WIGHT SALADS GROUP

World-class Speciality Growers

FINAL REPORT

To:
Horticultural Development Company
Agriculture and Horticulture Development Board

Organic tomato: Contingency plans
for the control of *Nesidiocoris tenuis*

January 2011

Commercial – In Confidence

Project title	Organic tomato: Contingency plans for the control of *Nesidiocoris tenuis*
Project number:	PC 302a
Project leader:	Dr P Morley, Wight Salads Group
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Project coordinator:	Mr P. Pearson, Chairman, TGA Technical Committee
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Key words:	tomato, organic production, *Nesidiocoris tenuis*, IPM, spinosad, natural pyrethrins,

The results and conclusions in this report are based on investigations 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.

Dr P Morley
Senior Company Agronomist
Wight Salads Group

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Report authorised by:

Dr P Challinor
Technical Director
Wight Salads Group

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GROWER SECTION

HEADLINE

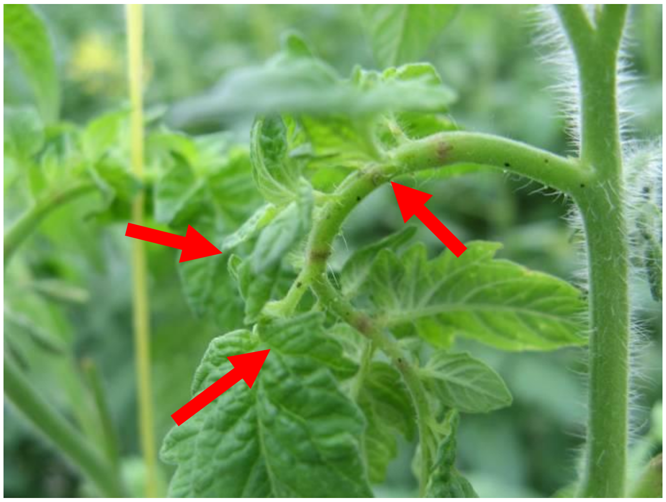
- A short-term strategy based on natural pyrethrins and spinosad has been developed for the control of *Nesidiocoris tenuis* in organic tomato crops.

BACKGROUND AND EXPECTED DELIVERABLES

Nesidiocoris tenuis originated in tropical regions but is now cosmopolitan in the Mediterranean basin. For many years, it has been known to be a voracious predator capable of attacking a wide range of pest species. During the 1990s and early 2000s, most researchers in the Mediterranean region focused on its potential as a biological agent, particularly against *Bemisia tabaci*. However, it is now known that it can also cause severe damage to tomato plants and, as a consequence, it has become a very controversial species.

In the absence of invertebrate prey, the predator will feed on tomato stems, leaf petioles and flower stalks. This initially shows as brown feeding marks, progressing to chlorotic leaf tissue, lost growing points and premature flower / fruit drop as illustrated in the figures below:

Brown feeding marks on leaf petiole



Chlorotic leaf tissue beyond feeding puncture. Similar damage to a stem near the head of the plant can result in loss of the growing point.

Damaged flower stalk leading to premature flower / fruit drop

Furthermore, broad spectrum insecticides, applied to limit direct damage by the pest, disrupt IPM and lead to secondary problems with other pest species. *Nesidiocoris* has been particularly damaging in organic crops because there has been no effective treatment that is allowed under this growing regime. In some situations, the loss in marketable yield has exceeded £100k per ha.

There is evidence that *Nesidiocoris* is moving further north in Europe. It is known to have become established in one conventional tomato crop in the UK in 2007. The infestation was controlled with acetamiprid (Gazelle); a broad spectrum neonicotinoid insecticide which is neither IPM compatible nor allowed in organic production. Established populations have also been confirmed in All Year Round (AYR) crops in Finland, where it is proving difficult to eradicate.

Organic tomato crops in the UK are particularly vulnerable to *Nesidiocoris* because growers will not be allowed to use synthetic pesticides and retain their organic status, even under Plant Health and Safety Inspectorate (PHSI) instruction. The objective of this study was to begin to prepare a contingency plan to manage this pest in organic tomato crops in the UK.

SUMMARY OF THE PROJECT AND MAIN CONCLUSIONS

Preliminary studies

A preliminary desk study investigated options for the control of *Nesidiocoris tenuis* in organic tomato crops in the UK. The following products were given consideration:

Trade name	**Active ingredient**	**Approval status**
Savona	fatty acids	This product has on-label approval for this use
Eradicoat	glucose polymer	This product has on-label approval for this use
Eradicoat T	glucose polymer	This product is not approved
Mycotal	*Verticillium lecanii*	This product has on-label approval for this use
Naturalis-L	*Beauveria bassiana*	This product has on-label approval for this use
BugOil	A mixture of plant oils extracted from thyme, tagetes and wintergreen	This product is not approved
Conserve	spinosad	This product has a specific off-label approval (SOLA) for use on this crop
Pyrethrum 5EC	natural pyrethrins	This product has on-label approval for this use and has a specific off-label approval (SOLA) for use on this crop

There was no published data referring to efficacy studies in which this range of products had been tested against *Nesidiocoris*. However, all except BugOil and Conserve had been tested by the authors against the related Mirid bug, *Macrolophus caliginosus*. *Nesidiocoris* and

Macrolophus are taxonomically similar and have comparable life cycles and life styles. It was therefore reasonable to assume that products that were effective against one species may also have an effect against the other.

Savona, Eradicoat / Eradicoat T, *Verticillium lecanii* and *Beauveria bassiana* were all eliminated based on previous experience of their use against *Macrolophus.* The available evidence suggested that both BugOil and spinosad could have potential and were worthy of evaluation in small scale trials. The most promising option appeared to be natural pyrethrins and their efficacy was investigated in crop-scale trials.

Evaluation of BugOil and spinosad

BugOil and spinosad (as Spintor 480SC) were evaluated against adults and nymphs of *Nesidiocoris* at normal and double the application rates recommended for other pests. All treatments were compared to untreated controls.

Adults and nymphs were collected from a natural population of *Nesidiocoris* which had become established in a mature tomato crop. They were sorted into batches of twenty and each batch was placed on a filter paper in a ventilated dish. Treatments were applied with a hand-held sprayer fitted with a fine nozzle which gave a light covering of the target equivalent to spraying foliage the ‘point of run off’. After treatment, the insects were moved from the wet filter papers to dry filter papers in similar ventilated dishes. They were provided with crumpled tissue paper for refuge, a damp pad for moisture and then kept in the dark at approximately 20^{o}C for five days. The numbers of live insects were recorded daily.

Survival of adults following treatment with BugOil was similar to the untreated control. In comparison, numbers of adults in the spinosad treatments were reduced by 56%. The results were less consistent for nymphs but BugOil and spinosad reduced numbers by 50% and 61% respectively compared to the untreated controls at day 3. Overall, the level of control provided by both products was considered to be insufficient to warrant further evaluation under the less ideal conditions in commercial crops.

