, as you don't want to damage the TPP (cutting too close can result in additional pressure placed on the TPP by the cover slip, i.e. squashing the TPP and losing gut contents which are needed for molecular analysis).
Also, check both sides of the YST to make sure you are not cutting through or too close to any TPP on the other side. If there is a group of TPP close together, cut them out as one – they don't need to be cut out individually. Record the number of TPP cut out. Wipe scissors with 25% bleach solution between YSTs using a new paper towel.

4) Add enough De-Solv-It to the Petri dish (~1 cm depth) to submerge the cut YST sections.

5) Place the cut sections of one YST in a Petri dish with TPP facing down in the De-Solv-It. If you have YST sections with TPP on both sides, try to submerge the entire section. If you can't fit all the cut sections from one YST in one Petri dish then set up a second Petri dish, ensuring all Petri dishes are labelled. Either label the lid of the Petri dish or on a piece of paper tucked under the Petri dish, but avoid using marker pen on the underside of the Petri dish as the De-Solv-It will remove it if any spills.

6) Add Petri dish lid and check after 30 minutes. Most of the coverslips (i.e. Gladwrap/cling film) should have detached from the YST and TPP should be floating at the bottom of the Petri dish. If any cover slips or TPP remain attached, they should come off very easily if you gently move the section of YST through the solution using tweezers (if not, soak in De-Solv-It for longer). Wipe tweezers down with 25% bleach solution between YSTs using a new paper towel.

7) Remove each TPP from a Petri dish carefully with a toothpick (use a new toothpick for each TPP). Place in a collection tube either individually or per five TPP (please check protocol by your provider). Check if the number of TPP in the collection tubes matches the number noted down under bullet point 3.

8) Place all tube/s related to one YST in a small plastic ziplock bag and write YST and collection date on the outside of the bag (please check protocol by your provider). It is also a good idea to include an additional paper copy of the YST and collection information inside the bag.

9) De-Solv-It can be reused. Sieve solution to remove any debris and store in glass bottles. De-Solv-It can be reused 3–4 times, until the soaking time required increases. Petri dishes are used only once.

10) The collection tubes holding TPP can be stored in the fridge (4°C) before being sent for extraction, or before extraction occurs (1–2 days maximum), otherwise they need to be stored in the freezer (-20°C). Ideally the tubes containing TPP are couriered at the end of the day, using an overnight courier. Samples need to be packaged carefully and ideally with a chilly block included.

APPENDIX 4. NUMBER OF TOMATO POTATO PSYLLIDS, APHIDS AND BENEFICIAL INSECTS DURING THE SEASON FOR EACH GROWER

In the following graphs, which are presented per grower (crop), the total number of tomato potato psyllids (TPP) on the four yellow sticky traps (YST) per crop are presented over the duration of the trapping period (black bars). The red coloured bars in these graphs indicate whether one or more of the TPP samples tested for the presence of *Candidatus* Liberibacter solanacearum were positive. In addition, there are also graphs presenting the total number of trapped aphids and beneficial insects.

There are two sections for each grower:

1. Tomato potato psyllid trapping data

Graph A for each grower indicates total number of TPP on the four traps over the growing season.

Graph B presents the total number of TPP on each of the YST (east (E), north (N), south (S), and west (W)) in that crop for the whole growing season.

Graph C is a composite of four graphs and presents the total number of TPP on each of the YST (east (E), north (N), south (S), and west (W)) over the growing season.

2. Aphids and beneficial insects trapping data

Graph A for each grower indicates total number of aphids on the four YST over the growing season.

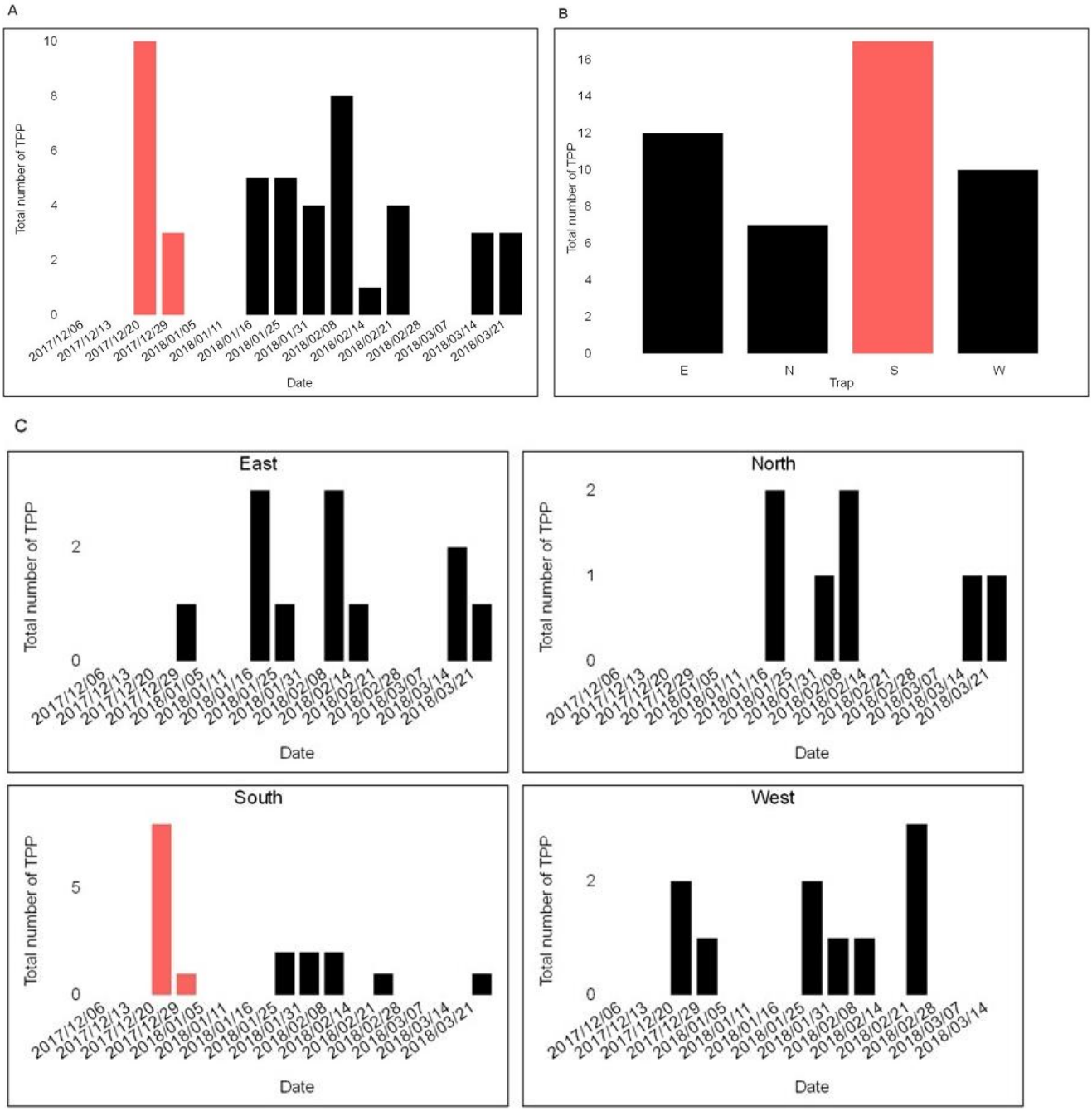
Graph B for each grower indicates total number of beneficial insects on the four YST over the growing season.

Graph C for each grower presents the total number of aphids on each of the YST (east, north, south, and west) over the whole growing season.

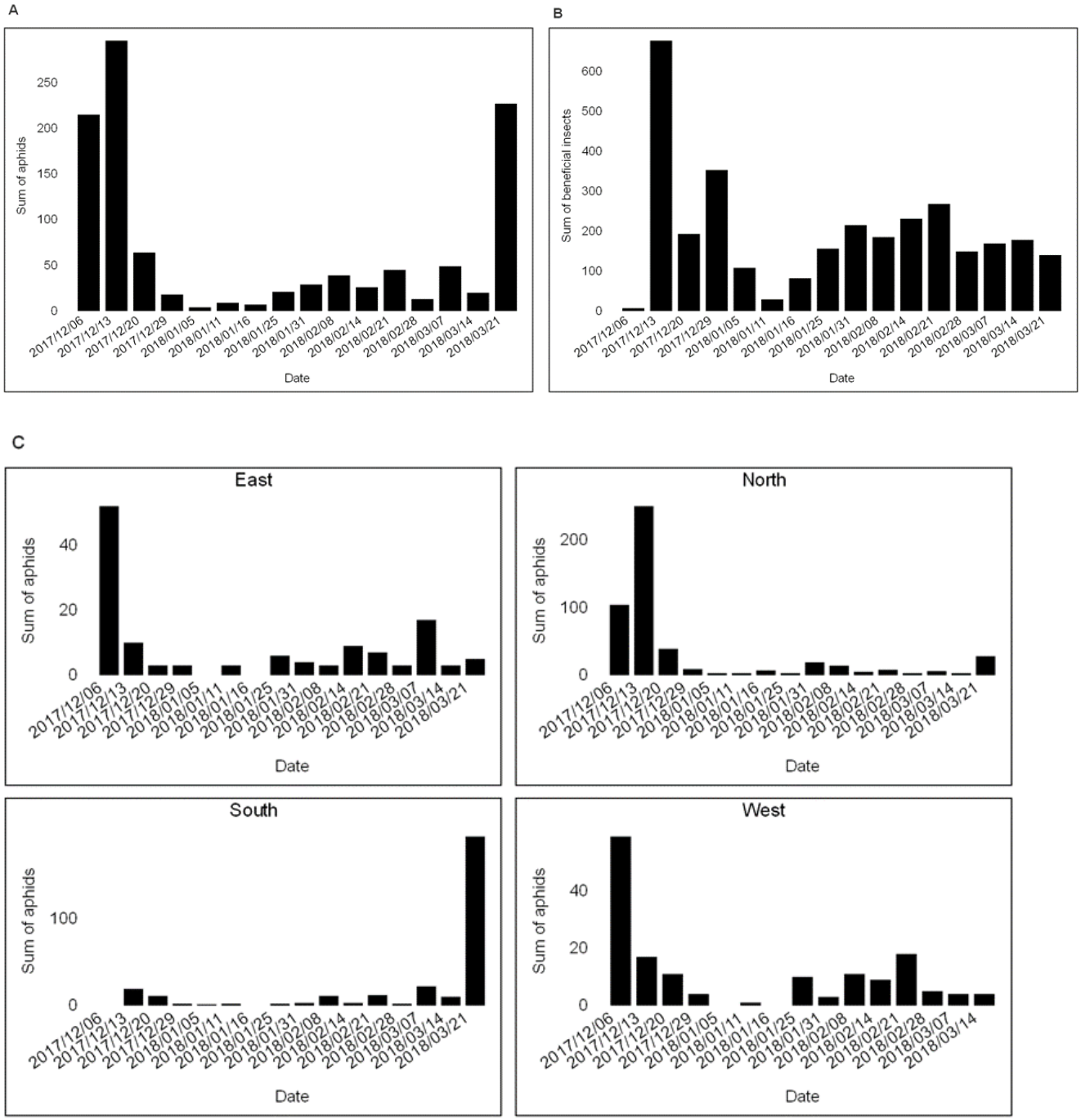
Graph D for each grower presents the total number of beneficial insects on each of the YST (east, north, south, and west) over the whole growing season.

