Project title:	Control of spider mite (*Tetranychus urticae*) on protected cherry using the predatory mite *Ambyseius andersoni*
Project number:	TF 219
Project leader:	Dr Michelle Fountain, East Malling Research, Kent, ME19 6BJ Tel: 01732 523749 Email: michelle.fountain@emr.ac.uk
Report:	Annual Report 2015
Previous report:	None
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Date project commenced:	1 April 2014
Date project completed (or expected completion date):	31 March 2017

DISCLAIMER

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Michelle Fountain

Research Leader in Entomology

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CONTENTS

GROWER SUMMARY

Headline

- *Amblyseius andersoni* introductions made at a rate of one Gemini sachet per five cherry trees could aid control of pest mites.

Background and expected deliverables

Growing cherries under protection brings benefits of consistency of supply by reducing splitting from frost and rain. However, the increased temperature and humidity under tunnels also causes problems including attacks from pests and diseases which thrive in these conditions.

Pest mites on cherry include two-spotted spider mite (TSSM, *Tetranychus urticae*) and the European red mite (*Panonychus ulmi*). Due to the warmer conditions in protected cherry there has, in recent years, been a build-up in *T. urticae* close to harvest causing bronzing of the leaves and webbing, making harvest difficult or impractical. This was particularly problematic in 2013 when warmer, drier weather conditions promoted the population growth of *T. urticae* on cherry trees in tunnels. There was concern by agronomists that this may affect the subsequent years' bud growth.

Approved pesticides on cherry for spider mite control are either damaging to natural enemies, have short persistence or have harvest intervals which are too long.

Many species of predatory mites occur naturally and/or are available commercially. Naturally occurring predators offer some control of spider mites, but there is often a lag between the population build-up of the pest and the predator, resulting in spider mites overwhelming the trees before the predator can gain control.

Amblyseius andersoni is a generalist predator and will feed on many mite species including *P. ulmi*. Commercial trials have shown promising results using *A. andersoni* Gemini sachets to control spider mites in outdoor apple trees.

The aims of the research in the first year of this project were to evaluate the effectiveness of two release densities of *A. andersoni* in Gemini sachets in cherry orchards to control spider mites in protected cherry and to test the efficacy of laboratory mite extraction and counting methods on cherry leaves.

Summary of the project and main conclusions

Two orchards with a history of *T. urticae* infestation in 2013 were selected for a field trial to test high density (one Gemini sachet per tree) and low density (one Gemini sachet per five trees) releases of *A. andersoni* for spider mite control. The trial was a randomised replicated block, with six replicates in each of the two orchards. Gemini sachets were stapled into the canopy of the trees on 16 April. Assessments included a pre-assessment to measure background levels of predatory and phytophagous mites, a near harvest assessment (2 June) and a spring assessment (the latter to be conducted in spring 2015). Leaves were collected and then mites extracted using an ethanol extraction method, shown to be the most effective (see report for comparisons).

At the pre-assessment (before the introductions of *A. andersoni* were made), the numbers of mites on cherry leaves did not significantly differ between treatments or orchards. This included numbers of *A. andersoni*, *Euseius finlandicus*, (predatory mites), predatory mite eggs, *T. urticae*, *P. ulmi* or other, probably saprophytic, mites.

At harvest there were significantly more *A. andersoni* on leaves in the cherry trees where Gemini sachets had been deployed compared to the untreated, control trees. The numbers of pest mites in the different treatments were not significantly different between treatments and, unlike 2013, the pest mites did not increase to damaging levels in 2014 in either orchard. This may have been because there were background levels of *A. andersoni* already present. There was no difference in the numbers of *A. andersoni* in the high or low density Gemini sachet release plots suggesting that, in this one year trial, there is no added advantage to deploying a Gemini sachet in every tree.

Financial benefits

The economic damage caused by *T. urticae* feeding on cherry has not been estimated, but it led to economic losses in 2013 when some fruit was discarded. Supermarkets demand consistency of supply from year to year and many, e.g. Sainsbury's, are aiming to sell

double the volume of UK fruit by 2020. Reliable control of *T. urticae* from early in the season would help to reduce the risk of damaged fruit nearer to harvest.