Practical evaluation of natural pyrethrins.

The label rate for Pyrethrum 5EC is 20ml product per 5 litres of water (*i.e.* equivalent to 400ml product per 100 litres water). However, the lower rate of 100ml product [5g active ingredient] per 100 litres of water had been shown to be effective against *Macrolophus*. Our preliminary practical studies with natural pyrethrins used an alternative product, Serv-Crisant (this product is not approved in the UK), because Pyrethrum 5EC was not available in Portugal at that time. We tested Serv-Crisant at standard, double and quadruple rates, which spanned the rates for Pyrethrum 5EC in the UK. Each product was applied high volume, to the point of run off, to approximately 350m^2 of a mature organic tomato crop (cv Piccolo). Numbers of *Nesidiocoris* adults and nymphs were recorded immediately pre-treatment and 24 hours post-treatment. Numbers of adult *Nesidiocoris* were reduced by 39%, 91% and 95% following the standard, double and quadruple rate treatments respectively. Numbers of nymphs were reduced by 6%, 27% and 48% respectively. Numbers did not decline in the untreated control.

The maximum overall reduction of 56% at 28.4g active ingredient per 100 litres water was disappointing compared to previous results with natural pyrethrins against *Macrolophus*. This may have been at least in part due to different histories of exposure to insecticides. The *Macrolophus* populations in UK greenhouses originate from biological products that were raised in insecticide-free cultures for many generations and have since had little selection pressure from synthetic insecticides in tomato crops. In contrast, the *Nesidiocoris* were a natural Portuguese population that invaded from local agro-ecosystems where previous generations had been subjected to a wide range of synthetic insecticides over many years. The preliminary practical studies indicated that natural pyrethrins were unlikely to provide adequate control of *Nesidiocoris* with quarantine status in the UK. However, a chance observation suggested that their performance may be enhanced when applied in a tank mix with spinosad and this was followed up with a crop-scale trial.

The tank mix study was done in two mature organic tomato crops; cvs Roturno and Piccolo. The treatment comprised a tank mix of natural pyrethrins as Pyrethrum 5EC (80ml per 100 litres) and spinosad as Spintor 480SC (25ml per 100 litres) (this product is not approved in the UK). The spray was applied with the nursery’s robotic sprayer calibrated to provide cover to the point of run off, which was equivalent to 2,991 litres per hectare. Assessments were done immediately pre-treatment and one day post-treatment. There were 10 sample stations in each of 10 plots within the treated area (*i.e.* 100 in total) and 10 sample stations in each of 4 plots within the untreated area (*i.e.* 40 in total). Two leaves, positioned 1-2 and 3-4 leaves down from the top of the plant, were chosen at random at each sample station. Each leaf was tapped 4 times over a white plastic tray to dislodge adults and large nymphs. The leaf was then scanned for remaining individuals. The numbers of adult and nymphs found on the two leaves were recorded separately for each sample station.

In the untreated control, the mean numbers of *Nesidiocoris* adults and nymphs increased by 6% and 29% respectively. The increase in numbers of adults was probably due to nymphs maturing while the much larger increase in numbers of nymphs was no doubt due to continuous egg hatch. Overall, there was a 17% increase in numbers in the untreated plots between assessments. In contrast, numbers of *Nesidiocoris* adults and nymphs decreased in the treated plots by 89% and 94% respectively.

This treatment can form the basis of a short-term strategy for the control of *Nesidiocoris* in UK tomato crops and as such satisfies the main objective of this project. A single application of the tank mix would be adequate if we were following a 'culling' strategy similar to that employed against populations of *Macrolophus* in the UK. However, as a non-indigenous pest, it is more likely that growers will be instructed to eradicate *Nesidiocoris*. In that case, it is highly likely that at least one further application will be required 7-10 days after the first. Multiple applications to the whole plant will inevitably harm biological control agents used against other pest species.

FINANCIAL BENEFITS

The damage caused by *Nesidiocoris* is similar to that caused by *Macrolophus* but more severe. Prior to HDC project PC 240 (Organic tomato: Development and implementation of a robust IPM programme), it was estimated that the cost of *Macrolophus* infestations in speciality organic tomato crops was over £100k per ha per season (Starkey, Grotek, unpublished data, 2004). Based on this information, the cost of uncontrolled infestations of *Nesidiocoris* in 10ha of organic tomato crops would exceed £1m per season. Effective control measures for organic crops will minimise such losses and allow growers to retain their organic production status. Furthermore, the new control measures will have knock-on benefits to conventional tomato production (particularly those growers attempting 'synthetic pesticide free' production) and will therefore be advantageous to the whole UK tomato industry.

ACTION POINTS FOR GROWERS

- Seek specialist help immediately if you suspect that *Nesidiocoris* is present in your crop.
- Any action must be taken under the instruction / guidance of the PHSI.
- Acetamiprid (Gazelle) has provided control of a population of *Nesidiocoris* in a conventional tomato crop in the UK but it must be used with care within an IPM programme.
- This HDC project has developed a control measure based on a tank mix of natural pyrethrins and spinosad which can be used in organic and conventional crops. The precise rates of use may require some further fine tuning to take into account the discrepancies between recommended dilution rates, the amount of active ingredient allowed per hectare and the quantity of diluted spray required to provide cover of tomato foliage to the point of run off. This issue has previously been addressed for the application of Pyrethrum 5EC against *Macrolophus* on mature tomato crops (SOLA 3026 / 2006) but not, as yet, for products containing spinosad.

GUIDELINES FOR GROWERS

Background:

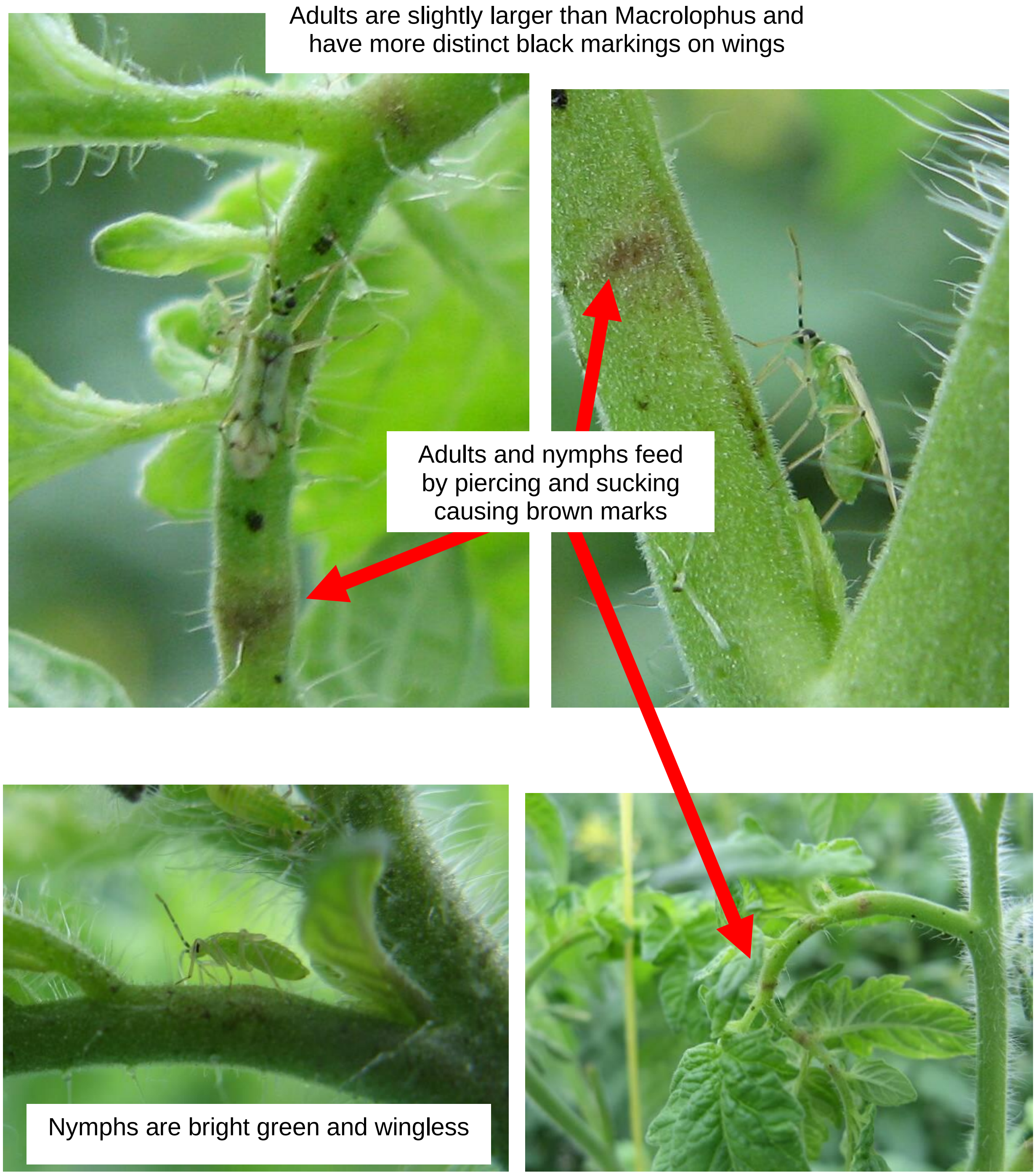
- *Nesidiocoris tenuis* is related to *Macrolophus caliginosus* and has a broadly similar life cycle and life style.
- It is slightly larger than *Macrolophus* and the adult has distinct black markings on its wings and 'knees'.
- Both adults and nymphs predate upon a wide range of insect hosts and can make a significant contribution to an IPM programme.
- In the absence of insect prey, they feed on the plants and are capable of causing very serious damage:
    - Feeding causes characteristic brown markings on stems, leaf petioles and flower stalks.
    - The plant tissue beyond the feeding mark often dies resulting in yellow leaves, lost growing points and premature flower / fruit drop.
    - In some circumstances, localised swelling develops on stems around the feeding mark.

Previous incidence in the UK:

- *Nesidiocoris* is common throughout the Mediterranean basin but not in northern Europe.
- It was found on a nursery in the UK in 2007 where it produced a large and damaging population. It was eradicated with a broad spectrum insecticide.
- It has become established in Finland where it is proving difficult to control.
- *Nesisdiocoris* remains a very real threat to UK growers and it is important that growers are aware of the damage symptoms.

Action:

- Seek specialist help immediately if you suspect that *Nesidiocoris* is present in your crop.
- Any action must be taken under the instruction / guidance of the Fera Plant Health and Seed Inspectorate.
- Acetamiprid (Gazelle) has provided control of a population of *Nesidiocoris* in a conventional tomato crop in the UK but it must be used with care within an IPM programme.
- An HDC project has developed a short-term control measure based on a tank mix of natural pyrethrins and spinosad which can be used in organic and conventional crops.


Adults are slightly larger than Macrolophus and have more distinct black markings on wings
Adults and nymphs feed by piercing and sucking causing brown marks
Nymphs are bright green and wingless

Note that the leaf is yellowing beyond the feeding marks

Damaged flower stalks become yellow and swollen before fruit drop prematurely

Some tomato varieties react to feeding with localised swellings

SCIENCE SECTION

SECTION 1: DESK STUDY TO IDENTIFY SHORT-TERM SUSTAINABLE CONTROL MEASURES FOR *NESIDIOCORIS TENUIS*

The problem

Nesidiocoris (*Cyrtopeltis*) *tenuis* Reuter (Heteroptera: Miridae) originated in tropical regions but is now cosmopolitan in the Mediterranean basin (Goula *et al*, 2002). For many years, it has been known to be a voracious predator capable of attacking a wide range of pest species (Malausa & Henao, 1988). During the 1990s and early 2000s, most researchers in the Mediterranean region focused on its potential as a biological agent (*e.g.* Alomar *et al*, 1991), particularly against *Bemisia tabaci* (Calvo *et al*, 2008). However, it is now known that it can also cause severe damage to tomato plants and, as a consequence, it has become a very controversial species.

The phytophagous habits of *Nesidiocoris tenuis* were initially masked because its population growth in commercial crops in Mediterranean countries was suppressed by broad spectrum insecticides applied against the traditional pest species, such as whiteflies. However, in the early 2000s damage was noted in crops being grown using IPM techniques by WSG in both Spain and Portugal (Morley, unpublished data, 2002). In the absence of invertebrate prey, the predator was seen to feed on tomato stems, leaf petioles and flower stalks. This initially showed as brown feeding marks (Figure 1), progressing to chlorotic leaf tissue (Figure 2), lost growing points and premature flower / fruit drop (Figure 3) (Jacobson, unpublished data, 2005). Furthermore, broad spectrum insecticides, applied to limit direct damage by the pest, disrupted IPM and led to secondary problems with other pest species. *Nesidiocoris tenuis* has been particularly damaging in organic crops because there has been no effective treatment that is allowed under this growing regime (Pettersson, Horticilha, pers.com., 2006). In some situations, the loss in marketable yield has exceeded £100k per ha (Jacobson, 2009). As the use of IPM has become more common throughout the Mediterranean region, the damaging effects of *Nesidiocoris tenuis* have become more widely accepted and reported (*e.g.* Arno *et al*, 2006;

Arno *et al*, 2009; Sanchez & Lacasa, 2008; Calvo *et al*, 2008). It is now universally accepted that *Nesidiocoris tenuis* has both positive and negative characteristics.