Grower 1

Tomato potato psyllid trapping data



Aphids and beneficial insects trapping data



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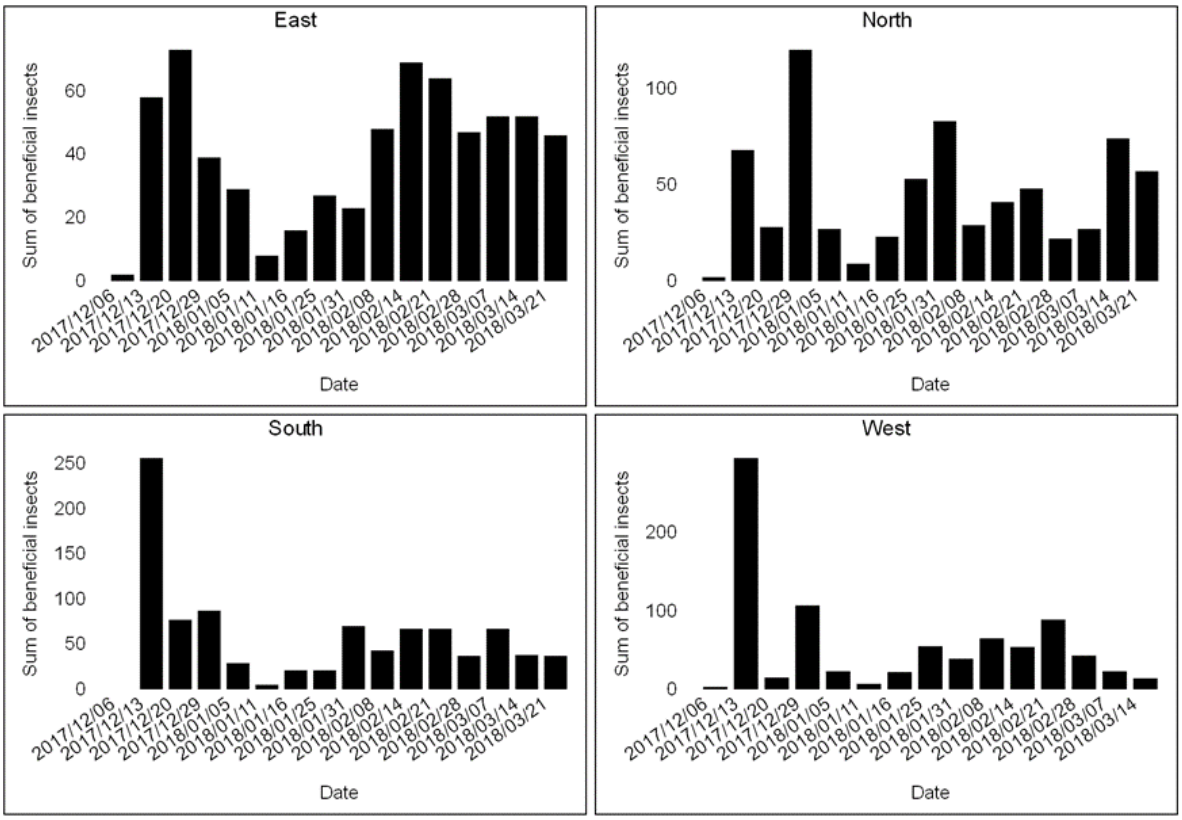
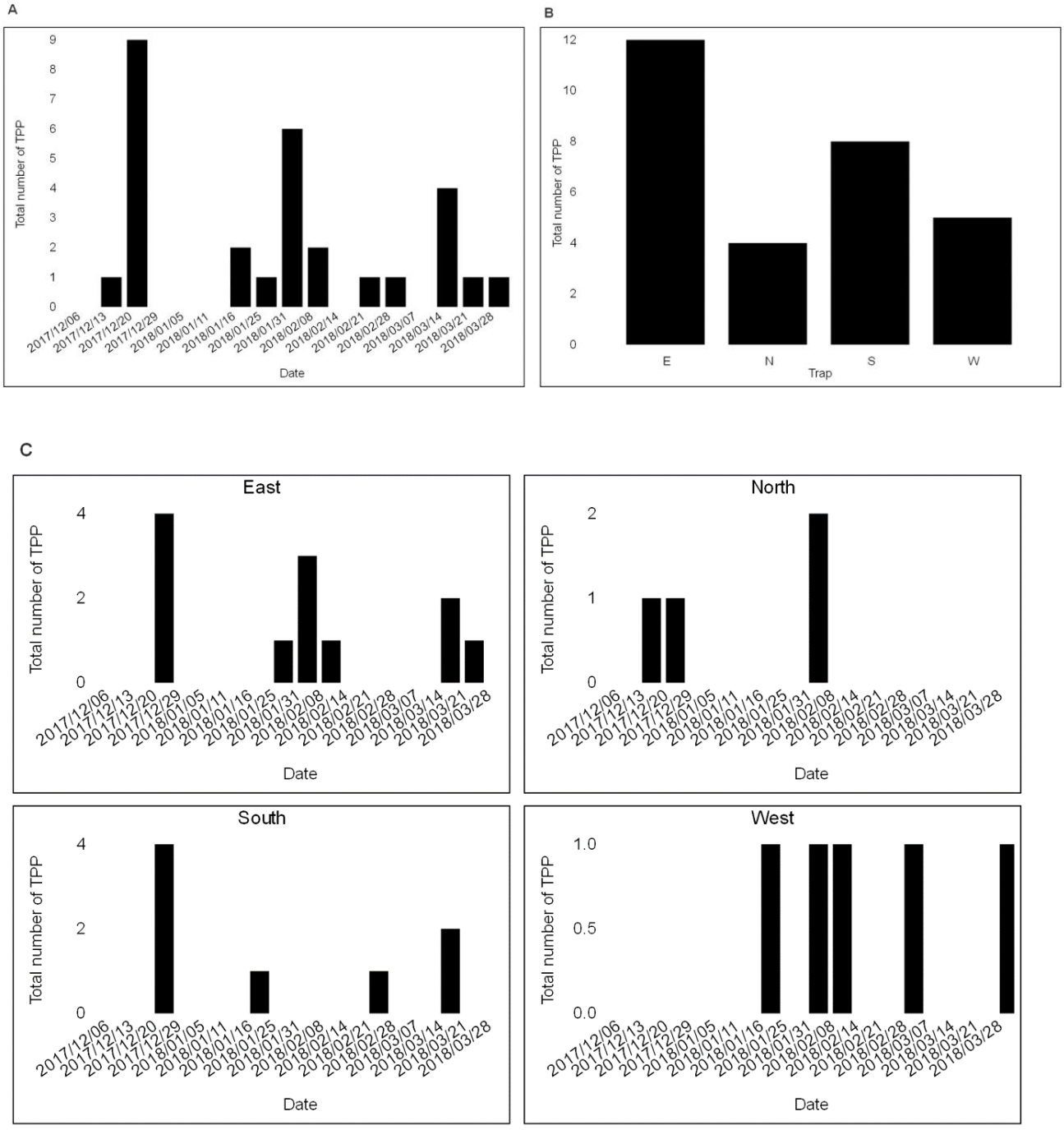
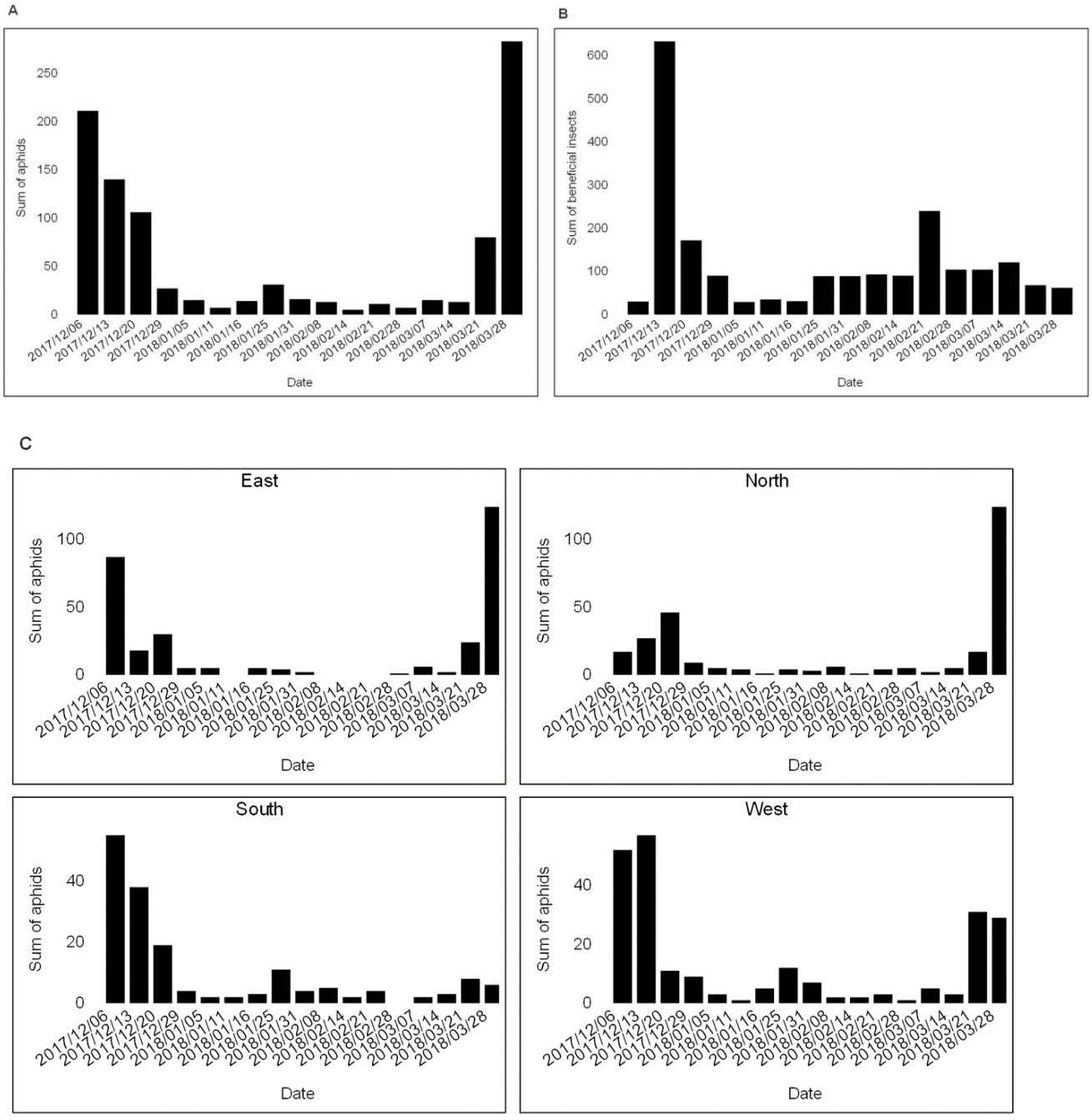


Grower 2

Tomato potato psyllid trapping data



Aphids and beneficial insects trapping data



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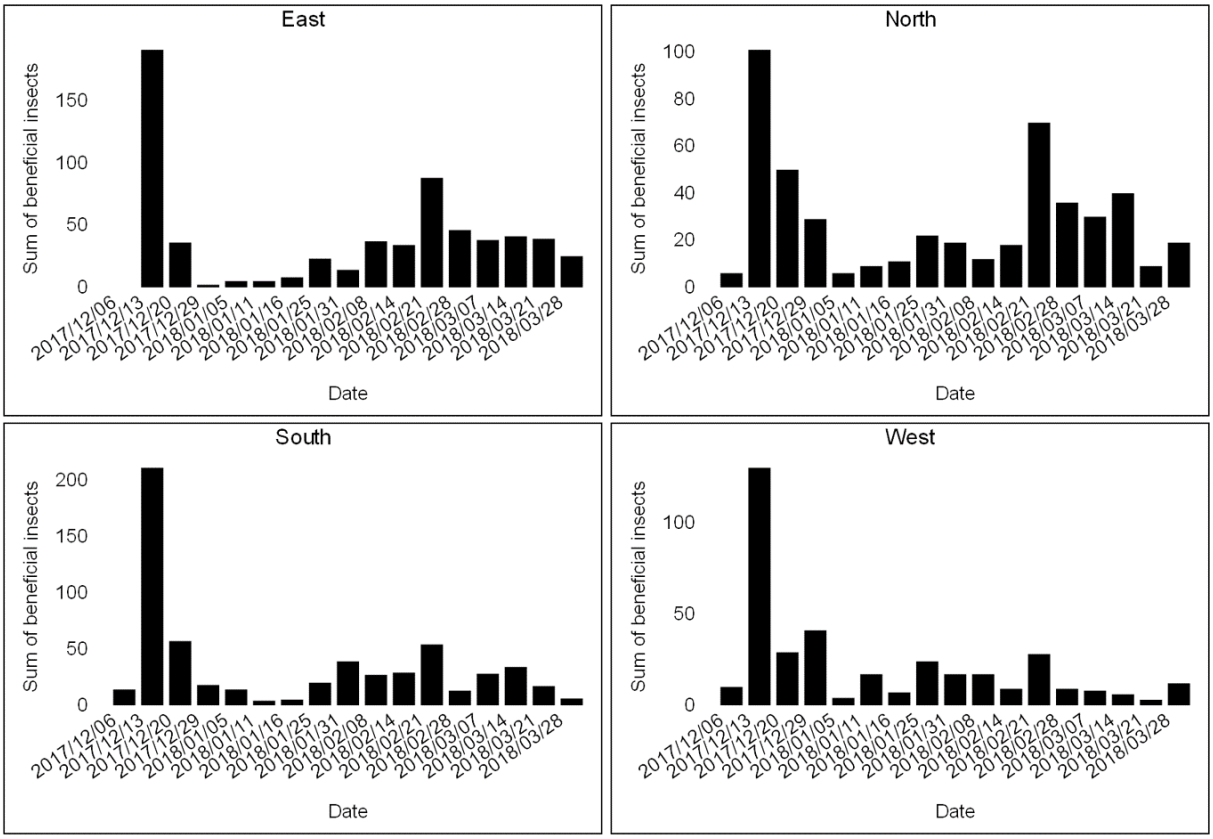
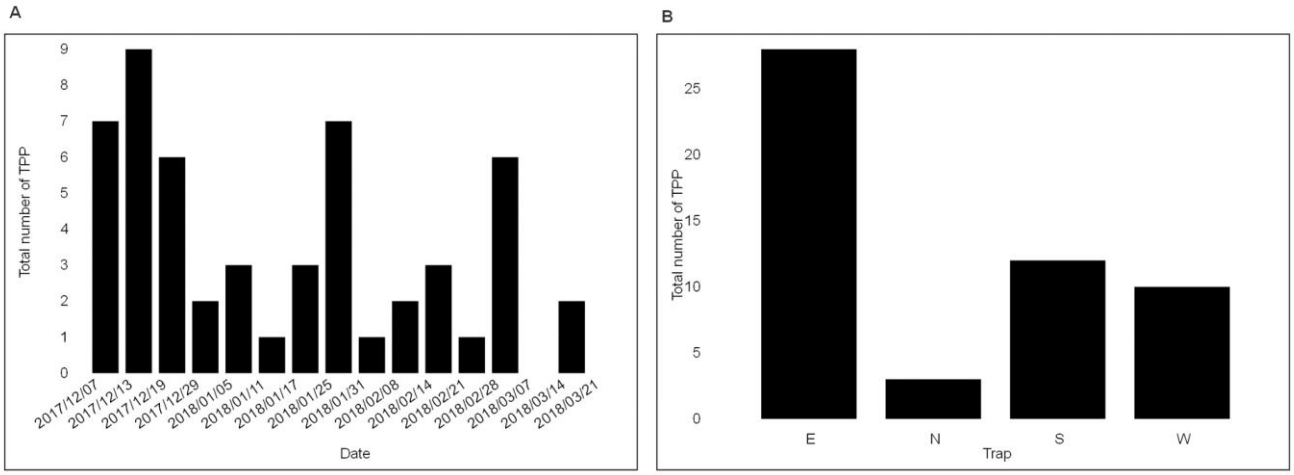
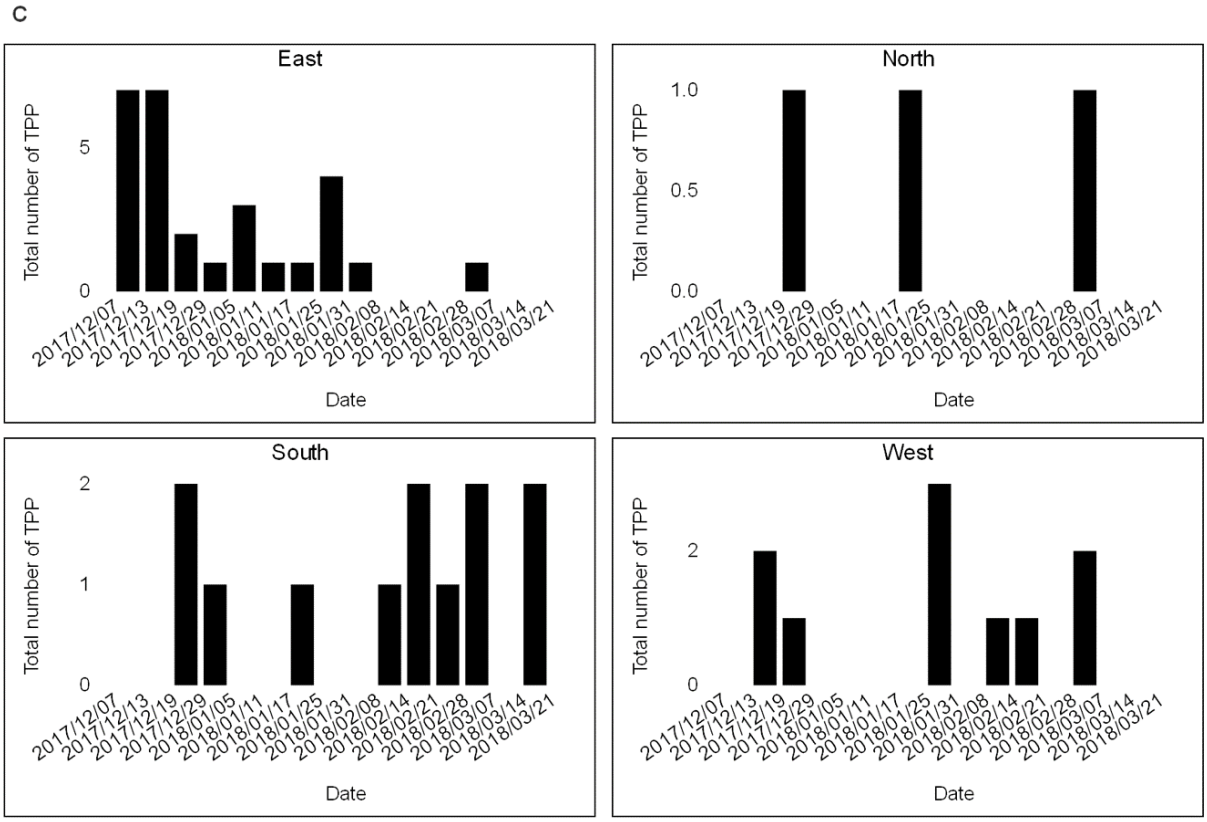