Action points for growers

- Releases of *A. andersoni* in Gemini sachets can be made at one sachet per five trees to supplement natural predatory mites for spider mite control in cherry orchards.
- Growers should avoid the use of plant protection products where possible to preserve predatory mites in trees.
- Consideration of sprays applied for spotted wing drosophila (SWD) management are likely to interfere with spider mite control, so supplementing with early, but well-timed predatory mite releases may be necessary to control pest mites before SWD becomes a problem.

SCIENCE SECTION

Introduction

Growing cherries under protection brings benefits of consistency of supply by reducing splitting from frosting and rain. However, the increased temperature and humidity under tunnels also causes problems, including attacks from pests and diseases which thrive in these conditions.

Pest mites on cherry include two spotted spider mite (TSSM, *Tetranychus urticae)*, and the European red spider mite (*Panonychus ulmi*). *T. mcdanieli* was also recorded in Europe in 1981, but is probably currently of minor importance compared to the two former species. Due to the warmer conditions in protected cherry there has, in recent years, been a build-up in *T. urticae* close to harvest. *T. urticae* reduce the photosynthetic ability of cherry tree leaves (Wise *et al*. 1999) by feeding on them causing stippling, bronzing and in severe cases cause webbing and eventually early defoliation (Fig. 1). This makes harvest difficult or impractical. In 2013 warmer dryer weather conditions promoted the population growth of *T. urticae* on cherry trees in tunnels to such an extent, there was concern by agronomists that this may have affected the subsequent years' bud growth. In one orchard in 2013 the fruits were shrivelled as a result of spider mite attack and had to be destroyed. *T. urticae* overwinters as a diapausing (red) adult female, probably in the cracks and crevices of the trees and the post and wire structure. This allows reproduction and population growth to begin early in the spring of the following season.

Figure 1. Spider mite webbing and characteristic feeding damage on protected cherry leaves in 2013

The infestation builds up close to harvest when there are no reliable options for plant protection products. Pesticide controls need to ensure full coverage and it is especially important to target the underside of leaves. Very few insecticides that are effective against plant feeding mites are approved for use on cherry. Clofentezine (Apollo 50) has a harvest interval of 56 days and only one application can be made per season. Pyrethrins are damaging to natural enemies in the crop and are of short persistence. Mitochondrial electron transport inhibitor products (e.g. tebufenpyrad (Masai) and fenpyroximate (Sequel)) are probably effective at controlling *T. urticae* but have not been approved for use on protected cherry. Spirodiclofen (Envidor), another effective acaride, is also not approved for use in protected cherry. Hence, building up levels of predatory mites on cherry trees early in the season will help to keep spider mites in check.

Many species of predatory mites occur naturally and/or are available commercially. *Typhlodromus* sp. and *Neoseiulus fallacis* (not commercially available and the latter not present in the UK; http://www.lea.esalq.usp.br/phytoseiidae/) do offer some control of spider mites, but there is often a lag between the population build-up of the pest and the predator, resulting in spider mites overwhelming the trees before the predator can gain control. In addition one of the most common predator species, *T. pyri,* is not common on cherry, probably because the leaves are smooth and hairless and the mite is unable to survive on these surfaces.

The two most promising commercially available predatory mites for outdoor use for control of spider mites on cherry trees are *Phytoseiulus persimilis* and *Amblyseius andersoni*.

Phytoseiulus persimilis is used against *T. urticae* in apple orchards in Israel at a release rate of half a million / acre (maintained populations) until the spider mite is under control – below economic threshold. *P. persimilis* could disperse at least 90 m within 45 days of the original release site (Steinberg and Cohen 1992). *P. persimilis* is a spider mite specialist predator and may have good potential for curative control, but its reliance on spider mites makes it difficult to sustain on trees when the pest is not present, and because *P. persimilis* will only attack *T. urticae* other pest mites may persist and increase.

Amblyseius andersoni is a generalist predator and will feed on many mite species, including *P. ulmi* and also pollen grains. Commercial trials have shown promising results using *A. andersoni* Gemini sachets to control spider mites in outdoor apple trees.