There is now evidence that *Nesidiocoris tenuis* is moving further north in Europe. It is known to have become established in one conventional tomato crop in the UK in 2007 (Morley, unpublished data, 2007). The infestation was controlled with acetamiprid (Gazelle) – a broad spectrum neonicotinoid insecticide which is neither IPM compatible nor allowed in organic production. Established populations have also been confirmed in AYR crops in Finland (Vanninen, Agrifood Research Finland MTT, pers.com., 2009). In addition, the authors are trying to substantiate unconfirmed reports of infestations in crops in the Netherlands.

Organic tomato crops in the UK will be particularly vulnerable to *Nesidiocoris tenuis* because growers will not be allowed to use synthetic pesticides and retain their organic status, even under PHSI instruction. The objective of this study is to begin to prepare a contingency plan that can be used to manage this pest in organic tomato crops in the UK.

Figure 1. Brown feeding marks on leaf petiole

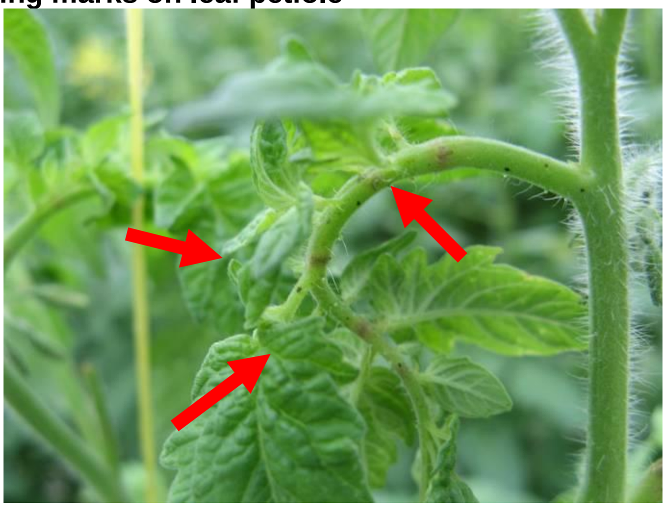


Figure 2. Chlorotic leaf tissue beyond feeding puncture. Similar damage to a stem near the head of the plant can result in loss of the growing point.

Figure 3. Damaged flower stalk leading to premature flower / fruit drop

Control options for organic crops

The options for control of *Nesidiocoris tenuis* in organic tomato crops in the UK are limited. The following products have been given consideration:

Trade name	Active ingredient	Approval status
Savona	fatty acids	This product has on-label approval for this use
Eradicoat	glucose polymer	This product has on-label approval for this use
Eradicoat T	glucose polymer	This product is not approved
Mycotal	*Verticillium lecanii*	This product has on-label approval for this use
Naturalis-L	*Beauveria bassiana*	This product has on-label approval for this use
BugOil	A mixture of plant oils extracted from thyme, tagetes and wintergreen	This product is not approved
Conserve	spinosad	This product has a specific off-label approval (SOLA) for use on this crop
Pyrethrum 5EC	natural pyrethrins	This product has on-label approval for this use and has a specific off-label approval (SOLA) for use on this crop

There is no published data referring to efficacy studies in which this range of products has been tested against *Nesidiocoris tenuis*. However, all except BugOil and Conserve have been tested by the authors against the related Mirid bug, *Macrolophus caliginosus*. *Nesidiocoris tenuis* and *Macrolophus caliginosus* are taxonomically similar and have comparable life cycles and life styles. It is therefore reasonable to assume that products that are effective against one species may also have an effect against the other.

Prior to 2006, control of *Macrolophus caliginosus* in approximately 10 hectares of organic crops on the Isle of Wight depended entirely on Eradicoat / Eradicoat T and Savona. All three products have a physical mode of action and had been shown to control the pest in small scale studies. However, the treatments were only partially effective when used on a large scale and it was necessary to apply them at weekly intervals. This intensive spray programme was expensive, harmful to biological control agents being used against other pests and was believed to be detrimental to plant growth (Howlett, WSG, unpublished data, 2005). Furthermore, it failed to entirely prevent premature fruit drop in the most vulnerable tomato cultivars. An alternative control measure based on natural pyrethrins was developed during the 2006 season (see below). This prevented damage and resulted in savings of over £6k per hectare in product alone (Howlett & Jacobson, 2006). As a consequence, neither Savona nor Eradicoat T will be further considered for the control of *Nesidiocoris tenuis* in this study.

Two strains of *Verticillium lecanii* (Mycotal and Vertalec) and one strain of *Beauveria bassiana* (Naturalis-L) were tested against *Macrolophus caliginosus* on caged tomato plants under favourable environmental conditions in HDC project PC 139 (Sampson & Jacobson, 1999). Mortality following treatment with Mycotal was approximately 40% greater than the untreated controls. Seventy nine percent of the dead insects collected from the Mycotal treated plants were subsequently found to be infected with *Verticillium lecanii*. Mortality following treatment with Vertalec and Naturalis-L was 10% and 12% greater than the untreated controls respectively. None of these products were considered to provide sufficient control of the pest to warrant further evaluation against *Macrolophus caliginosus* under less ideal conditions in commercial crops. Therefore, there is little justification for testing them against *Nesidiocoris tenuis*.