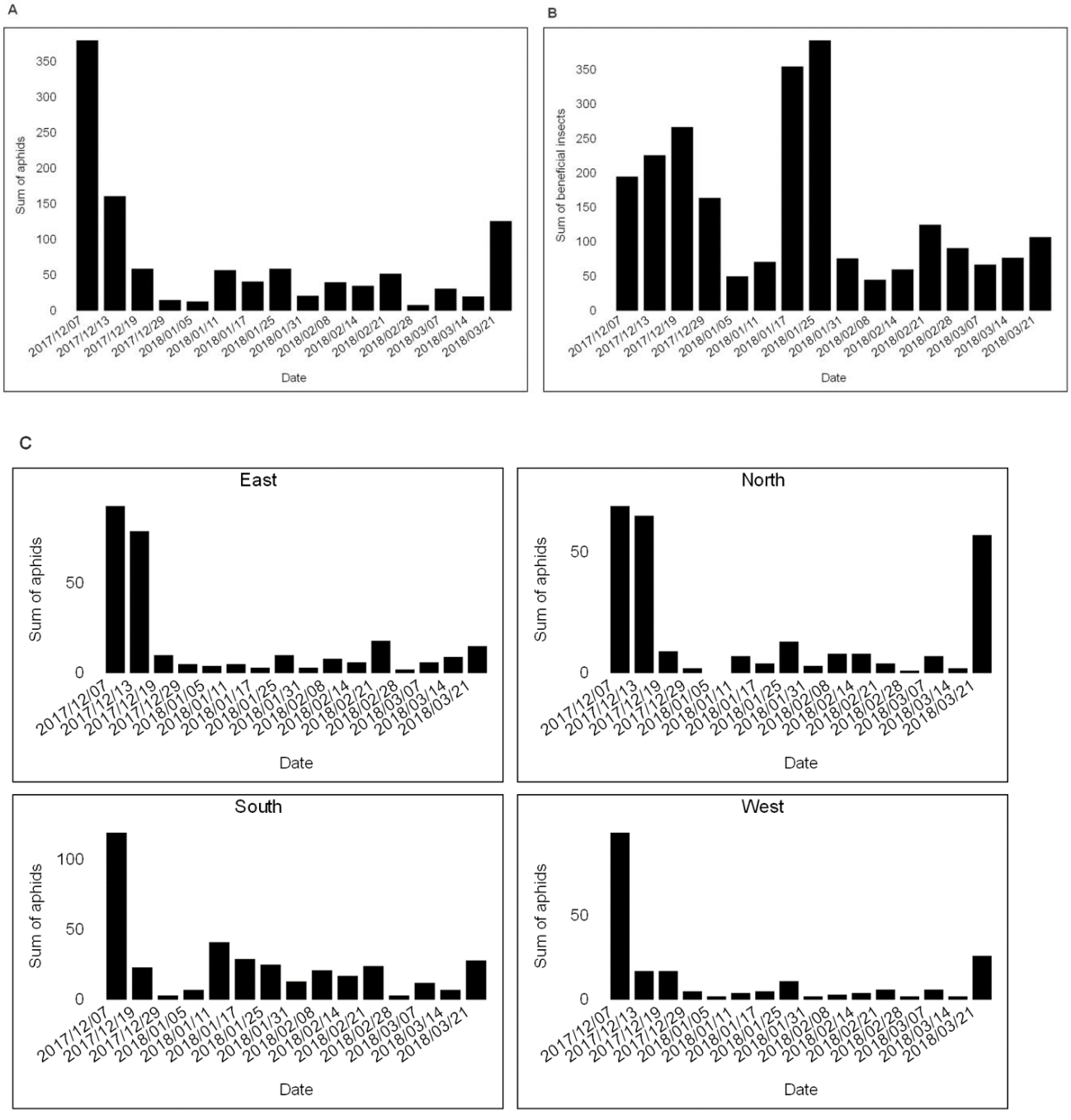
Grower 3

Tomato potato psyllid trapping data





Aphids and beneficial insects trapping data



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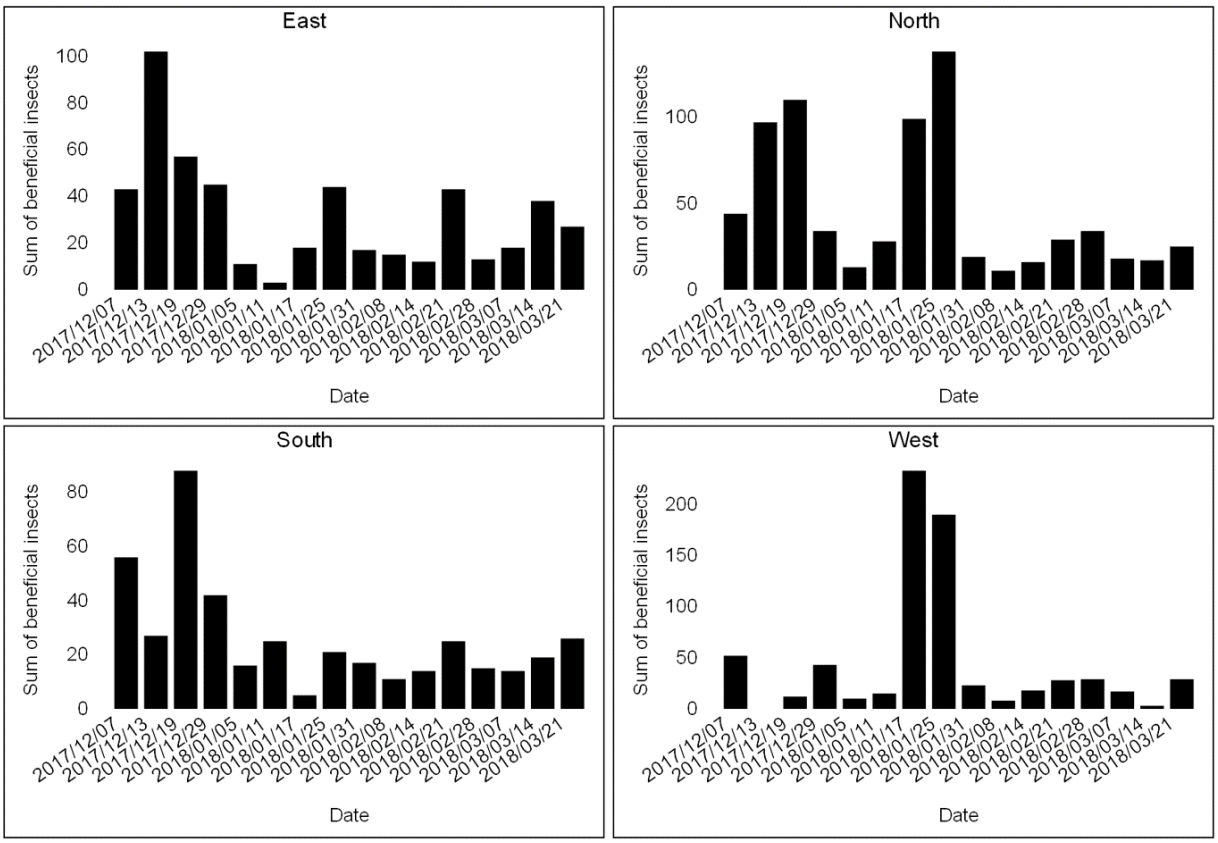
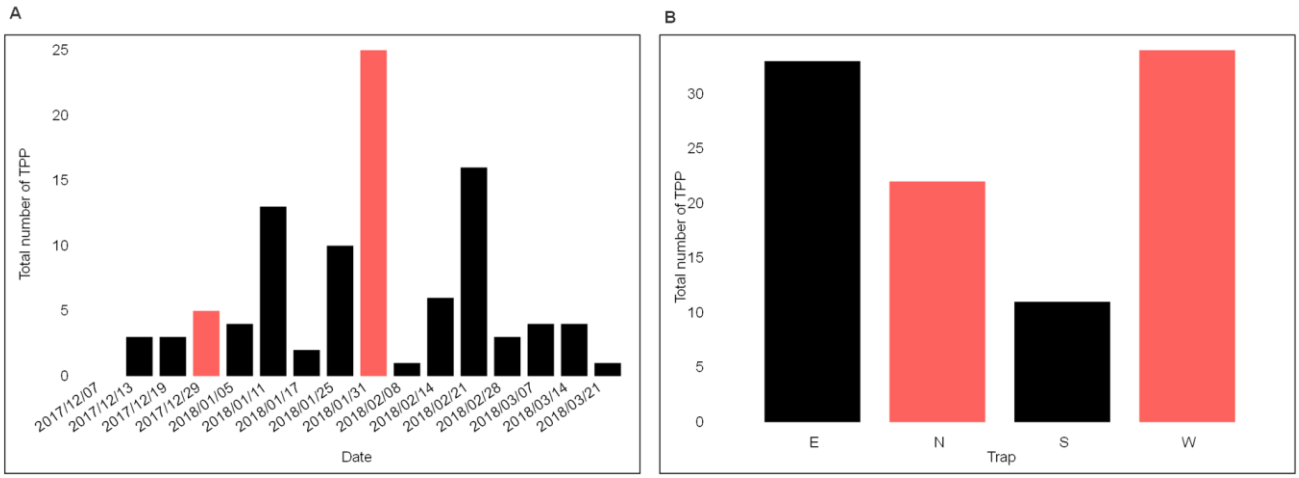
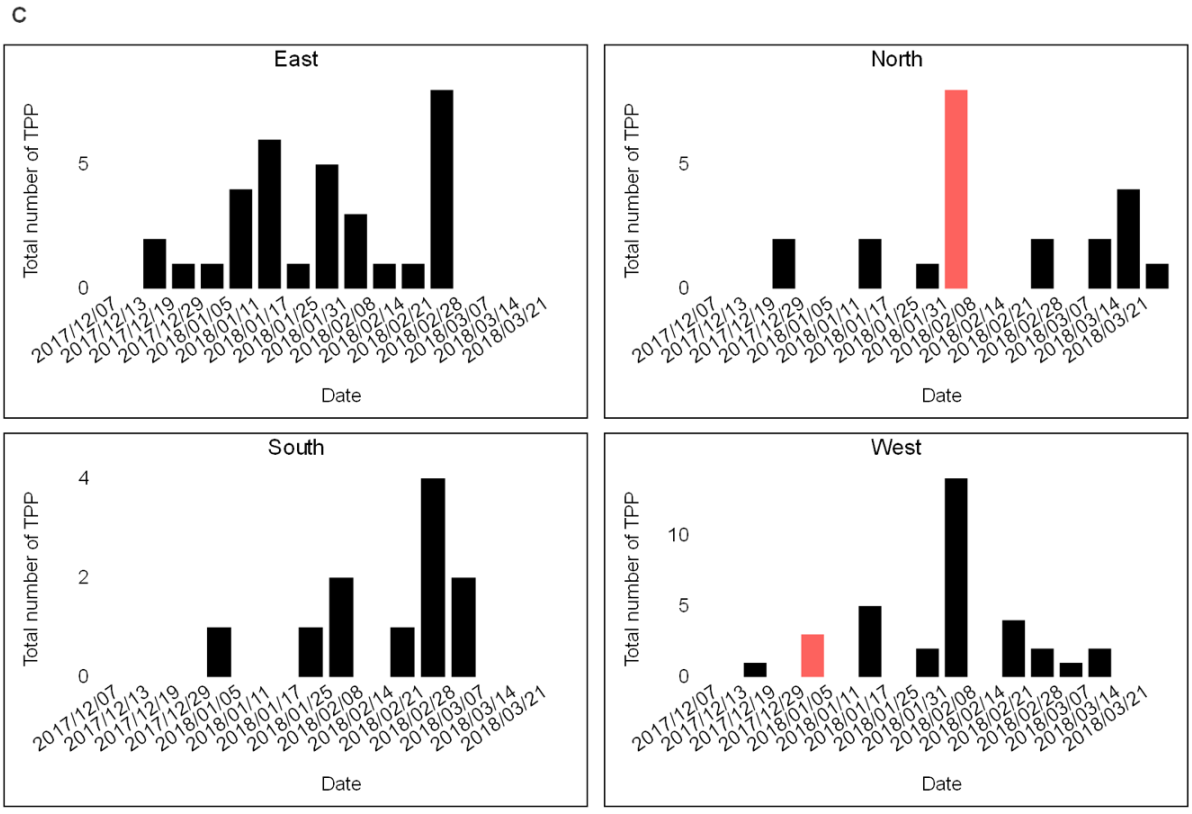