T. urticae is a widespread species that feeds on several crops including walnut, strawberry, blackcurrant, gooseberry, raspberry, apple, cherry, pear, and plum (Alford 2005).

Objectives

To evaluate the effectiveness of two release densities of *A. andersoni* to control spider mites in commercial protected cherry crops.

To test the efficacy of laboratory mite extraction and counting methods on cherry leaves.

Materials and methods

Field trial

Sites: Two orchards were selected which had a history of *T. urticae* infestation in 2013 (Table 1).

Table 1. Details of orchards

Farm	**Mt Ephraim ME13 9TX**	**Amery Court Farm CT2 9HF**
Orchard name	'Orchards'	'Wellington'
Size	2 ha	2 ha
Age	?	2007
No. rows	27 rows	13 rows
Row length	80 – 100 m	170 m
Distance between trees	2.1 x 1.8 m	3.0 x 2.7 m
Tunnel width	7 m	7.8 m
Planting	double rows	double rows
Overlapping branches	Yes	Mostly not
Varieties	1 Kordia, 2 Regina, 3 Sweetheart, 4 Inga, 5 Sasha, 6 Colney, 7-8 C, 9 Stella, 10 Sunburst, 11 C, 12 Summer sun, 13-14 Colney 15 Summer sun, 16 Penny 17 -18 Summer sun 19 -20 Colney, 21-22 Penny, 23-24 Summer sun 25 Colney, 26 Regina 27 Kordia (nb: half row of apricots)	1 and 2 = Summer Sun 3-5 = Penny 6-8 = Cordia 9-13 = Sweetheart
Row space	2.5	2.5
Tree space	2.0	3.0
Irrigation	Yes	Yes
Covers on	25 March	2nd week April

Experimental design and layout: A randomised replicated trial was conducted in each of the two orchards. There were six replicates of each treatment. Because the trees were in double rows each plot was 2 x 5 trees long (10 trees per plot). There were 2 x 5 tree guard plots between each treated plot (18 treated plots per orchard). Replicates were separated by a guard tunnel.

Treatments: Treatments were one release of two densities (one per tree or one per five trees) of *A. andersoni* Gemini sachets (Table 2) compared to an untreated control.

Table 2. Numbers of *A. andersoni* sachets used

Treatment	No. trees per plot	No. plots	No. orchards	No. sachets needed
1 Gemini sachet per tree	10	6	2	120
1 Gemini sachet per 5 trees	10	6	2	24
Untreated control	-	-	-	-

Treatment application: Mites were applied (16 April) once the tunnels had been covered and temperatures were >10°C, for good survival and distribution of *A. andersoni.* The Gemini sachets were comprised of two compartments separated by a paper hinge; this enables rapid deployment in glasshouse situations by hanging them over a wire. For field deployment the twin sachets were folded along this hinge so that the opening for the mites hung together to offer some protection for the exiting mites. The sachets were then stapled directly to the target tree through the paper hinge to a main branch in the centre of the tree at mid height (Fig. 2).

Figure 2. Deployment of Gemini sachets in one of the target orchards

Assessments: A pre-assessment was conducted before the sachets were deployed to establish the numbers and diversity of resident predatory mites in each orchard (16 April).

Twenty leaves were collected from the full canopy of the centre six trees of each plot (120 leaves per plot). The leaves were collected into two litre plastic jars containing 700 ml of 70% ethanol to kill the mites and preserve them for later analysis. An ethanol washing and filtering method was determined to be the most efficaint at the removal of mites from leaves to be counted (see below – Laboratory Trial).

The first assessment, post inoculation, was conducted on 2 June, but used 60 leaves per plot (10 leaves from each tree in a plot).

A final assessment will be conducted in April 2015 to ascertain the over wintering survival of *A. andersoni.*

Numbers of leaf feeding mites including spider mite and European red mite were also assessed including any other mites in notable numbers.