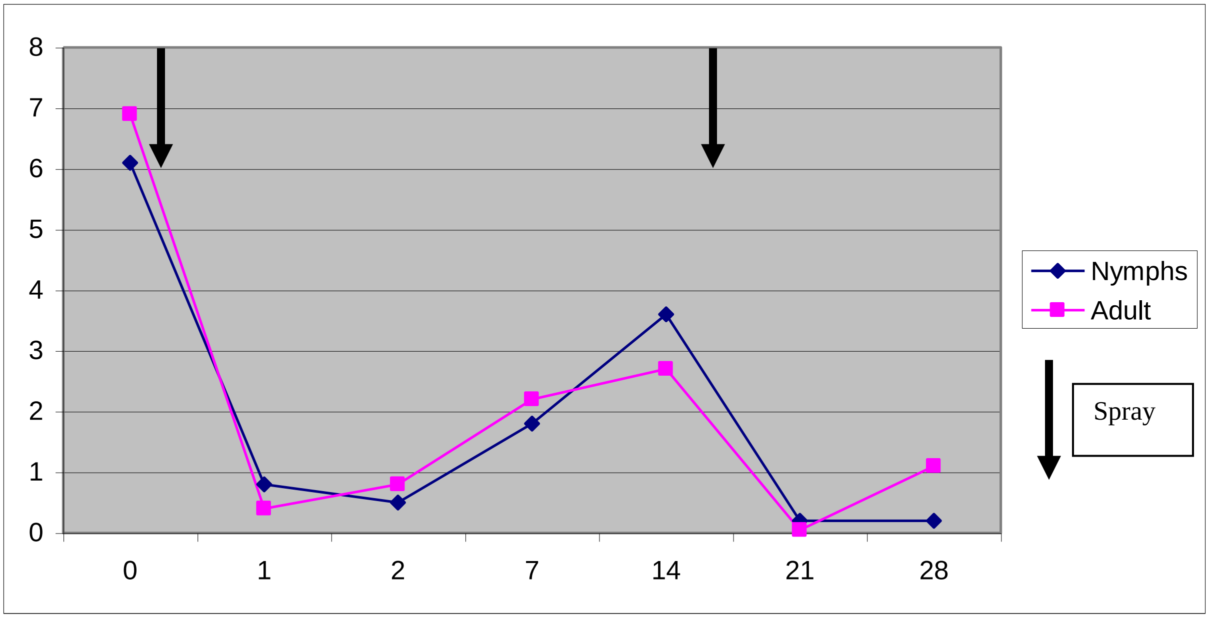
BugOil is undergoing registration and may become available to UK tomato growers during 2011. It is reported to have a broad spectrum of activity against invertebrates, including soft bodied sucking insects like *Nesidiocoris tenuis* (Pearce, Plantimpact, pers.com., October 2009). We have been told that there are no synthetic components in the formulation and it should be acceptable for use in organic production systems. It is not yet known whether it will be compatible with biological control agents used against other pests in the IPM programme. The product appears to be worthy of evaluation against *Nesidiocoris tenuis* in small-scale trials. However, crop-scale trials will not be possible until the product is approved for use in UK tomato crops unless the produce is destroyed.

There are mixed reports about the effect of spinosad on predatory bugs such as *Nesidiocoris tenuis* and *Macrolophus caliginosus*. In a trial in 2009, numbers of *Nesidiocoris tenuis* were reduced by 30% following treatment with spinosad as Spintor 480SC (Verissimo, Horticilha, pers.com., March 2009). Other practitioners have suggested that the effects on populations of both predators could be more devastating (Knight, Koppert UK, pers.com., November 2009). The Koppert BV website states that high volume sprays of spinosad are very harmful to *Macrolophus caliginosus* nymphs (Koppert BV, 2011). In contrast, the guidance notes provided by Fargro for the spinosad-based product Conserve, rate it as harmless to *Macrolophus caliginosus* (*i.e.* less than 25% reduction in numbers) but state that there may be a short term reduction in numbers following treatment. Those guidance notes also state that the predators may be introduced on the day of application, once spray deposits are dry. Given the variation in the available information, spinosad was evaluated against *Nesidiocoris tenuis* in a small-scale trial in this project.

The most promising of the available options appeared to be Pyrethrum 5EC; *i.e.* the product which replaced Eradicoat T / Savona in the *Macrolophus caliginosus* control strategy in 2006. Pyrethrum 5EC contains pyrethrins, which are natural active ingredients extracted from African chrysanthemums (*Chrysanthemum cinerarifolium*). The product is approved for both conventional and organic production in the UK. Pyrethrins act as both contact and stomach poisons. There is no vapour action, systemic activity or leaf penetration, so kill is dependent on direct contact. They have very short persistence under natural conditions and break down quickly under glass because degradation is not dependent on UV light.

The initial trials in commercial tomato crops in 2006 evaluated Pyrethrum 5EC applied as a one or two spray programme against *Macrolophus caliginosus* (Jacobson & Morley, 2006). The results from the time of treatment to 28 days post-treatment are shown in Figure 4. The impact of the first spray on the population, the subsequent recovery in numbers and the impact of the second spay are all quite clear. The recovery was due to the following two factors - i) migration of adults onto the plants from the surrounding untreated crop and ii) emergence of nymphs from eggs embedded in the plant tissue. The former should not happen where a whole crop is treated. The results suggested that treatments will be required in pairs with a 14 day interval to control nymphs hatching from unharmed eggs.

Figure 4. Numbers of *Macrolophus caliginosus* adults and nymphs per leaf up to 28 days post-treatment with Pyrethrum 5EC



In 2007, the emphasis changed from “controlling” to “culling” the *Macrolophus caliginosus* population so that some predators would survive to continue to suppress other pests (Jacobson & Morley, 2007). The key parameters governing the overall effect of applications of Pyrethrum 5EC were considered to be spray concentration, application rate, speed of passage of the robotic sprayer, proportion of plant sprayed and frequency of applications. Fifteen commercial crop scale applications of Pyrethrum 5EC were monitored in 2007 concentrating on one parameter on each occasion. Numbers of *Macrolophus caliginosus* were assessed immediately prior to each application and at varying intervals after application depending on the parameter under investigation. The percentage change between pre- and post-treatment counts was used to provide a simple means of comparing the effects of each treatment. Using this approach, a useful knowledge base was systematically constructed which was subsequently used to guide applications of the product in commercial tomato crops. When using a robotic pipe rail boom sprayer, an effective cull was achieved with 1 litre of Pyrethrum 5EC per 1000 litres of water, applied to the upper half of the plant canopy throughout the crop. It was unlikely that repeat sprays would be required at less than 4-5 week intervals. The same criteria was found to be appropriate when using a hand lance except the less precise spray was targeted to the top half to top two thirds of the plant canopy.

Natural pyrethrins against *Nesidiocoris tenuis*

When *Nesidiocoris tenuis* next arrives in the UK, it is probable that PHSI will insist that the populations be eradicated rather than culled. This study focused on finding an appropriate rate and method of application of Pyrethrum 5EC.

One grower was in a unique position in that they had crops in southern Europe which were infested with this pest and in which efficacy trials could be done on a large-scale. All trials work was done in commercial crops following the general approach that was successfully developed in HDC project PC 240 (Jacobson & Morley, 2007) and more recently used in HDC projects PC 251 (Jacobson, 2008) and PC 295a (Jacobson, 2009a; Jacobson, 2010). This immediately identified any important interactions with current agronomic practice and eliminated the need for an additional exploitation phase to transfer the technology to the commercial situation. In all four examples provided above, the results of the research were implemented by growers within the duration of the projects.

SECTION 2: EVALUATION OF BUGOIL AND SPINOSAD

Method

Insects

Nesidiocoris tenuis were obtained from the following two sources:

- From a natural population which had invaded the glasshouse and become established on a mature tomato crop. This population provided both adults and nymphs.
- As a purchased biological control product consisting entirely of adults.

The samples from the tomato crop were obtained using the method developed for the collection of *Macrolophus caliginosus* (Jacobson & Morley, 2007). Samples from both sources were chilled for approximately two minutes to reduce activity, sorted into twenty batches of twenty adults and placed on filter papers in ventilated Petri dishes. In addition, twenty batches of twenty medium-sized nymphs were collected from the natural population.