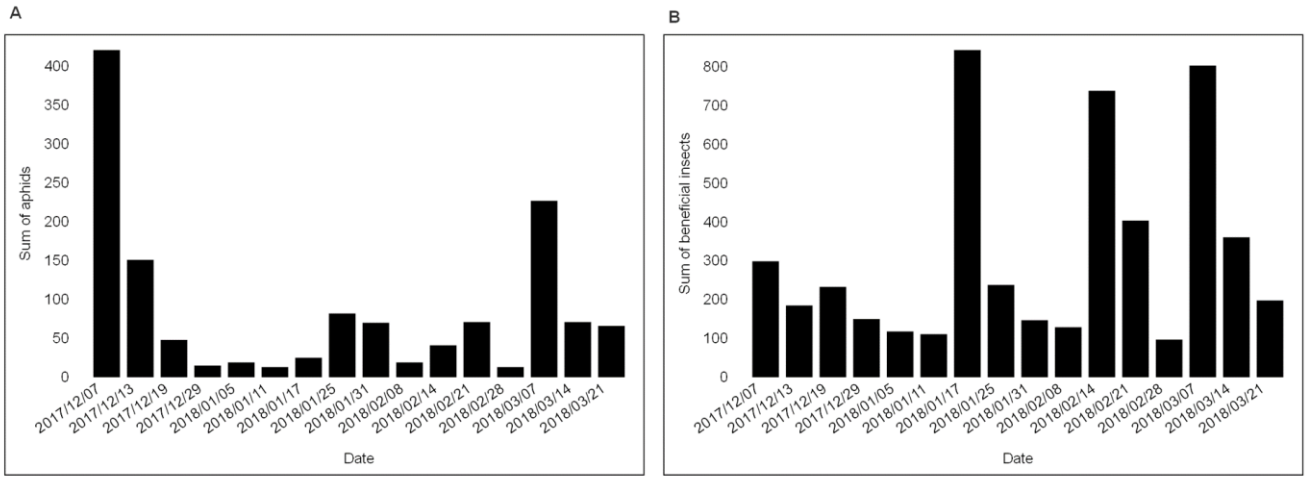
Grower 4

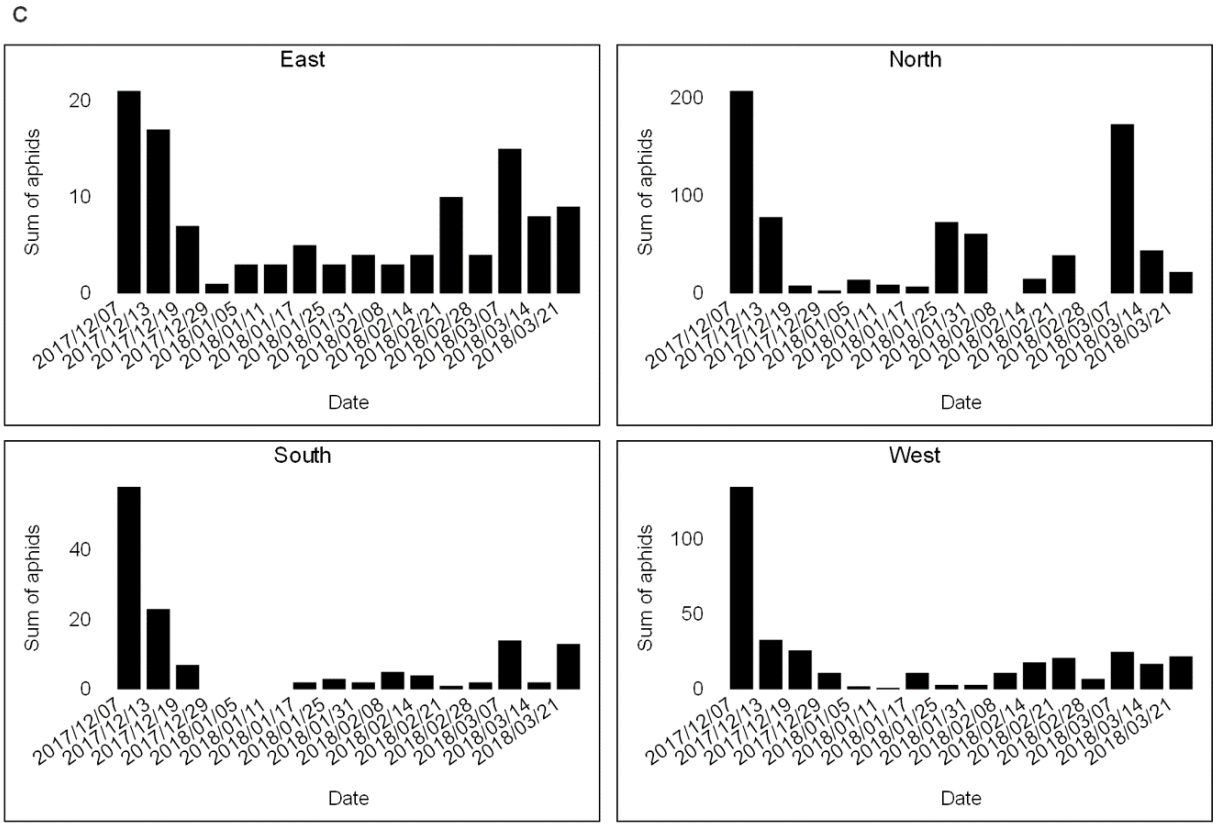
Tomato potato psyllid trapping data





Aphids and beneficial insects trapping data





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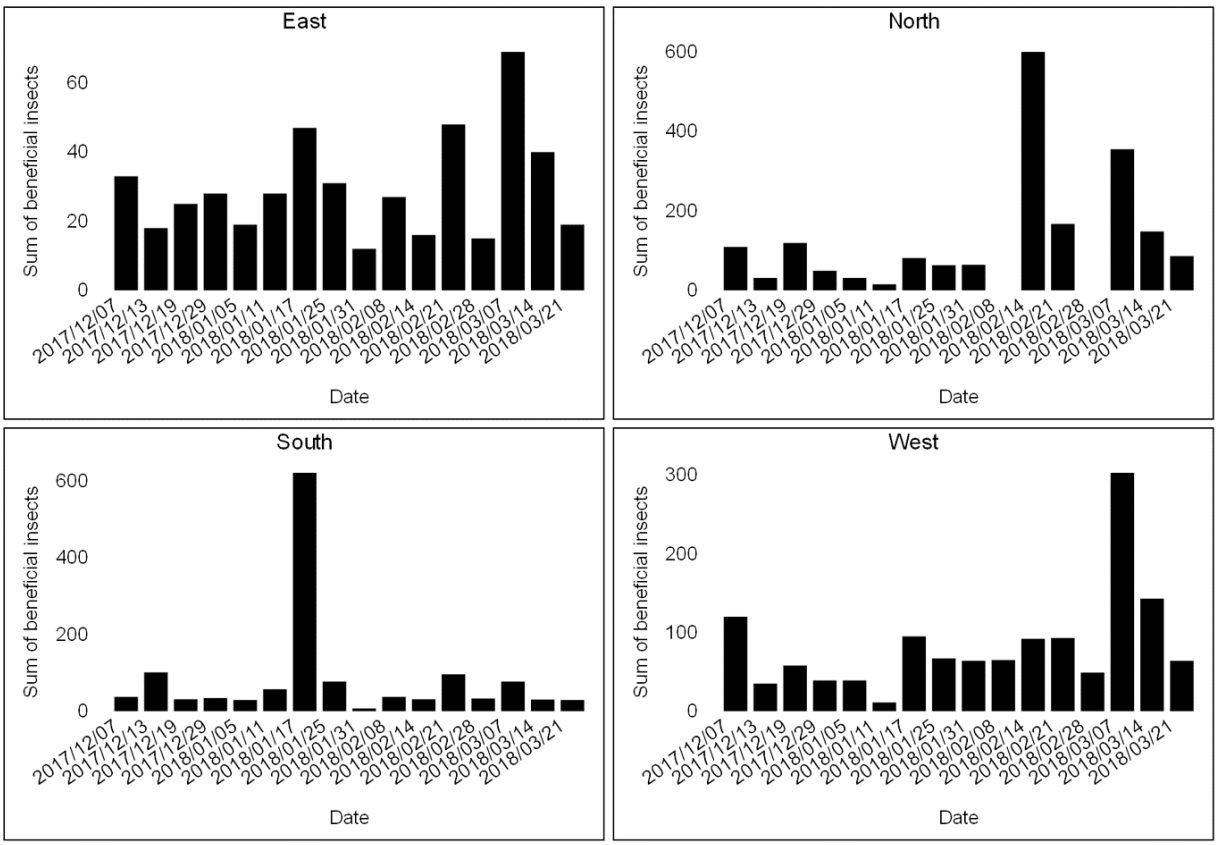
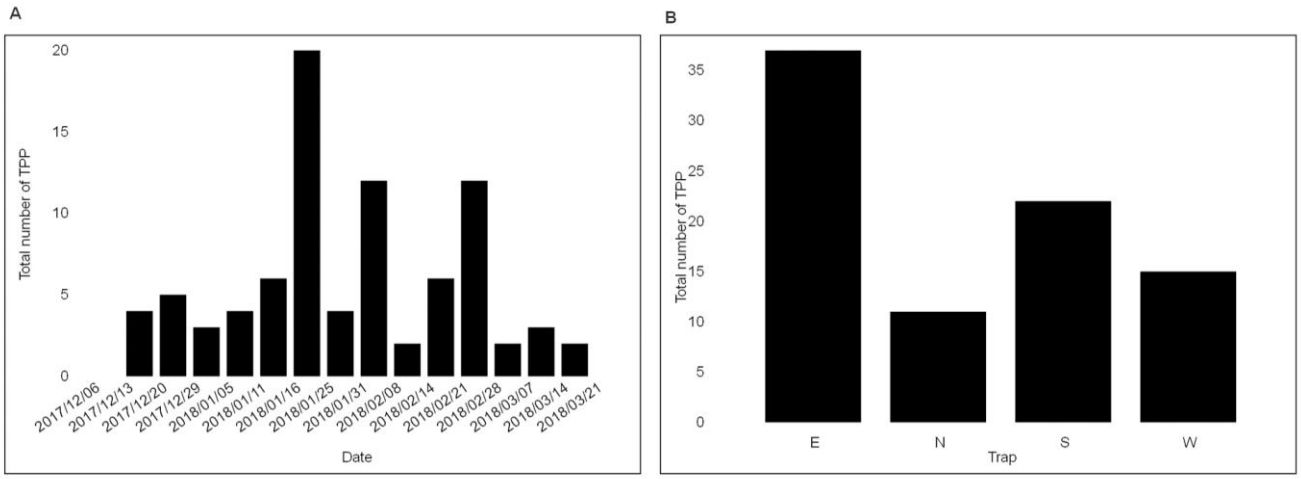
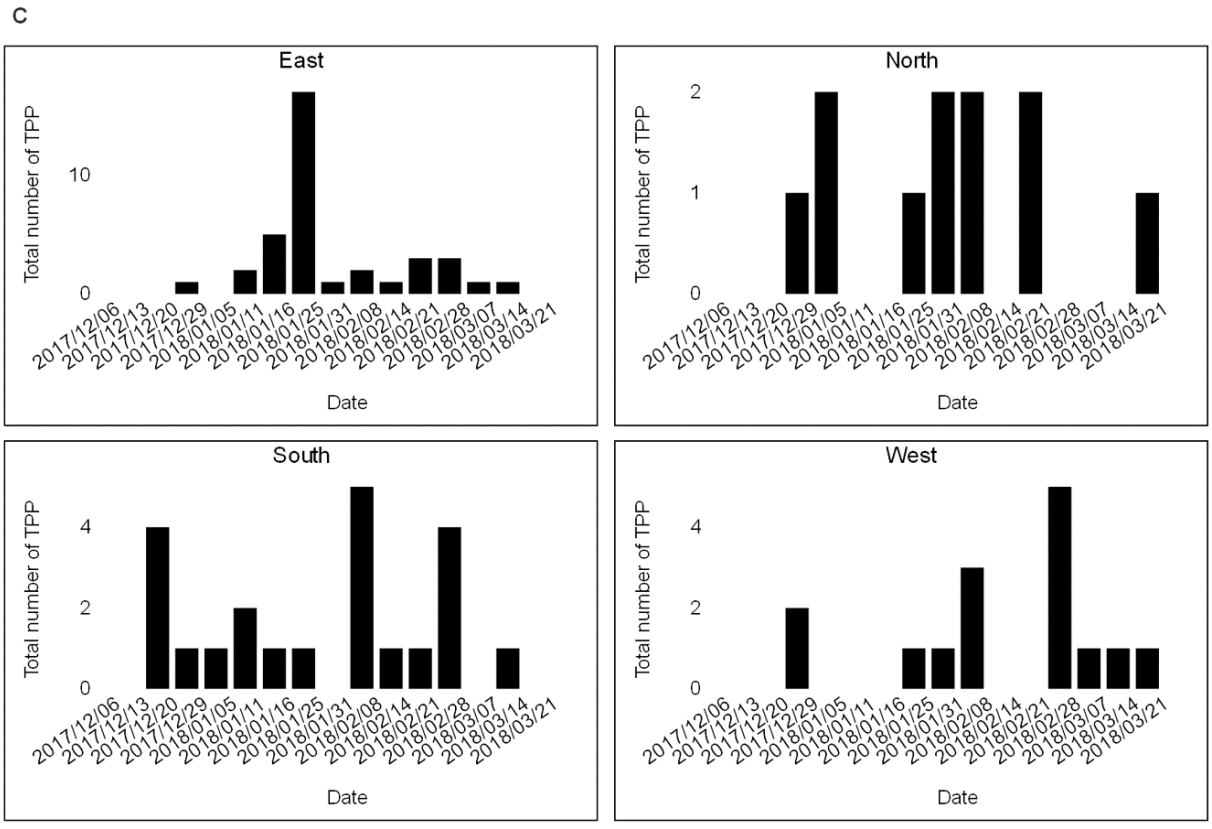


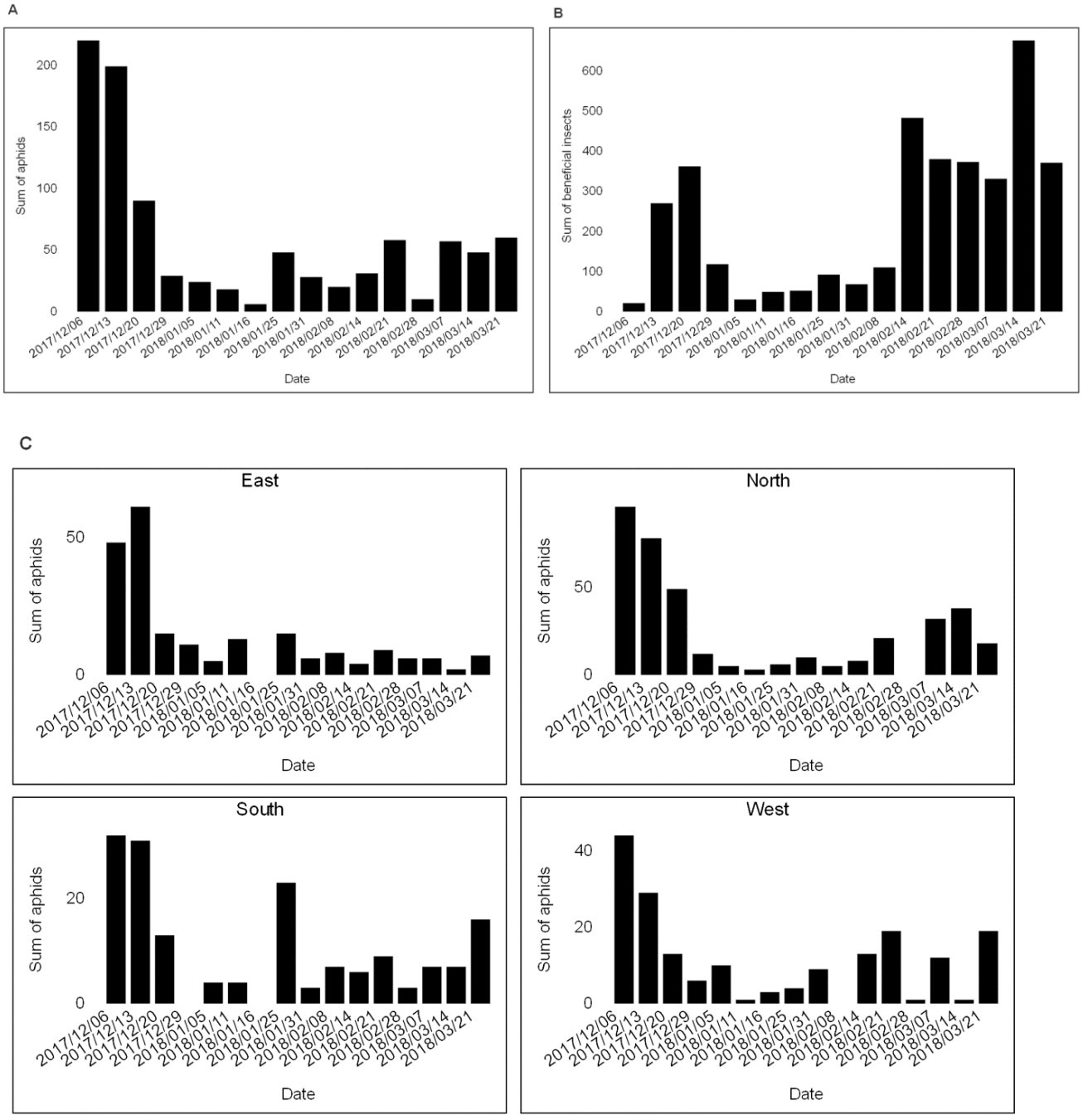
Grower 5

Tomato potato psyllid trapping data





Aphids and beneficial insects trapping data



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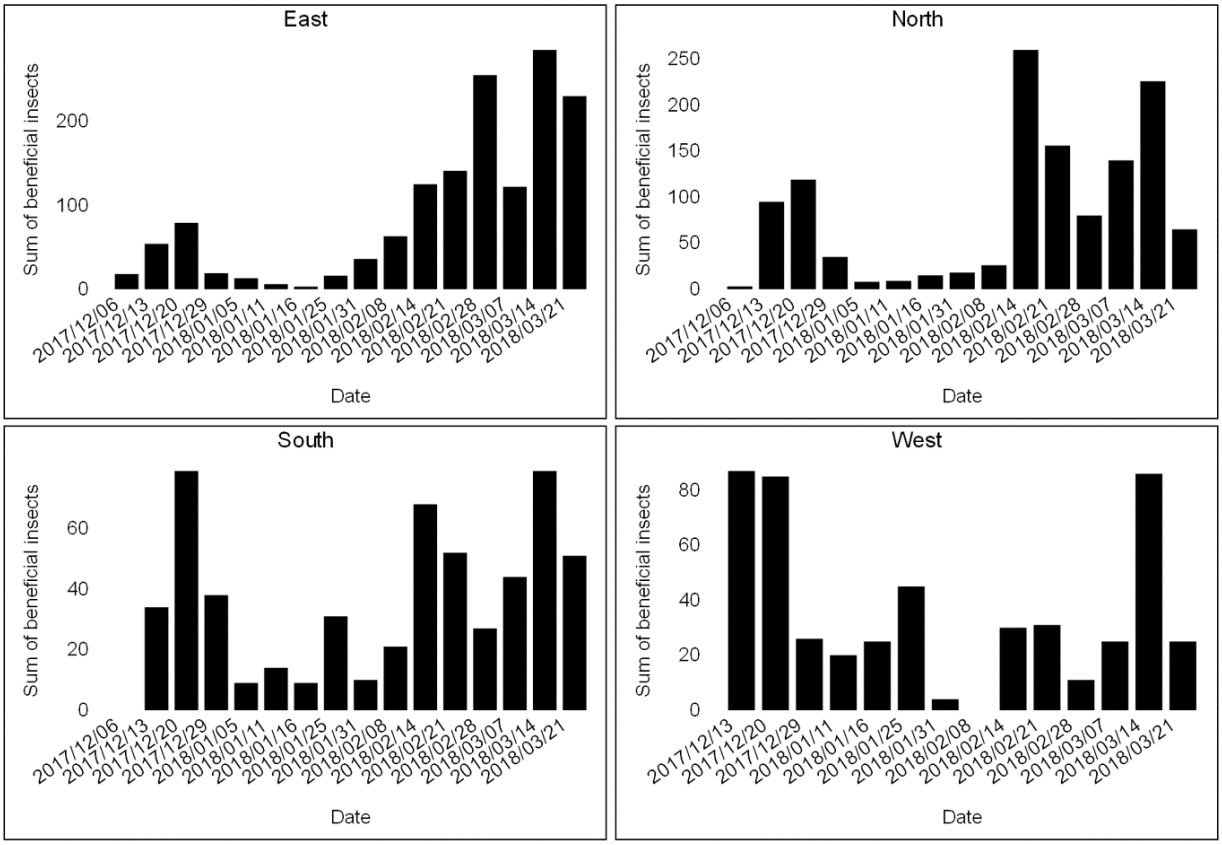