Plot maintenance: Trees received a routine protective spray programme against pathogens. 'Orchards' plot received Steward (210 g/ha) on 28 Mar 14, Aphox (560 g/ha) on 16 April 14 and Dipel DF (750 g/ha) on 19 May 2014. 'Wellington' orchard received Steward on 18 April 14 (0.428 kg,) and Calypso on 23 May 14 (0.652 L).

Meteorological record: Two lascar USB-502 loggers were deployed inside a Stevenson's screen within the crop to take hourly temperature and humidity readings inside the polytunnel and will be collected after the spring assessment.

Statistical analysis: Data was normally distributed and, therefore, analysed without data transformation using ANOVA in Genstat.

Laboratory trial

In order to determine an appropriate assessment method for cherry we evaluated four methods of mite assessment for cherry (glaborous) leaves in comparison to apple (setaceous) leaves, namely Tullgren extraction (e.g. Kranz 1978), ethanol washing (e.g. Hossain *et al*. 1991), paraffin washing (Farid Faraji 2004), ethanol extraction and the brushing method (Morgan *et al*. 1955). These methods were compared to direct counting of mites on leaf samples.

To test the most effective method for mite extraction 600 apple and 600 cherry leaves of the same variety were randomly sampled from orchards at East Malling Research. While

selection was random to reduce bias, consideration was taken to pick leaves with similar characteristics. Leaves of the same size, shape and level of damage were picked to keep variables constant.

The leaves were divided into 20 polythene sample bags for apple and cherry (20 leaves per bag). Each sample was then inoculated with a single runner bean leaf from glasshouse plants heavily infested with *T. urticae*. The bags were sealed with electrical tape to ensure that no mites escaped and left for 48 hours at 20°C to ensure the mites were given sufficient time to distribute across the leaf samples.

Bags of leaves were randomly assigned to different mite extraction methods (six cherry and six apple replicates per treatment). The bean leaf was left in the samples during extraction for the first experiment but removed for the second experiment. In the first experiment ethanol washing, paraffin washing, brushing and Tullgren extraction were compared to direct counts of mites on leaves. In the second experiment apple and cherry leaves were compared in the same test (excluding paraffin washing).

Ethanol washing: Each leaf sample was submerged in 700 ml of 70% ethanol in a 2 litre beaker. The sample was shaken thoroughly to dislodge mites from the leaves. In the first trial samples were left to soak for 24 hours and two hours in the second trial.

The solution was poured through a coarse sieve (2.5 mm mesh). This was repeated nine times and then the leaves were individually rinsed and submerged in clean ethanol and discarded. The new solution was then passed through the coarse sieve and added to the initial solution which had been collected. The container in which the leaves were initially soaked was rinsed with an ethanol washing bottle and sieved to ensure all mites were removed. Once the ethanol solution ‘containing the mites’ was collected, the solution was passed through a 50 µm sieve three times. The container was also rinsed and passed through the fine sieve.

A piece of black filter paper (9 cm dia.), segmented with a white chalk pencil, was placed into the Buchner funnel. The filter paper was then dampened with an ethanol washing bottle. The sieve contents were transferred into the Buchner flask using an ethanol washing bottle. A vacuum was then generated using a tap removing the ethanol from the funnel.

The filter paper was placed on a Petri dish and mites counted under a dissecting microscope.

Paraffin washing: A 2 litre plastic beaker was filled with 1.5 litres of water (plus 7.5 ml of detergent). Samples were left for 24 hours and then vigorously shaken and swirled to ensure the leaves did not stick together and mites were washed off.

Sieving was the same as for the ethanol method but the leaves were washed with water instead of ethanol. This rinsing technique was continued nine times. A drop of anti-foaming agent was added to remove foam to make mite recognition clearer later on.

After rinsing with ethanol, mites were transferred to a test tube via funnel and an equal amount of paraffin was added to the ethanol with three drops of methyl blue. Tubes were closed and shaken thoroughly and left to set into two layers with paraffin at the top, ethanol at the bottom and the mites preserved on the meniscus. Once the solution had settled into two distinct layers, the samples were carefully transferred into Petri dishes and mites counted as above.