Bioassay procedure

The dishes were placed on a cool pad to reduce insect activity while the lids were removed and treatments applied using a hand-held sprayer fitted with a fine nozzle. The sprays were applied to give a light covering of the target which was judged to be equivalent to spraying foliage to 'the point of run off'. After treatment, the insects were moved from the wet filter papers to dry filter papers in similar ventilated dishes. They were provided with a crumpled tissue paper refuge, a damp pad for moisture and then kept in the dark at approximately 20ºC for five days. The untreated controls were subjected to the same chilling and handling procedures but without any spray application.

Treatments

The three insect types (*i.e.* two strains of adults and one of nymphs) were subjected to the following five treatments with four replicates of each:

- Control (no treatment)
- 1% BugOil
- 2% BugOil

- Spintor 480SC (spinosad) at 50ml / 100 litres water
- Spintor 480SC (spinosad) at 100ml / 100 litres water

Assessments

The effects of BugOil usually become apparent within 24 hours (Pearce, Plantimpact, pers.com., December 2010) but spinosad may take longer to kill target organisms (*e.g.* Jacobson & Morley, 2010). Therefore, the numbers of live insects in each replicate were recorded daily over a five day period.

Analysis of data

The data were binomial, *i.e.* we were analysing the proportion of insects surviving at successive time points. Clearly the observations on successive days were not independent as we were observing the same groups of insects from day to day – this meant that observations on different days were correlated; *e.g.* the number of surviving insects on day t + 1 could not be greater than the number of survivors on day t. Nevertheless, we simply compared the survivors on each day, without attempting a formal analysis of the survival rate. A generalised linear model framework was used to compare the five treatments on those days where the numbers were sufficient. Analysis of deviance was used to test differences between the control and treatments, and to compare the two products, the two rates of application and any interaction between them. For adults on day 1, there was significant amount of over-dispersion; *i.e.* the response for different replicates within the same treatment were more variable than one would expect from the binomial distribution. This is normally dealt with in the analysis by using an F-test (as in analysis of variance). For all the other tests (days 2 and 3 for adults and all analyses for nymphs) the standard chi-squared method was used.

On day 1 only 16.25% of the biocontrol adults had survived compared to 78.75% of the crop-derived adults, and by day 2 only 1% of the former were still alive compared to 28.75% of the crop-derived adults. For day 1, a comparison has also been made of the survival of the two adult types as well as the between treatment comparisons. Otherwise, the biocontrol adults have been analysed for day 1; the crop-derived insects for days 1, 2 and 3; and the nymphs (crop-derived) for all five days, though the numbers are small for days 4 and 5.

Results and Discussion

The results are best examined as a series of charts (Figures 5 to 7), which effectively convey all the information. The statistical explanation that follows provides confirmation.

Figure 5. Mean numbers of adult *Nesidiocoris*, sourced from the biocontrol material, over five days following treatment with two rates of BugOil or spinosad.

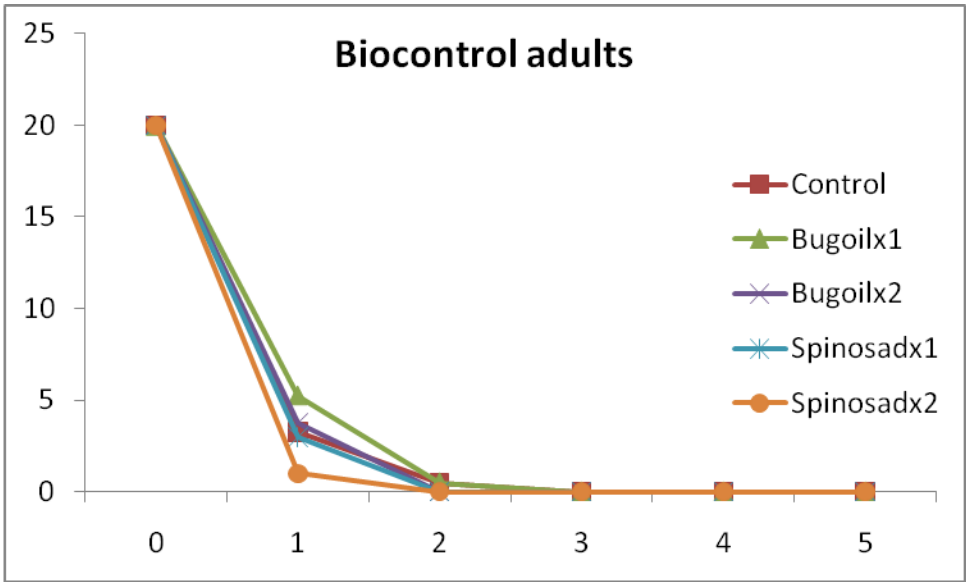


Figure 6. Mean numbers of adult *Nesidiocoris*, collected from the tomato crop, over five days following treatment with two rates of BugOil or spinosad.