Grower 6

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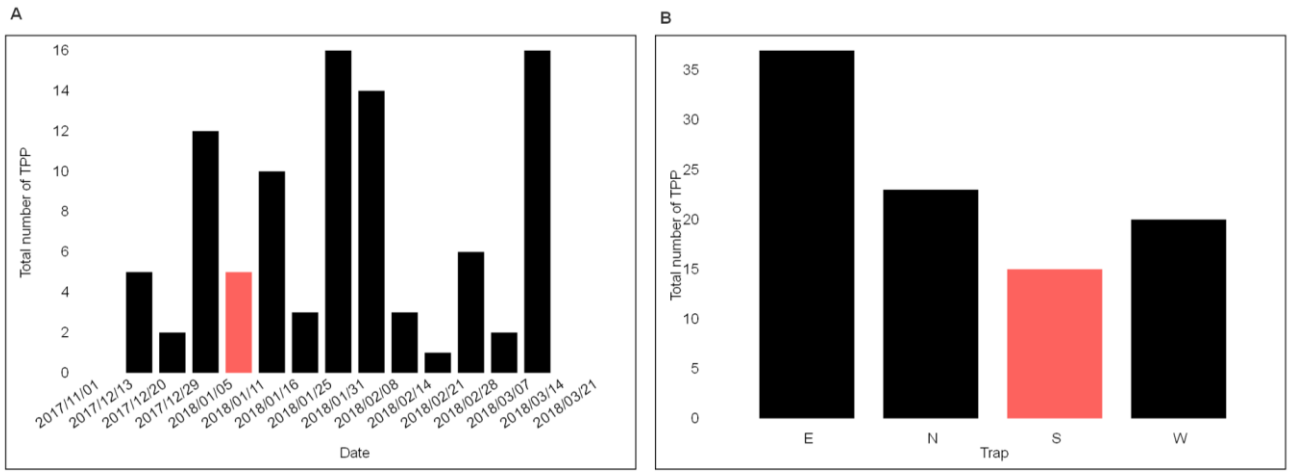
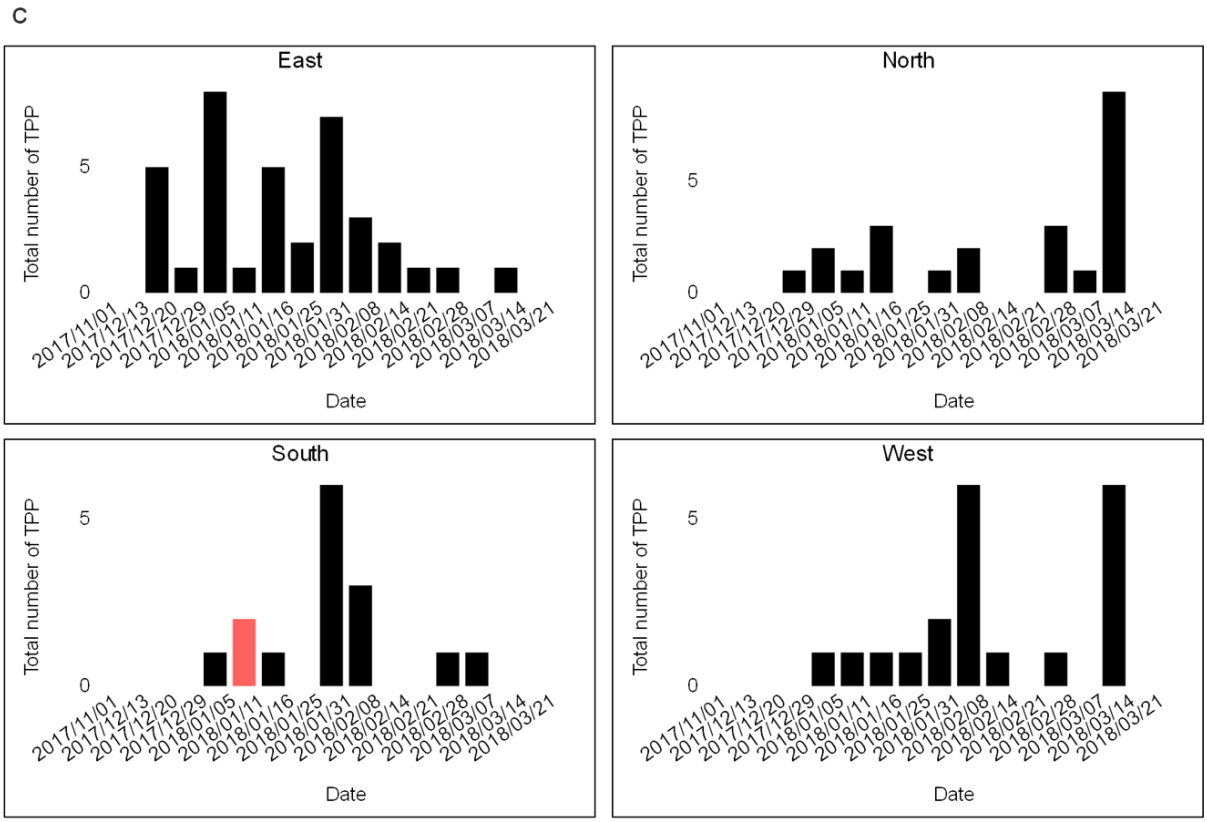
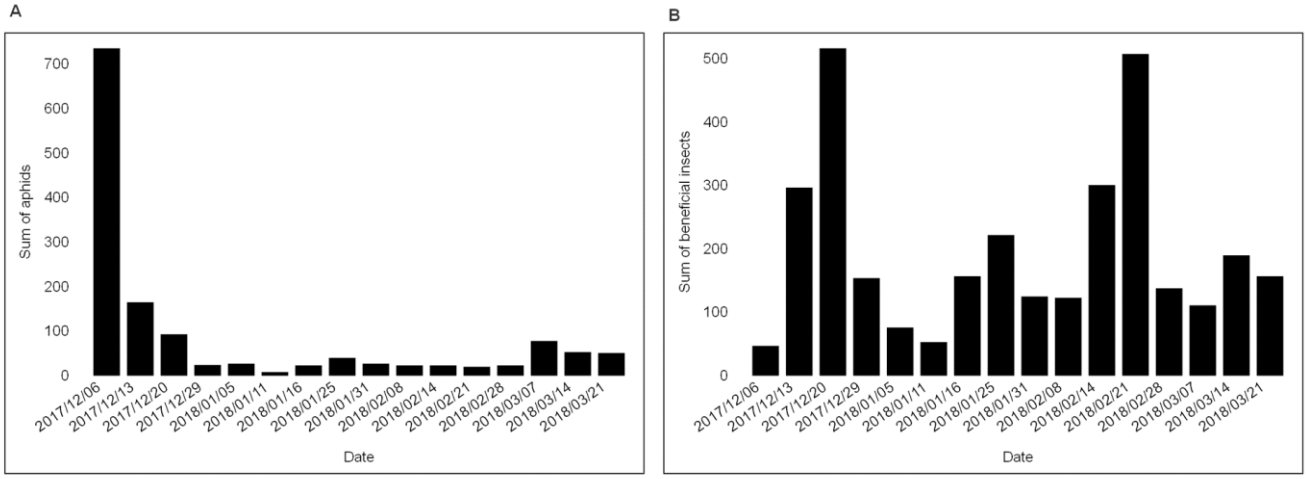
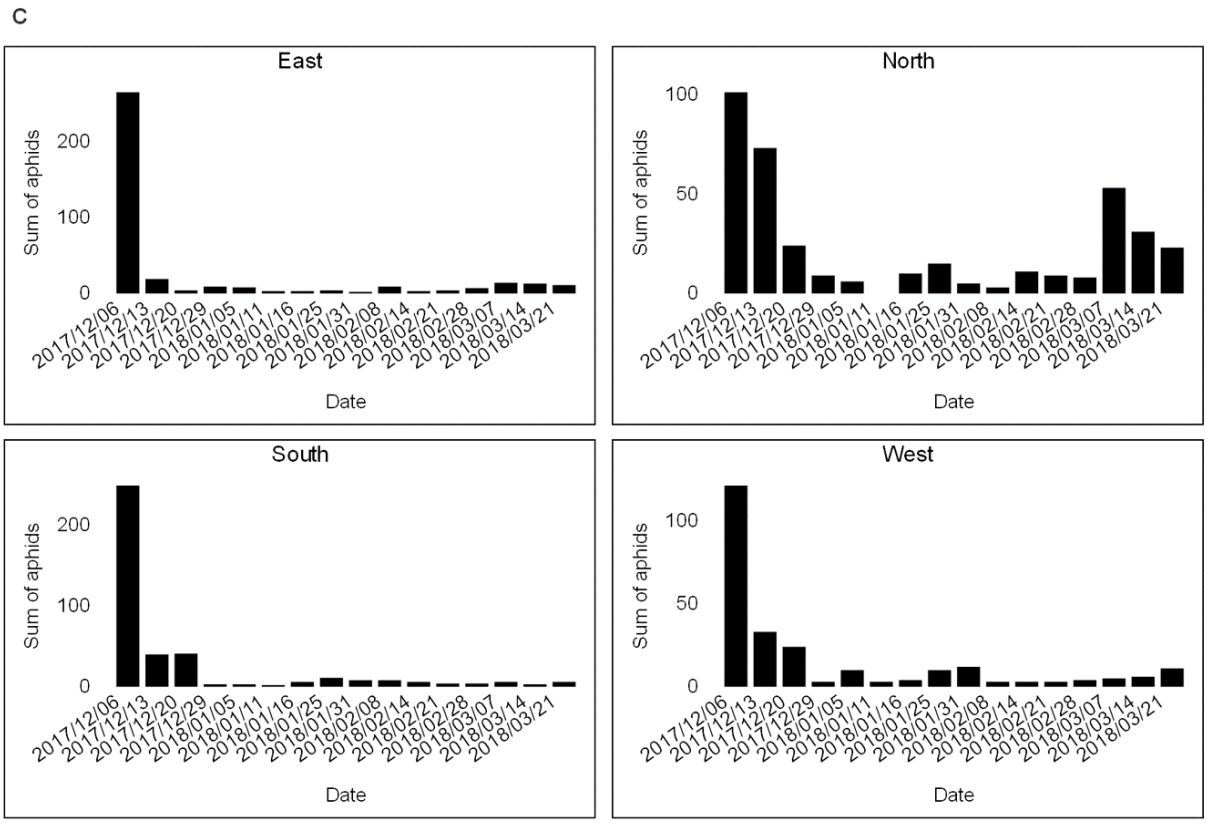




Aphids and beneficial insects trapping data





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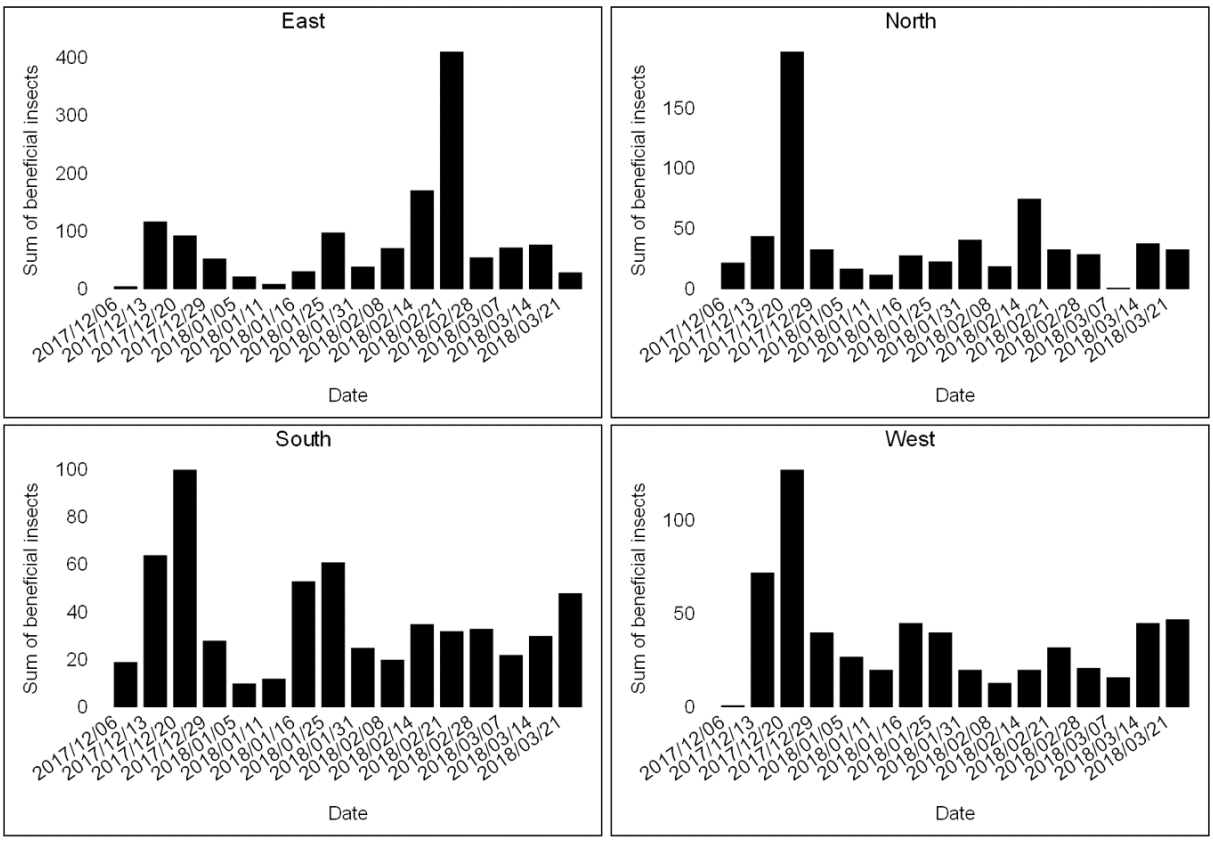
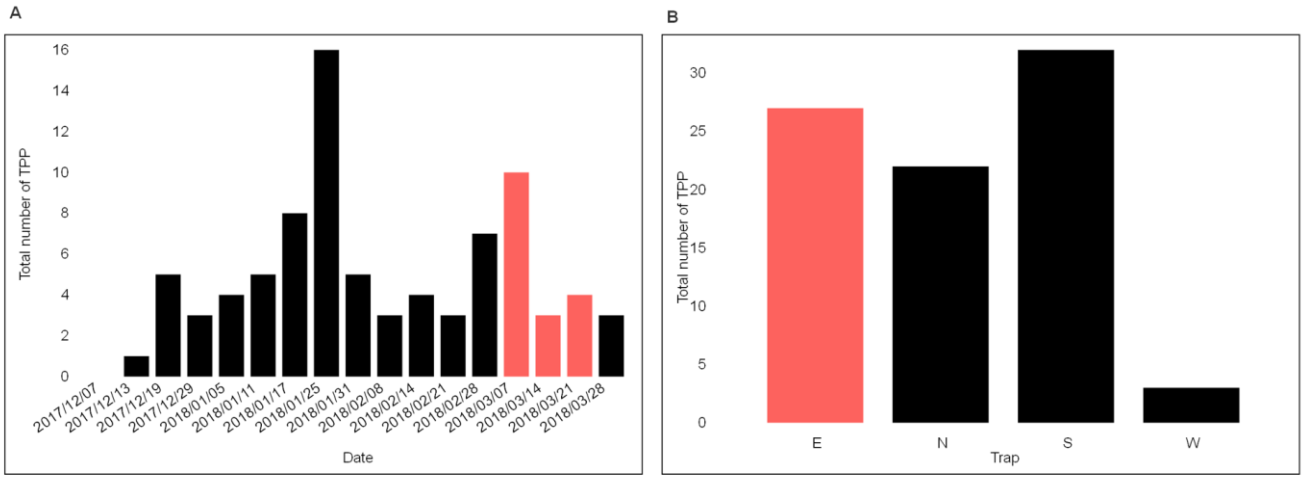
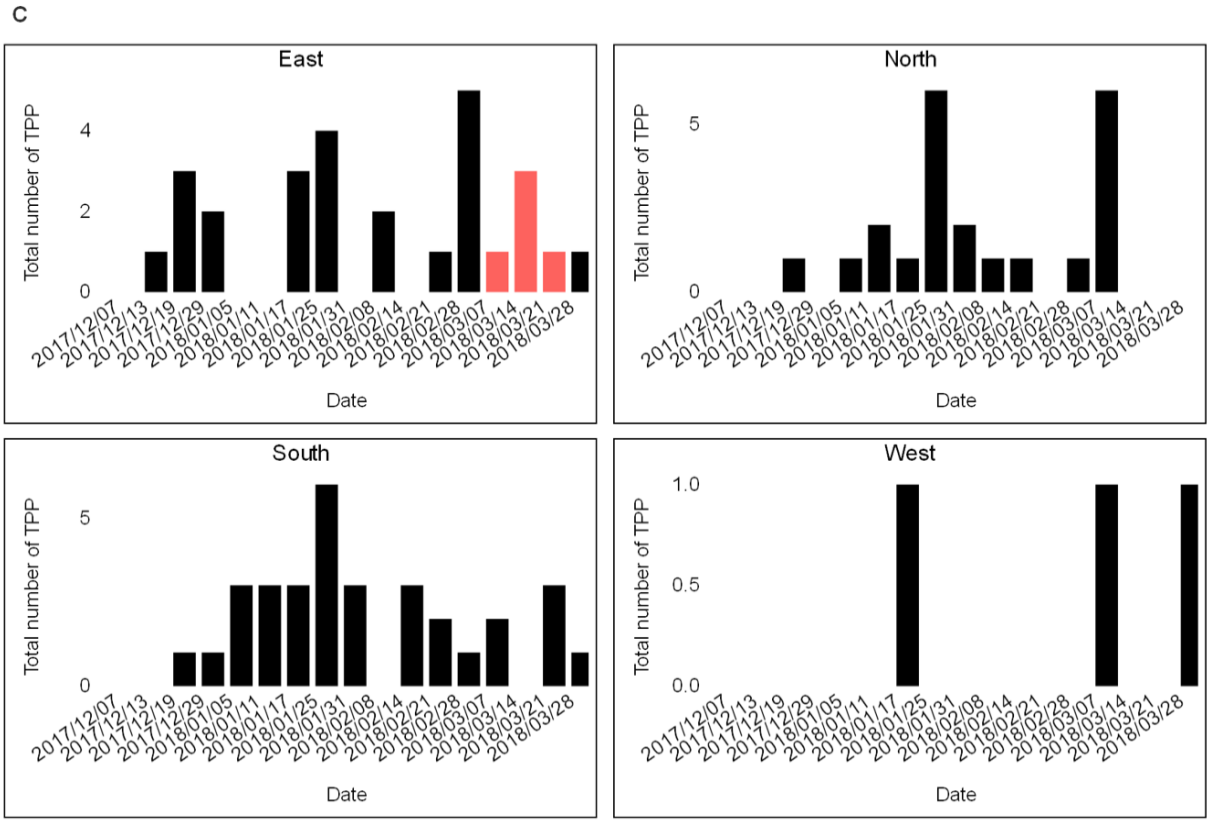