Brushing: 3 ml of detergent was spread onto a 12.5 cm glass disc and placed under the leaf brushing machine (Henderson and McBurnie 1943). The machine consists of two rotating brushes powered by a small electric motor. Each leaf from a sample was individually brushed at least three times on each side, held by the petiole (e.g. Herbert *et al*.1966). The leaves were also turned upside down to ensure that the hairs of the midrib, protected by the lateral veins, were thoroughly brushed, because many mites accumulate in this region of the leaf. This machine has proven effective at removing several species of mites (Morgan *et al*. 1955). A 132 square section slide was placed under the glass disk to aid counting under a microscope.

Tullgren extraction: Each leaf sample (20 leaves) was placed into a Tullgren sieve. A fixed light intensity (25 Watts) was positioned above the leaves for 48 hours with a test tube containing 10 ml of 70% ethanol underneath each funnel. The heat and light force the mites to retreat downwards into the test tube. A segmented Petri dish lid was used to assist mite counting.

Direct counting: Direct counting was used as a control to ensure the efficacy of extraction of the other methods (above). Both surfaces of each leaf were viewed under a microscope. Most mites situated themselves on the undersides of the leaves and under the leaf hairs of the mid vein of the leaf.

Results

Field trial

The pre-assessment (before the introductions of *A. andersoni* were made) of the numbers of mites on cherry leaves did not significantly differ between treatments or orchards. This included numbers of *A. andersoni*, *Euseius finlandicus*, (predatory mites), predatory mite eggs (Fig. 3), *T. urticae*, *P. ulmi* or other, probably saprophytic, mites (Fig. 4).

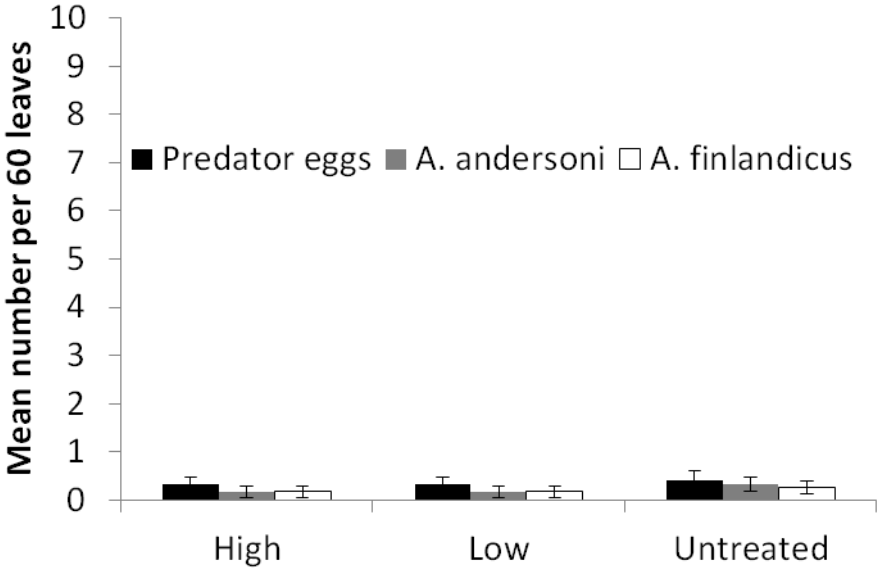

Figure 3. Numbers of predatory mite eggs, *A. andersoni* and *E. finlandicus* on 120 cherry leaves in the experimental orchards before releases of *A. andersoni* sachets were made