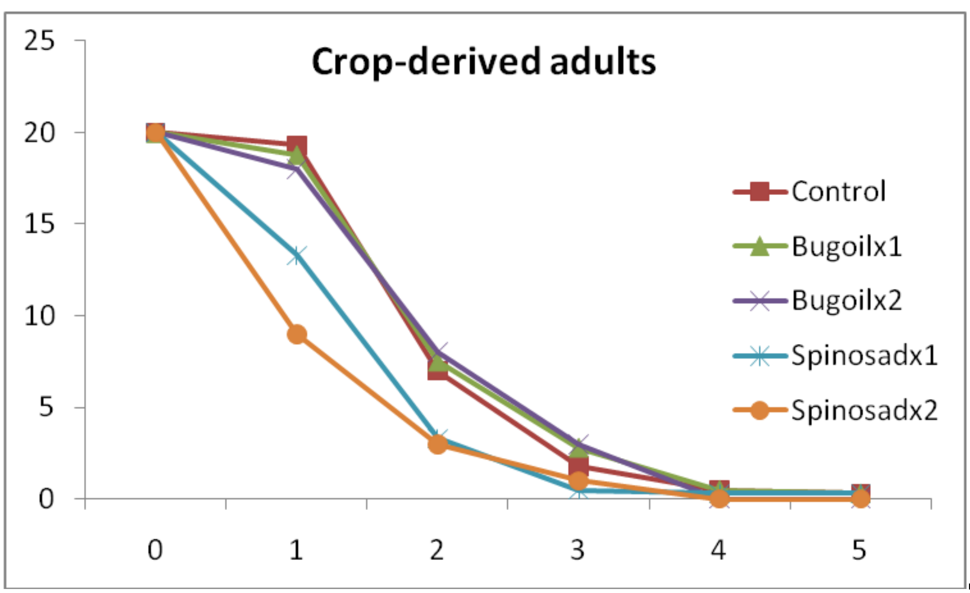
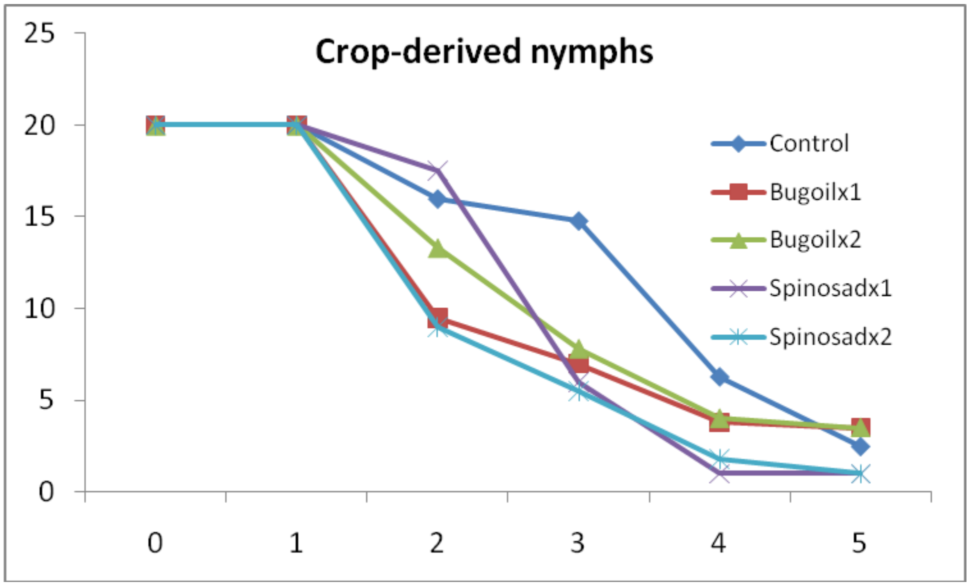


Figure 7. Mean numbers of *Nesidiocoris* nymphs, collected from the tomato crop, over five days following treatment with two rates of BugOil or spinosad.



Comparison of adults on Day 1

Table 1 shows the survival on day 1 for adults from the two insect types. The mean survival is significantly (*p<0.001*) higher in the crop-derived group indicating that the insects in the biocontrol material had been weakened by the rigours of storage and transit.

The difference between the five treatments is also highly significant (*p<0.001*) but there is no significant interaction between insect type and treatment. Breaking this down further, the average difference between the control and the four treatments is marginally significant (*p=0.052*), while that for the difference between products (BugOil and spinosad) is highly significant (*p<0.001*). The difference between rates is also significant (*p=0.041*).

If we take the biocontrol group separately, the analysis is essentially null; *i.e*. no difference between the five treatments. For the crop-derived group, however, there is a significant difference (*p=0.002*) between treatments. This breaks down into a significant difference (*p=0.006*) between control and treatment, and a significant difference (*p=0.002*) between products. Reference to Table 1 suggests a simpler reading with no difference between BugOil and the control, but a marked depression due to spinosad.

Table 1: Mean number of adult insects surviving to day 1 from the two different sources (out of 20)

	Crop	**Biocontrol**
Control	19.3	3.3
BugOilx1	18.8	5.25
BugOilx2	18	3.75
Spinosadx1	13.3	3
Spinosadx2	9	1

Analyses for days 2 and 3 (Crop-derived adults)

The mean survivors for days 2 and 3 are shown in Table 2. Beyond day 3 there is no point in formally analysing the data due to the high level of natural mortality. For days 2 and 3 there remains a significant difference between treatments (*p=0.003* for day 2 and *p=0.008* for day 3), but the only detailed significant effect is for the difference between products. In fact, for all three days it is quite evident that the spinosad treatments depress the survival rate compared to the control and BugOil treatments.

Table 2: Mean number of crop-derived adult insects surviving through the experiment (out of 20)

Day	0	1	2	3	4	5
Control	20	19.3	7.0	1.8	0.5	0.3
BugOilx1	20	18.8	7.5	2.8	0.5	0.3
BugOilx2	20	18	8	3	0	0
Spinosadx1	20	13.3	3.3	0.5	0.3	0.3
Spinosadx2	20	9	3	1	0	0

Analysis of nymph data (all days)

From Figure 7 we see that no nymphs died until the second day, and that the responses changed somewhat from day to day; *i.e.* the survival profiles are not entirely consistent. Overall, however, it does appear as if the treated nymphs survived less well than the controls, and that the spinosad-treated nymphs had higher mortality towards the end of the trial.

Table 3 shows that the responses on day 2 were very inconsistent with the lower rate of BugOil causing more mortality than the double dose. Formal analysis shows a highly significant difference (*$p<0.001$*) between treatments, but breaking this down shows that the most important component contrast is the interaction between product and rate confirming the comments above. For day 3, there is also a highly significant effect of treatments overall (*$p<0.001$*), but this is almost entirely due to the contrast between the control and the mean of the treatments – this can be clearly seen in Figure 7. By day 4, there is a clear difference between the spinosad treatments and the other three treatments. This is reflected in a significant effect of control *vs* treatments (*$p=0.0014$*) with a smaller effect of products (*$p=0.0275$*). On day 5, the only significant effect is that of products (*$p=0.0032$*) with spinosad having eliminated nearly all of the nymphs and 17.5% of BugOil-treated nymphs still surviving.

Table 3: mean number of crop-derived nymphs surviving through the experiment (out of 20)

Day	0	1	2	3	4	5
Control	20	20	16	14.8	6.3	2.5
BugOilx1	20	20	9.5	7	3.8	3.5
BugOilx2	20	20	13.3	7.8	4	3.5
Spinosadx1	20	20	17.5	6	1	1
Spinosadx2	20	20	9	5.5	1.8	1

Conclusion

There was a high level of natural mortality, which had to be separated from the effects of the treatments. This reflects the fragility of these soft bodied insects and was so severe for the biocontrol material that the results have been discounted. The overall profile of the crop-harvested adults was quite consistent with BugOil-treated insects similar to the control and both surviving better over the first three days than those treated with spinosad. At days 2 and 3, numbers of adults in the spinosad treatments had been reduced by an overall mean of 56% compared to the untreated controls. Although the results were less consistent for the nymphs, BugOil and spinosad had reduced numbers by about 50% and 61% respectively when compared to the untreated controls at day 3. Overall, the level of control provided by both products was considered to be insufficient to warrant further evaluation under the less ideal conditions in commercial crops.