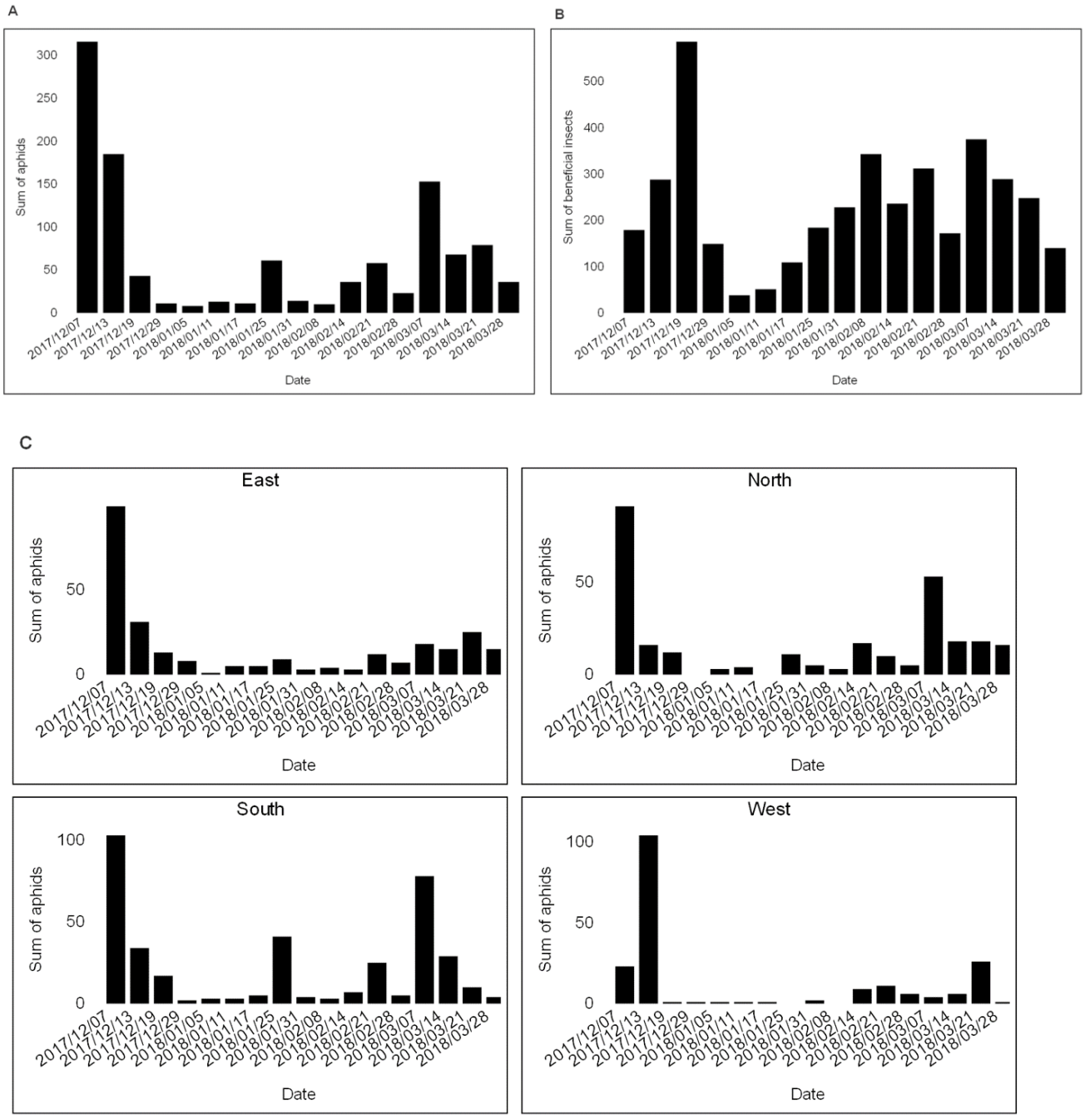
Grower 7

Tomato potato psyllid trapping data





Aphids and beneficial insects trapping data



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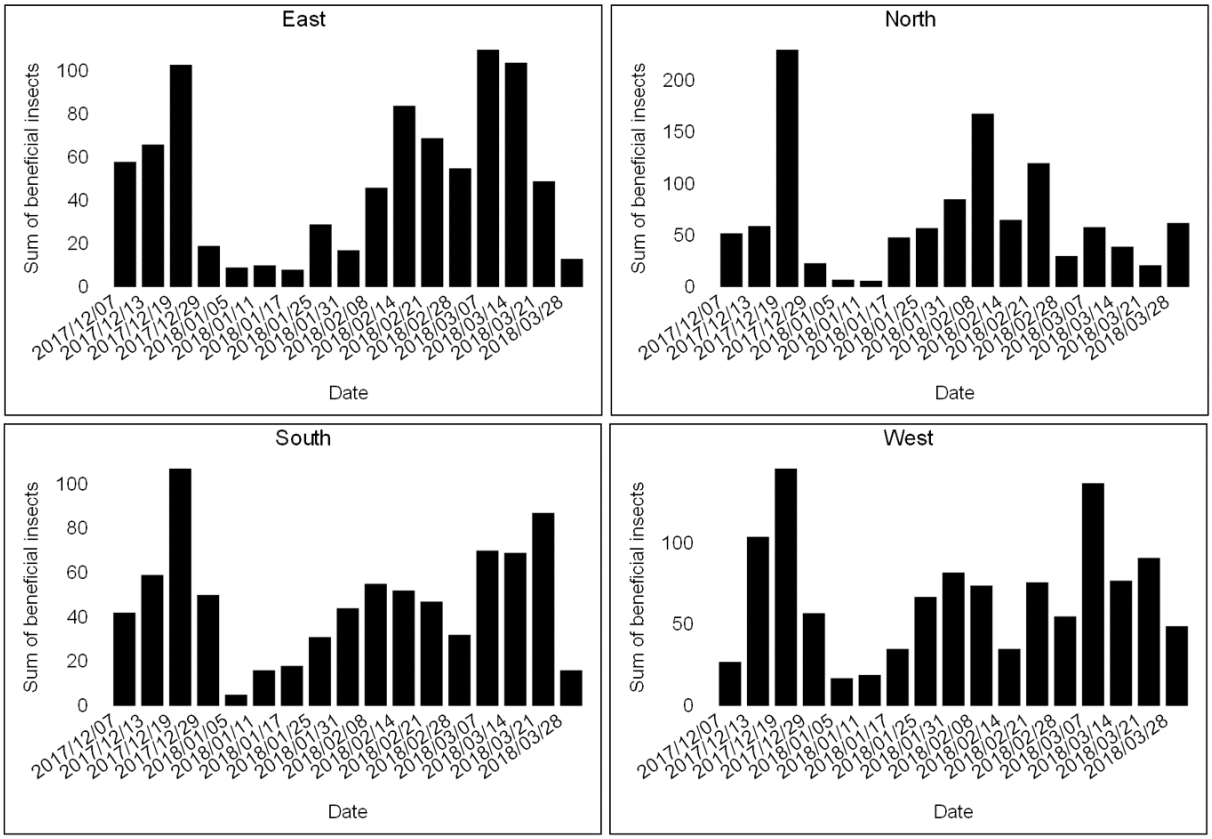
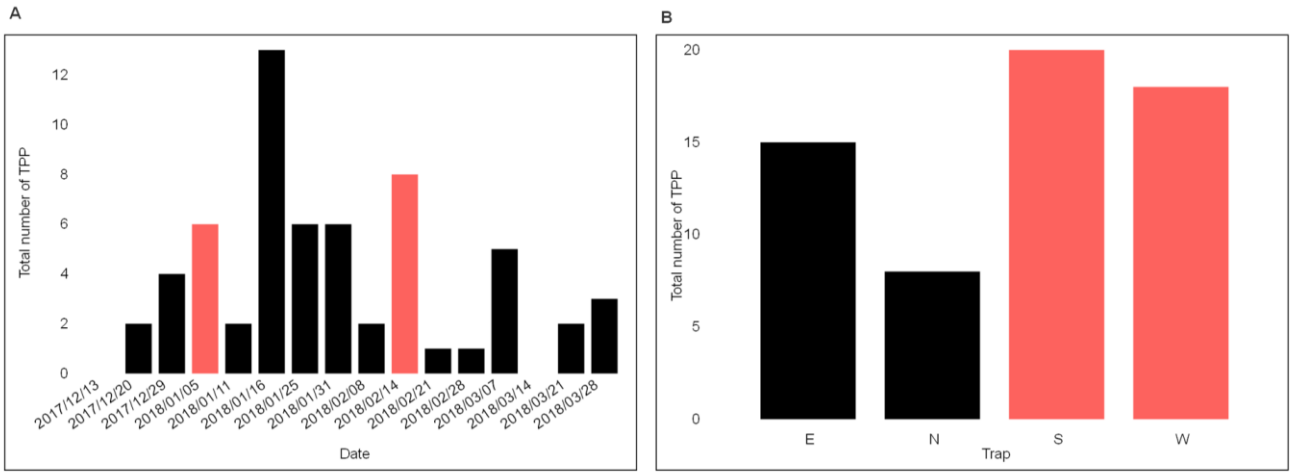
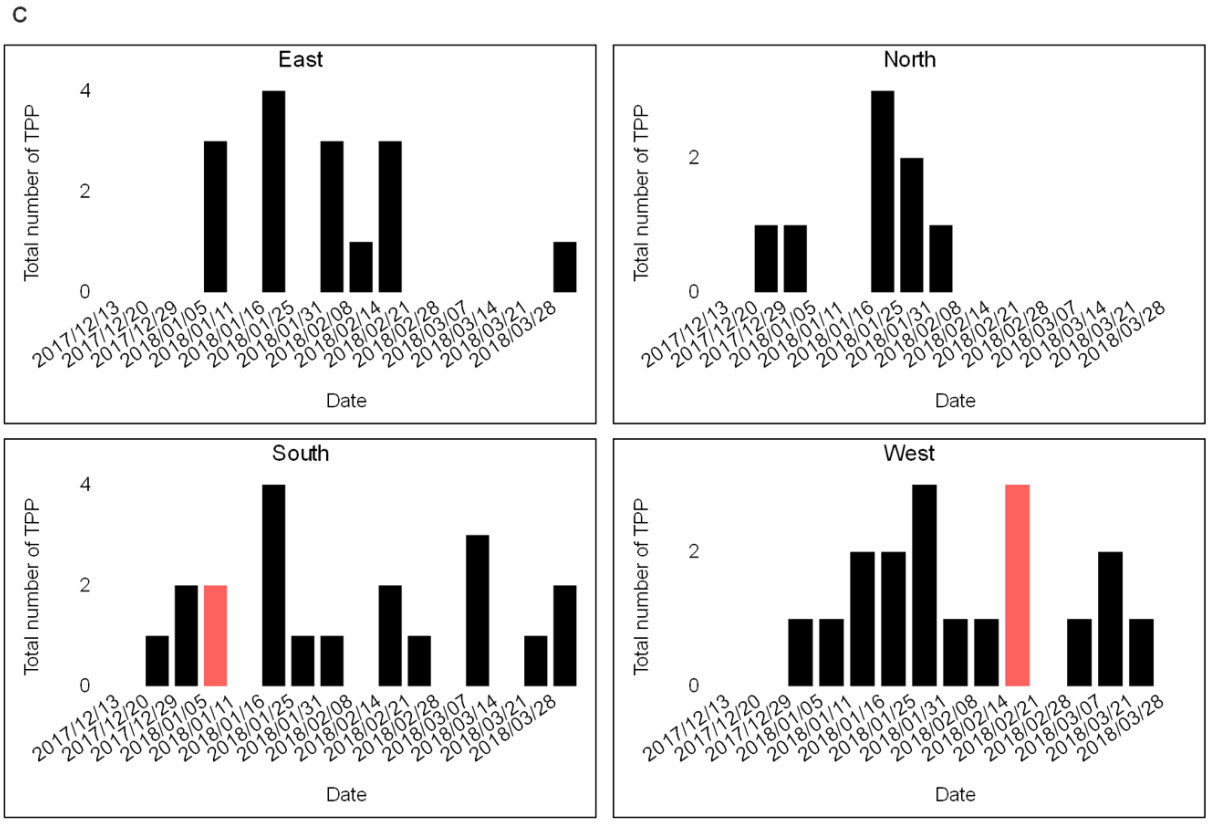