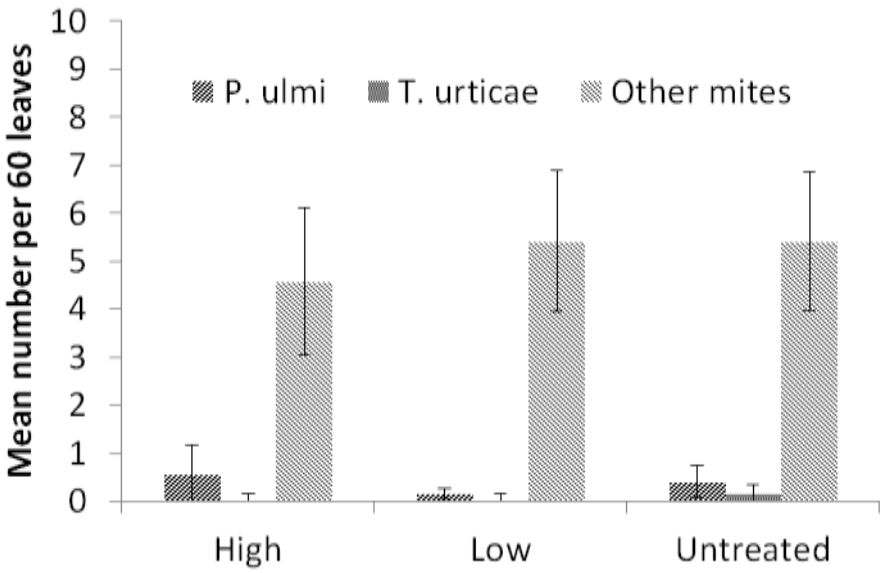

Figure 4. Numbers of *P. ulmi*, *T. urticae* and other mites on 120 cherry leaves in the experimental orchards before releases of *A. andersoni* sachets were made

Table 3. Mean numbers of mites per 60 leaves found in the high (one Gemini sachet per tree) and low (one Gemini sachet per 5 trees) density inoculated *A. andersoni* plots and two orchards compared to the untreated control. NSD = no significant difference. Different letters denote significant differences

		Predators			**Pests**	
Treatment	**Other mites**	**Predator eggs**	***A. andersoni***	***E. finlandicus***	***P. ulmi***	***T. urticae***
High	0.50	1.67	5.83 B	1.75	0.00	0.33
Low	0.25	2.50	7.17 B	0.58	0.08	0.33
Untreated	0.58	1.83	3.08 A	2.17	0.42	0.58
Orchards						
'Orchards'	0.17 A	2.50	6.67	2.17	0.00	0.33
'Wellington'	0.72 B	1.50	4.06	0.83	0.33	0.50
Treatment						
F pr	NSD		0.017	NSD	NSD	NSD
s.e.d.			1.311			
l.s.d. (p=0.05)			2.735			
Farm						
F pr	0.037		NSD	NSD	NSD	NSD
s.e.d.	0.230					
l.s.d. (p=0.05)	0.513					

Six weeks after the treatments were applied there were significantly more *A. andersoni* on leaves in the cherry trees where Gemini sachets had been deployed compared to the, untreated, control trees. The numbers of pest mites in the different treatments were not significantly different between treatments. Unlike 2013, pest mites did not increase to damaging levels in 2014 in the two cherry orchards used for the trial. However, the trend in mean numbers of pest mites was encouraging with *P. ulmi* and *T. urticae* numbers, overall, lower in the trees where predatory mite releases had been made. There was no difference in the numbers of *A. andersoni* in the high or low density Gemini sachet release plots.

Richard GreatRex and Nathan Medd (Syngenta Bioline) visited the site and confirmed the majority of the predatory mites on the plots as *A. andersoni*. Small numbers of *E. finlandicus* and *T. pyri* were also found on this visit (12 June).

Laboratory trial

There were significantly more mites recovered from apple leaves using the ethanol washing method compared to the other extraction methods and direct counting (ANOVA, $p<0.001$, sed 29.2, lsd 60.9, Fig. 5). In addition, direct counting and the mite brushing machine recovered more mites than either the paraffin or Tullgren funnel methods. For cherry leaves, the ethanol was equally as effective as for apple. The paraffin method recovered less and a quarter of the *T. urticae* from the leaves with direct counting, Tullgren extraction and leaf brushing recovering just over half the numbers of mites as ethanol extraction (ANOVA, $p<0.001$, sed 28.6, lsd 59.6, Fig. 6).