SECTION 2: A SHORT-TERM SOLUTION TO INFESTATIONS OF *NESIDIOCORIS TENUIS*

Background:

Of the products currently available, the desk study indicated that natural pyrethrins offered the most promising option for the control of *Nesidiocoris tenuis* in organic tomato crops in the UK. The label rate for Pyrethrum 5EC is 20ml product per 5 litres of water (*i.e.* equivalent to 400ml product per 100 litres water). However, the lower rate of 100ml product per 100 litres of water had been shown to adequately ‘cull’ populations of *Macrolophus caliginosus* (Jacobson & Morley, 2007). As Pyrethrum 5EC contains 5% natural pyrethrins, the above rates comprised 20g and 5g of active ingredient per 100 litres water respectively.

Our preliminary practical studies with natural pyrethrins used an alternative product, Serv-Crisant, because Pyrethrum 5EC was not available in Portugal at that time. Serv-Crisant contains 3.16% natural pyrethrins, plus the synergist PBO, and the standard recommended application rate is 225ml per 100 litres of water. We tested the product at standard, double and quadruple rates, which contained 7.1g, 14.2g and 28.4g product per 100 litres water respectively. This range spanned the rates for Pyrethrum 5EC in the UK. Each product was applied high volume to the point of run off to approximately 350m^2 of a mature organic tomato crop (cv Piccolo). There were 22 sample stations per treatment area and numbers of *Nesidiocoris tenuis* adults and nymphs were recorded immediately pre-treatment and 24 hours post-treatment at each station. Numbers of adult *Nesidiocoris tenuis* were reduced by 39%, 91% and 95% following the standard, double and quadruple rate treatments respectively. Numbers of nymphs were reduced by 6%, 27% and 48% respectively. Numbers did not decline in the untreated control.

The maximum overall reduction of 56% at 28.4g active ingredient per 100 litres water was disappointing compared to previous results with natural pyrethrins against *Macrolophus caliginosus*. This may have been at least in part due to different histories of exposure to insecticides. The *Macrolophus caliginosus* populations in UK greenhouses originate from biological products that had been raised in insecticide-free cultures for many generations. In contrast, the *Nesidiocoris tenuis* were natural populations that invaded from local agro-ecosystems where previous generations will have been subjected to a wide range of insecticides.

The preliminary practical studies indicated that natural pyrethrins were unlikely to provide adequate control of *Nesidiocoris tenuis* with quarantine status in the UK. However, a chance observation suggested that their performance may be enhanced when applied in a tank mix with spinosad. This was explored in more detail in the trial described below.

Materials and Methods

The study was done in two mature organic tomato crops (cvs Roturno and Piccolo) which had been planted in August 2009 at Horticilha, Cilha Queimada, Alcochete, Portugal. The condition of the plants at the start of the trial is shown in Figure 8.

The treatment comprised a tank mix of natural pyrethrins as Pyrethrum 5EC (80ml per 100 litres) and spinosad as Spintor (25ml per 100 litres). The spray was applied between 2000 hrs and 2300 hrs on 15 May 2010 with the nursery's robotic sprayer (Figure 9). The sprayer was calibrated to travel at 100m of row per 283 seconds which provided spray cover to the point of run off. This was equivalent to 2,991 litres of diluted spray per hectare.

The pre-treatment and post-treatment assessments were completed on 15 and 16 May respectively. There were 10 sample stations in each of 10 plots within the treated area (*i.e.* 100 in total) and 10 sample stations in each of 4 plots within the untreated area (*i.e.* 40 in total). Previous studies have shown that the majority of *Nesidiocoris tenuis* are active within the upper third of the plants and so the assessments focused on that part of the crop canopy. Two leaves, positioned 1-2 and 3-4 leaves down from the top of the plant, were chosen at random at each sample station. Each leaf was tapped 4 times over a white plastic tray to dislodge adults and large nymphs. The leaf was then scanned for remaining individuals. The total numbers of adult and nymphs found on the two leaves were recorded separately for each sample station.

Figure 8. Condition of plants at the start of the trial

Figure 9. The robotic sprayer used to apply the spray

Results and discussion

The mean numbers of *Nesidiocoris tenuis* adults and nymphs in each treatment on each assessment date are shown in Table 4.

In the untreated control, the mean numbers of *Nesidiocoris tenuis* adults and nymphs increased by 6% and 29% respectively. The increase in numbers of adults was probably due to nymphs maturing while the much larger increase in numbers of nymphs was no doubt due to continuous egg hatch. Overall, there was a 17% increase in numbers in the untreated plots between assessments.

In contrast, numbers of *Nesidiocoris tenuis* adults and nymphs decreased in the treated plots by 89% and 94% respectively. The effect on adults was probably an underestimate because there was known to be some reinvasion from heavily infested adjacent crops. Nonetheless, there was an overall 93% reduction in numbers in the treated plots between assessments. The differences between treatments were so clear that no further analysis of data was deemed necessary.

This treatment can form the basis of a short-term strategy for the control of *Nesidiocoris tenuis* in UK tomato crops and as such satisfies the main objective of this project. Both active ingredients are available in the UK as products that are acceptable in organic production and could be used to control this pest without compromising a grower's organic status.

A single application of the tank mix would be adequate if we were following a 'culling' strategy similar to that employed against populations of *Macrolophus caliginosus*. However, as a non-indigenous pest, it is more likely that growers will be instructed to eradicate the pest. In that case, it is highly likely that at least one further application will be required 7-10 days after the first. Multiple applications to the whole plant will inevitably harm biological control agents used against other pest species.

The precise rates of use may require some further fine tuning to take into account the discrepancies between recommended dilution rates, the amount of active ingredient allowed per hectare and the quantity of diluted spray required to cover tomato foliage to the point of run off. This issue has previously been addressed for application of Pyrethrum 5EC against

Macrolophus caliginosus on mature tomato crops (Notice of extension of use number 3026 of 2006) but not, as yet, for products containing spinosad.

Table 4. Mean numbers of *Nesidiocoris tenuis* adults and nymphs in each treatment on each assessment date.

	Treated		Untreated	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Adults	5.5	0.6	3.3	3.5
Nymphs	17.3	1.1	8.3	10.7
Total	22.7	1.7	12.1	14.2

TECHNOLOGY TRANSFER

- Presentation to TGA Technical Committee, 3 June 2009

- Presentation to Tomato Conference 2009 (24 September 2009, Coventry); 'Two more threats: *Tuta absoluta* and *Nesidiocoris tenuis*'.

- Presentation to Tomato Pest and Disease Seminar (14 January 2010, Stoneleigh); 'Tuta absoluta: Biology and control'.

- Article in HDC News (May 2010, Vol 163, 18-19); 'Research catching up with Tuta'.

- PC 302 Project Update to TGA Technical Committee, 2 June 2010

- Invited presentation to Tomato Conference 2010 (September 2010, Coventry); 'Update on *Tuta absoluta* and *Nesidiocoris tenuis*'.

Further technology transfer planned for 2011:

- Article in HDC News

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