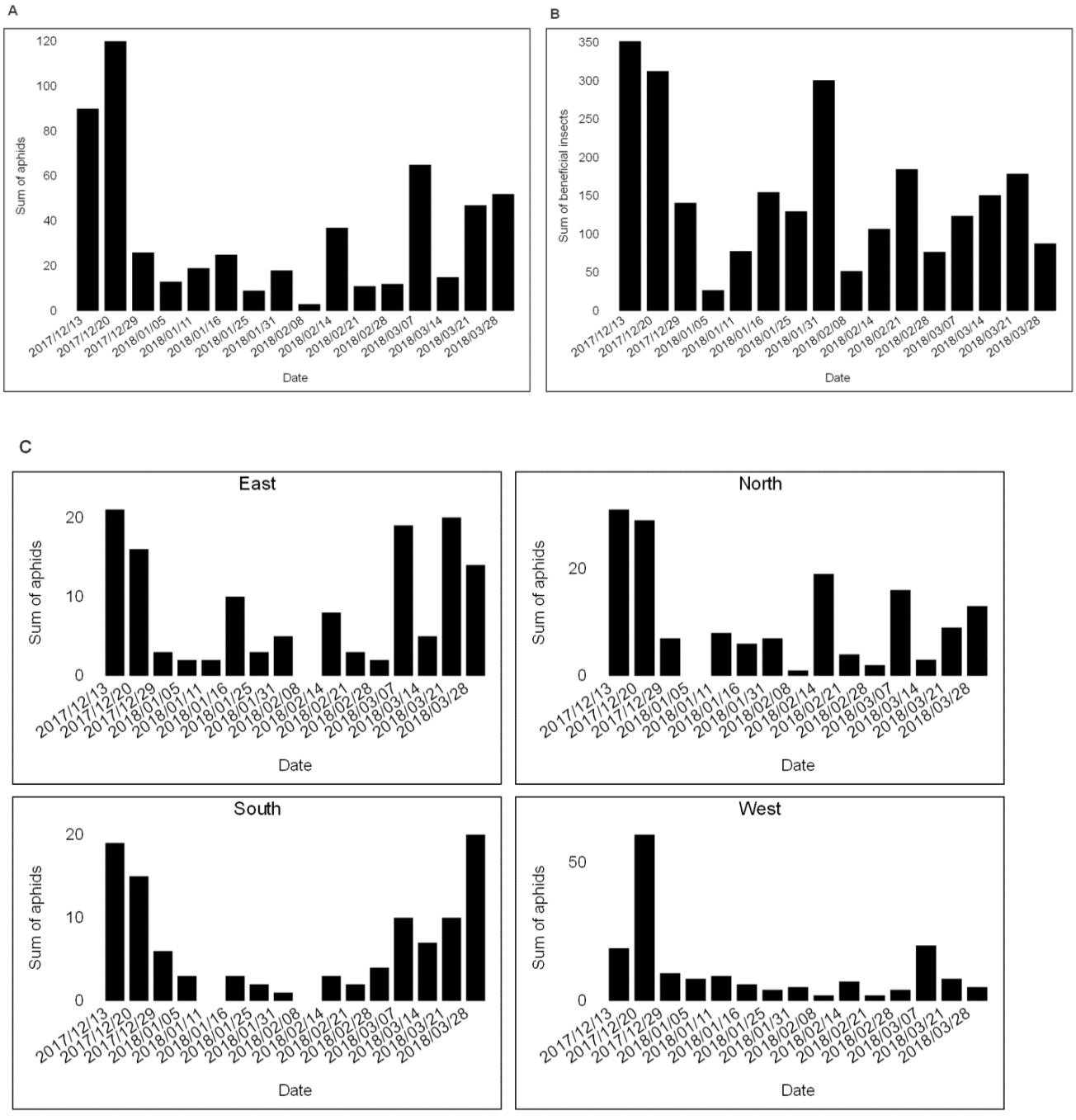
Grower 8

Tomato potato psyllid trapping data





Aphids and beneficial insects trapping data



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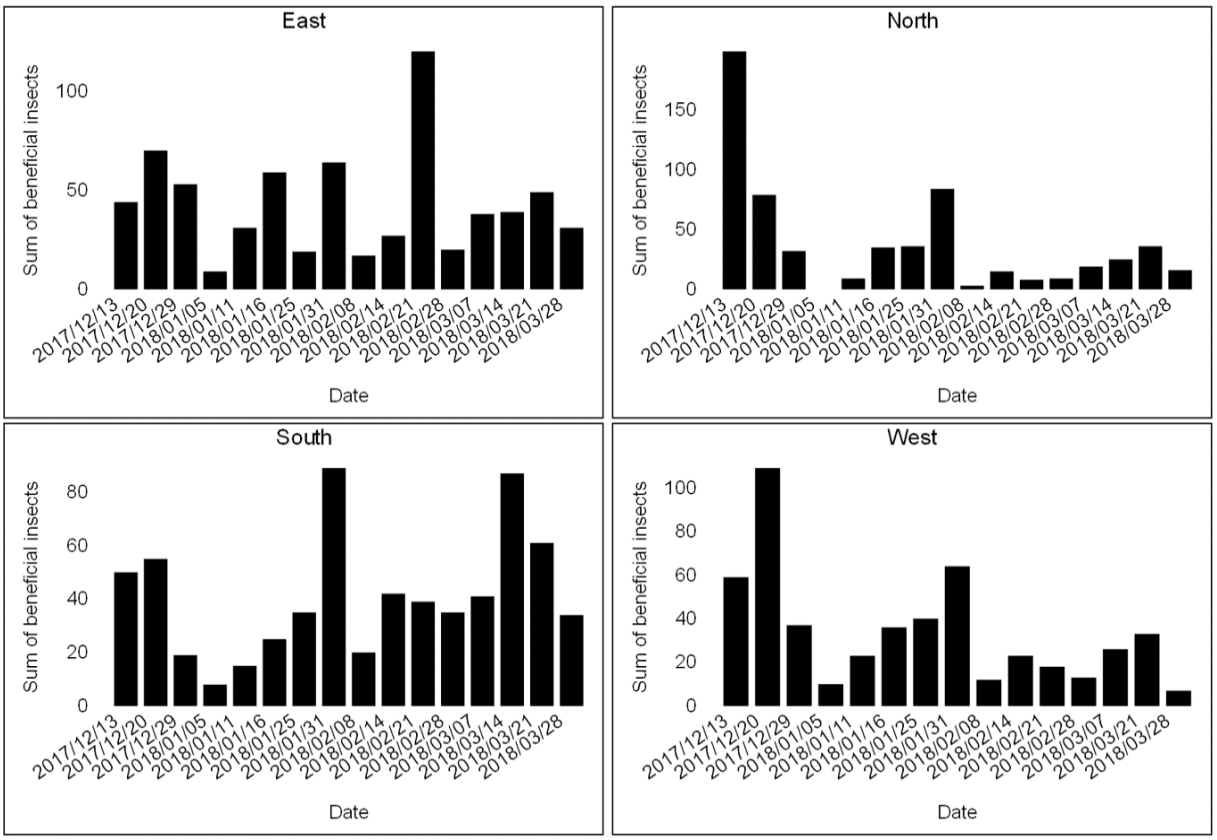
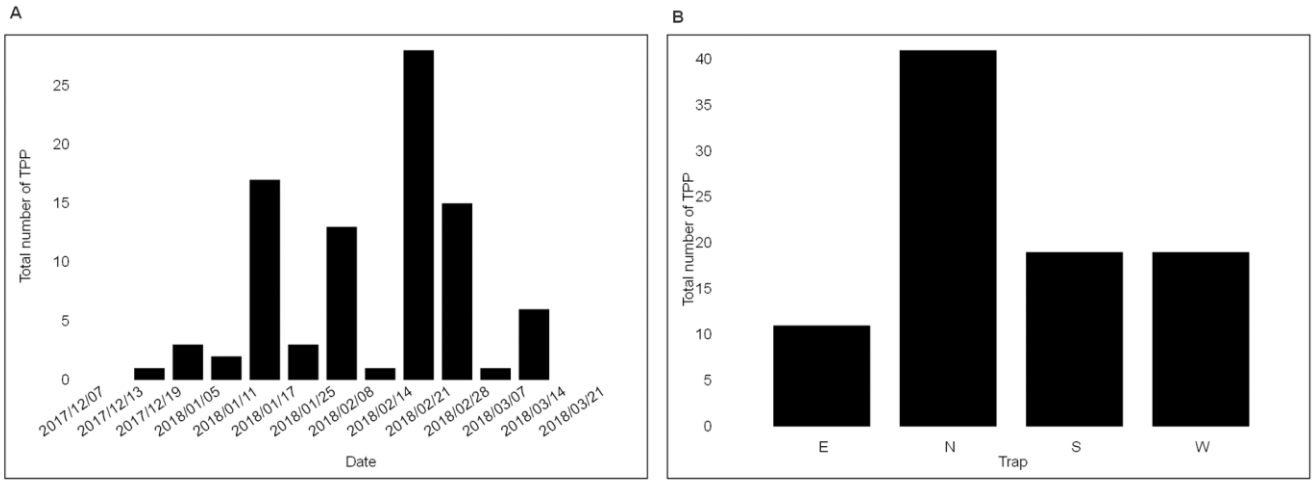
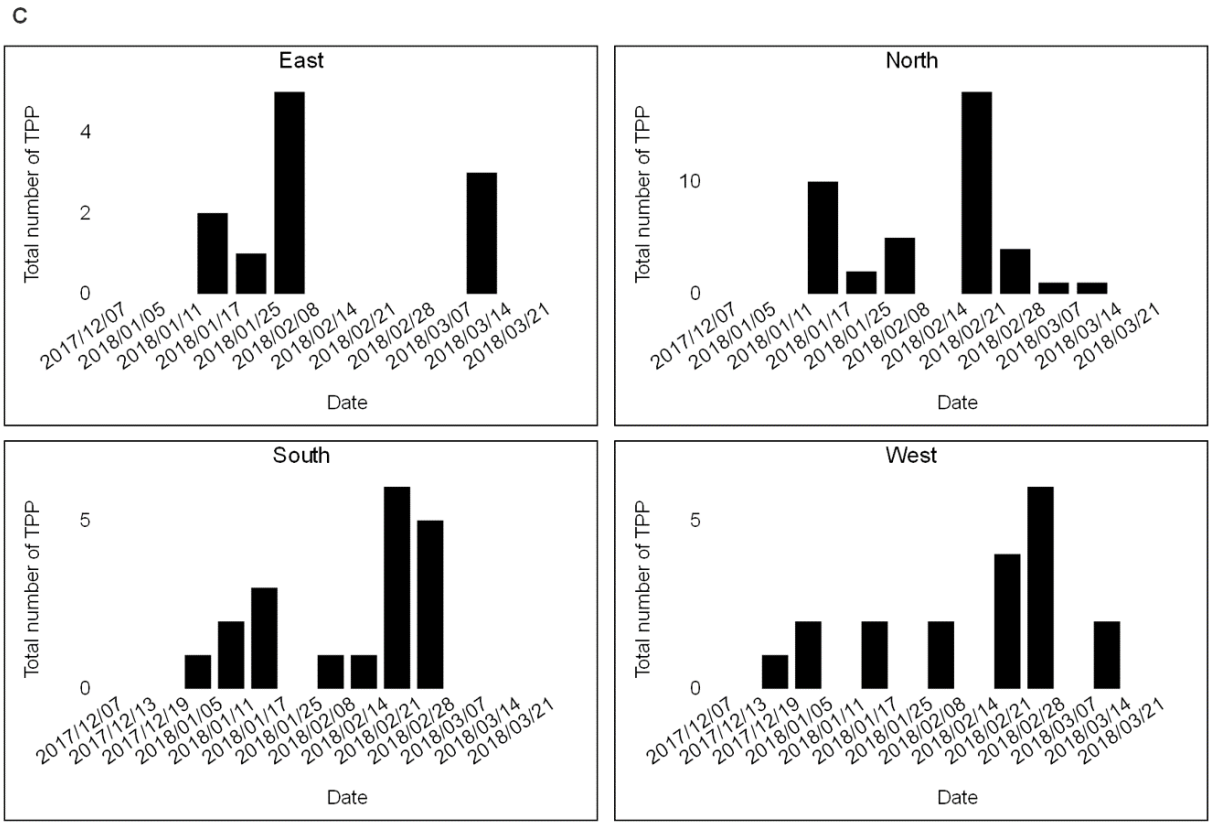