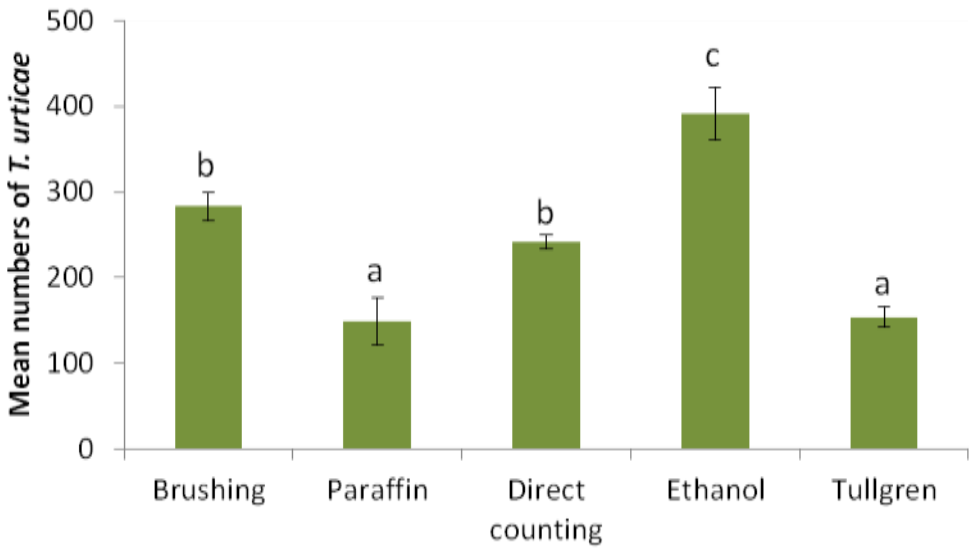

Figure 5 Mean numbers (n=6, ±SE) of *T. urticae* recovered per 20 apple leaves for each extraction method. Different letters indicate a significant difference between extraction methods (p<0.05)

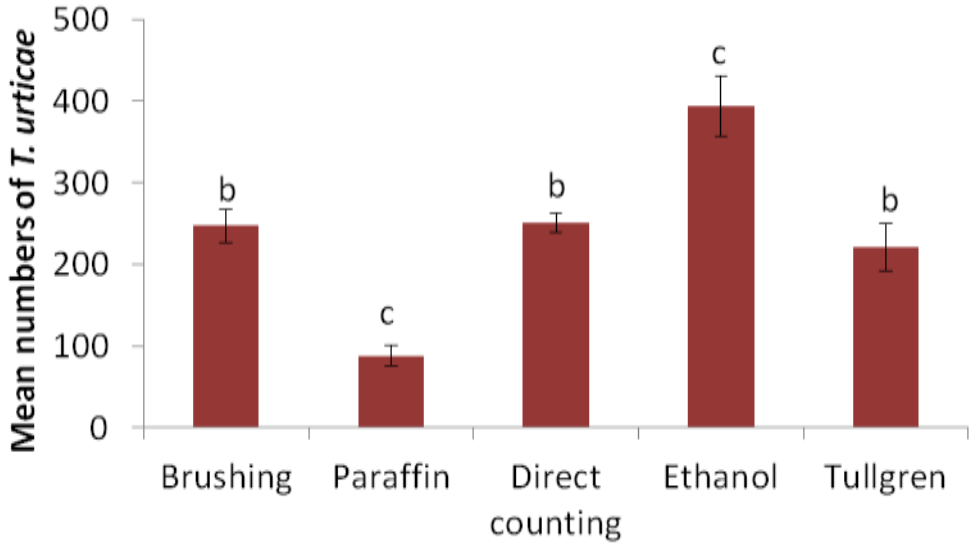

Figure 6. Mean numbers (n=6, ±SE) of *T. urticae* recovered per 20 cherry leaves for each extraction method. Different letters indicate a significant difference between extraction methods p<0.05)

In the second test, which focused on the difference in extraction techniques between the two leaf types (apple and cherry), more mites were recovered from apple compared to cherry when using brushing, ethanol or Tullgren methods. Fewer mites were observed directly on the leaves in apple compared to cherry (ANOVA, p<0.001, sed 8.1, lsd 20.8). Overall, leaf brushing, ethanol washing and direct observation of the leaves yielded more mites than Tullgren extraction (ANOVA, p<0.001, sed 19.7, lsd 40.2).

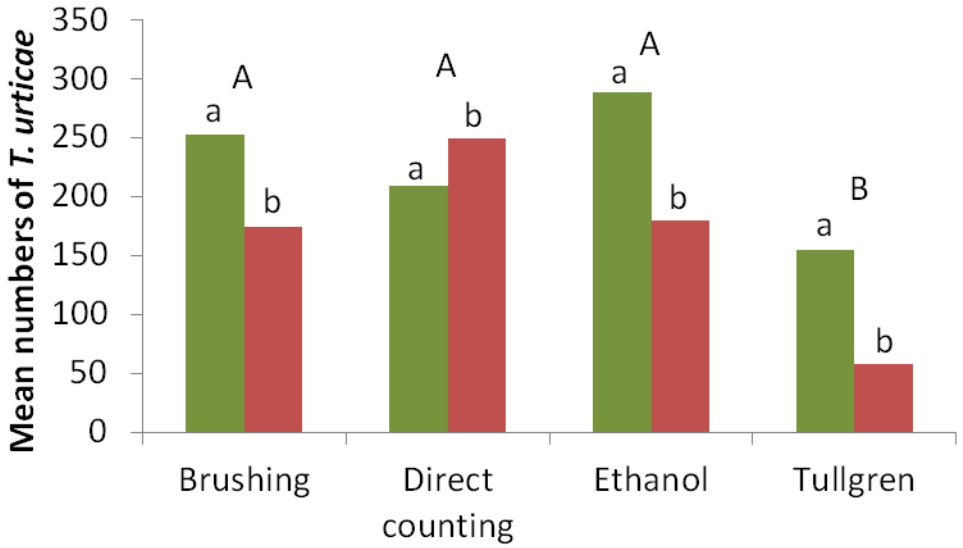

Figure 7: Mean number (n=6, ±SE) of *T. urticae* recovered per 20 cherry (red bars) and apple (green bars) leaves for each extraction method in the second test. Different small letters indicate a significant difference between leaf type and capital letters significant differences between extraction methods ($p<0.05$)

Overall, for cherry leaves, ethanol washing seemed to be the most effective method for *T. urticae* extraction. It was also less time consuming than direct counting mites on leaves and the paraffin method. The time taken to analyse the samples was similar to leaf brushing.

Table 4. Time estimates (minutes) of preparation of each mite extraction method and subsequent counting for 12 replicate samples

	Direct counting	**Brushing**	**Paraffin**	**Ethanol**	**Tullgren**
Preparation	0	120	310	240	30
Counting	540	240	200	140	240
Total time	540 (9 h)	360 (6 h)	510 (8.5 h)	380 (6.20 h)	270 (4.5 h)

Discussion and Conclusions

The number of numbers of *P. ulmi* and *T. urticae* in the trial cherry crops in 2014 was low compared to the previous year and statistics did not reveal any significant differences in pest numbers. However, no European red mites (*Panonychus ulmi*) were found in the plots treated with one *A. andersoni* Gemini sachet per tree. The average numbers of both pest species (*P. ulmi* and *T. urticae*) was 0.17, 0.21 and 0.5 per leaf, for one Gemini sachet per tree, one Gemini sachet per five trees and no added predators, respectively. Hence the trend was for fewer pest mites where Gemini sachets were added.

The numbers of *A. andersoni* mites on the Gemini sachet treated trees was not significantly different at high or low density releases. Hence, one sachet per five trees is adequate for good tree coverage with predatory mites.

Several mite extraction methods were evaluated for reliability, labour and accuracy. Of the methods tested the ethanol washing technique resulted in the highest recovery of *T. urticae* from cherry leaves.

Knowledge and Technology Transfer

None so far

Acknowledgements

We are grateful to the managers and owners of the two farms for allowing us to carry out these trials. We would also like to thank Jamie Stozka, Bethan Shaw, Maria Diez, Rebeca Conde-Ruiz, Maddie Cannon and Eduardo Jimenez for their help with treatment application and assessments. Thanks are owed to Syngenta Bioline for providing the Gemini sachets and guidance on their use.

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