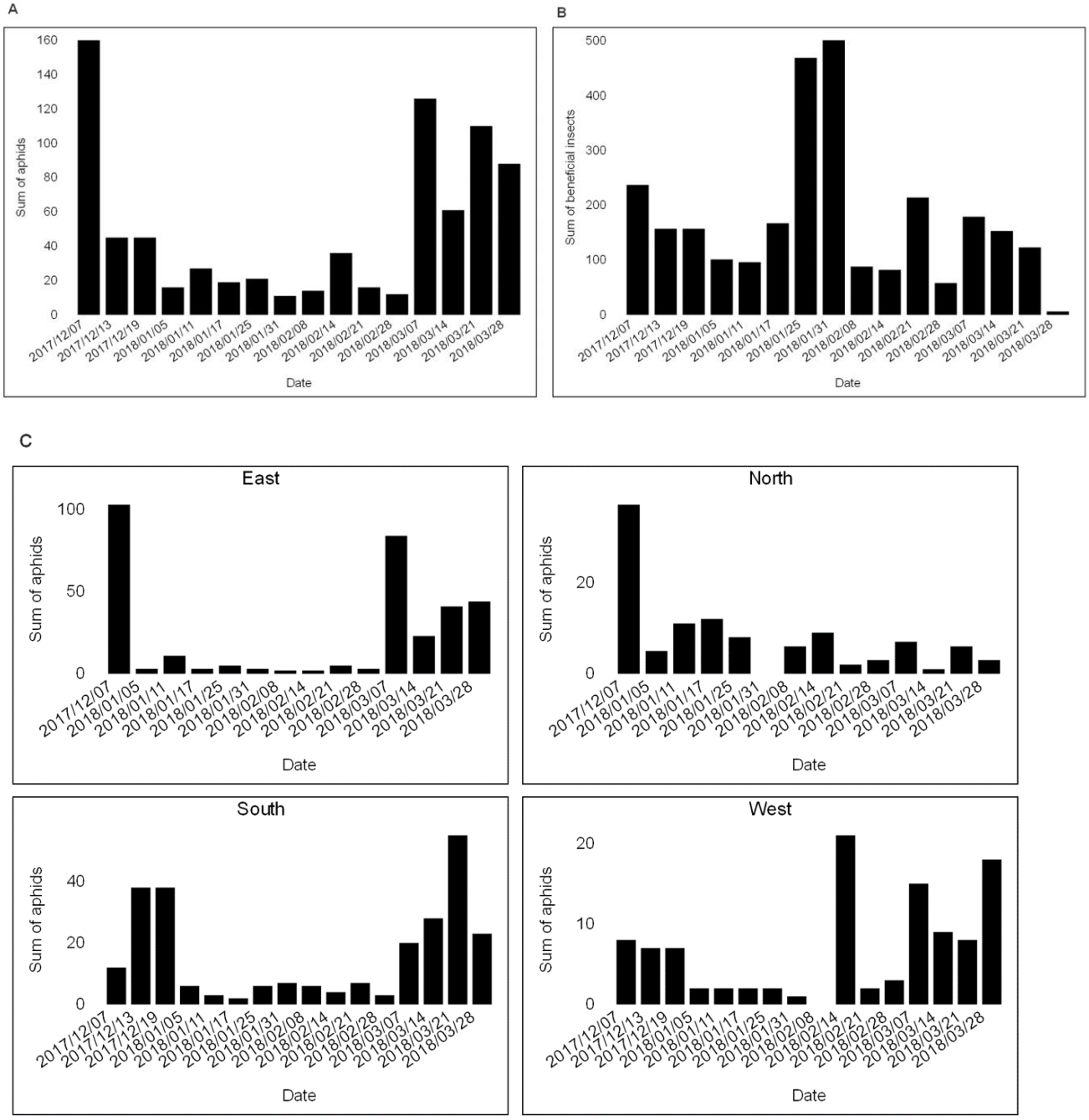
Grower 9

Tomato potato psyllid trapping data





Aphids and beneficial insects trapping data



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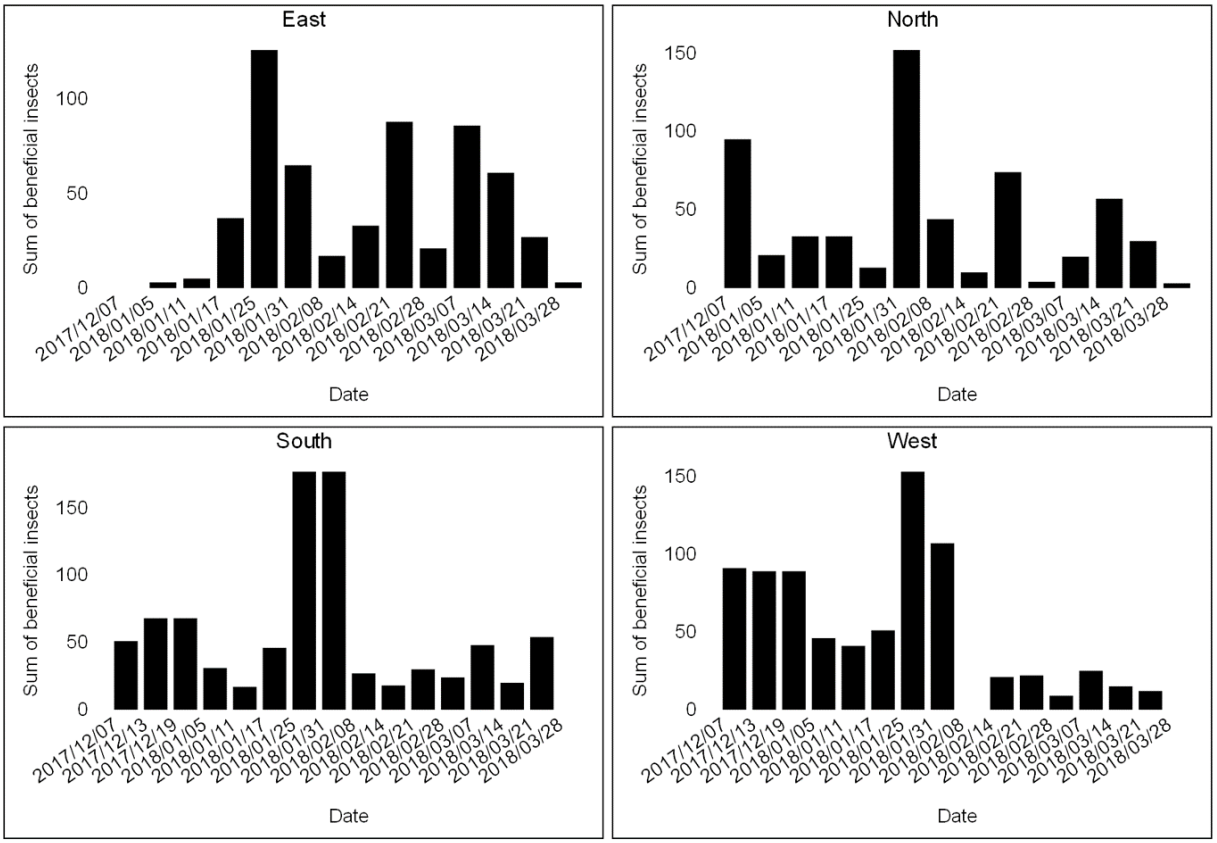
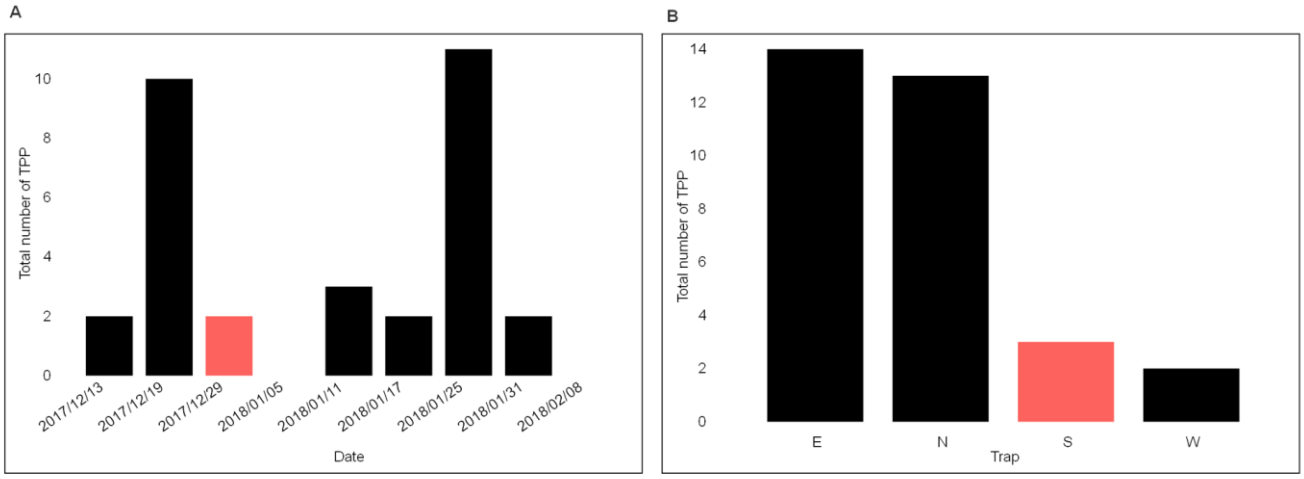
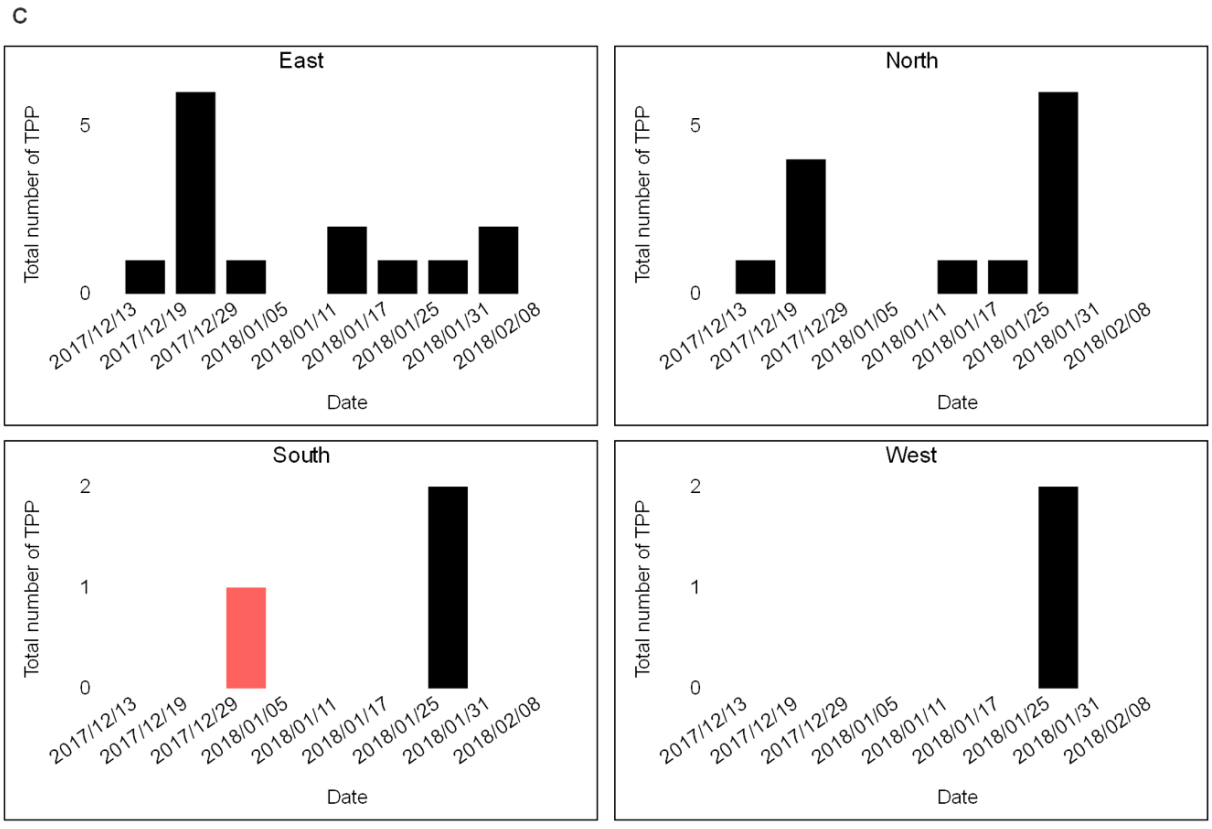


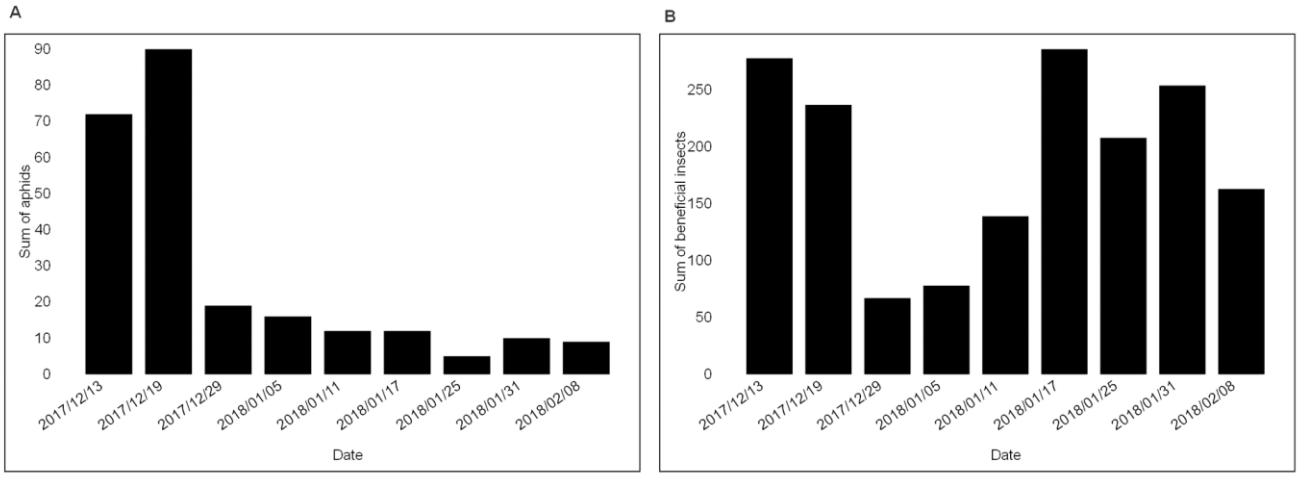
Grower 10

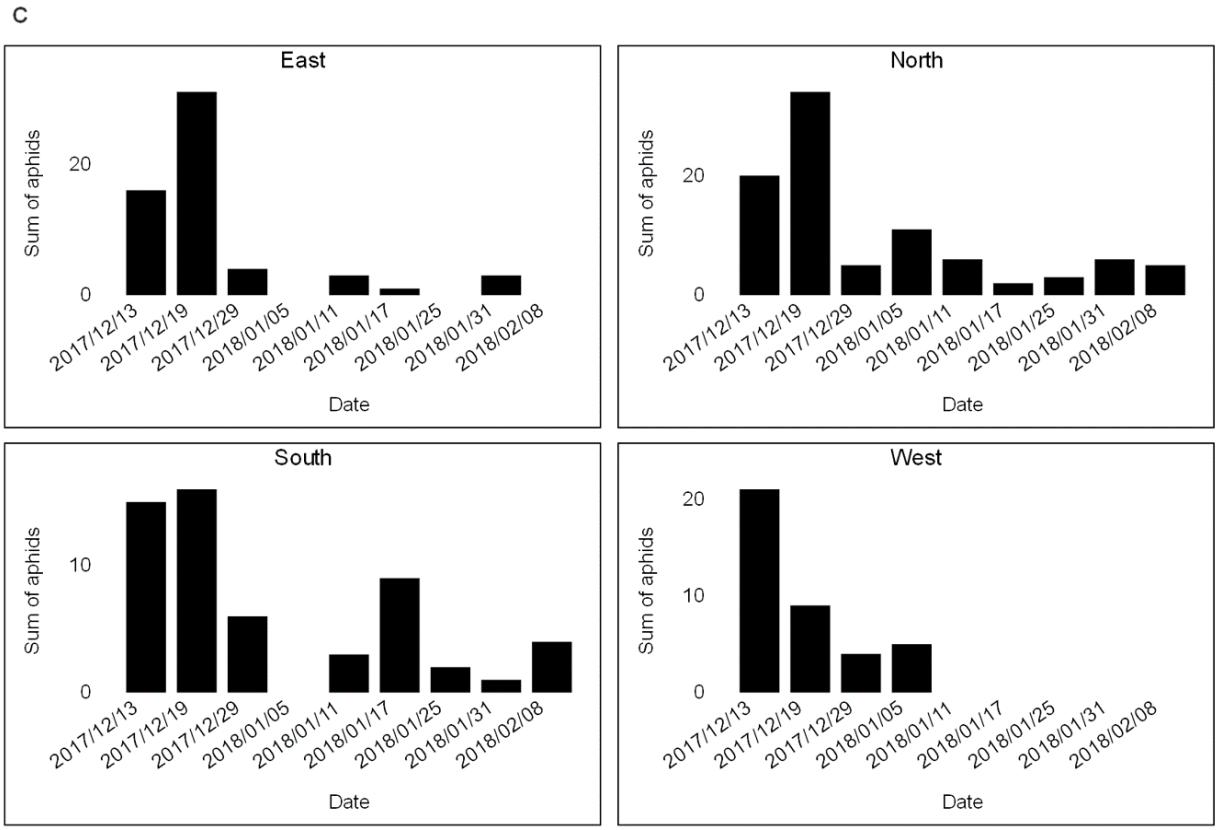
Tomato potato psyllid trapping data





Aphids and beneficial insects trapping data





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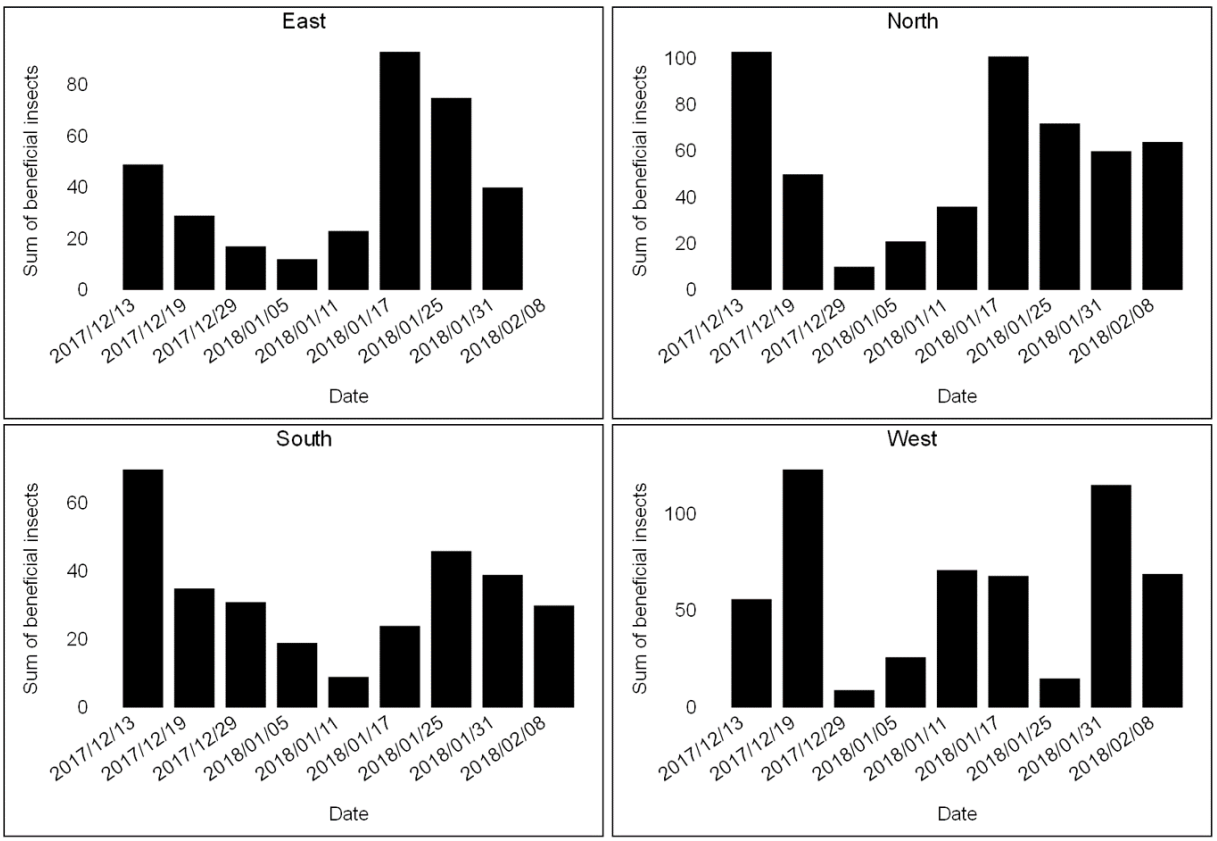

DISCOVER. INNOVATE. GROW.