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[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

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

Dr R J Jacobson

Director

Rob Jacobson Consultancy Ltd

RJaudsion Date 22/12/14 Signature ....

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## **GROWER SUMMARY**

#### Headline

A pheromone has been shown to be attractive to the parasitic tachinid, *Trichopoda pennipes*, which attacks *Nezara viridula*. If *T. pennipes* were present in the local ecosystem, then the pheromone could be used to draw it into crops infested by *N. viridula*.

### Background

*Nezara viridula* (southern green shieldbug) is believed to be native to Ethiopia but is now widely distributed across tropical and subtropical regions of the world. It feeds on a wide range of plants and is a serious pest of many important food crops. It has been imported into the UK on fruit and vegetable products for many years but was not found in the wild until 2003. *Nezara viridula* has now found a favourable niche in heated glasshouses in the north London area and it seems highly likely that it will eventually be transported to other parts of the country on produce and packing materials. It also seems likely that the pest would survive year round anywhere in the UK if it were within a glasshouse with frost protection between crops.

Sexually mature males of *N. viridula* have been shown to release a pheromone which is said to be attractive in the field to females, males, and late-stage larvae of the same species. Formulated pheromone was obtained by Russell IPM from a source in Japan, where it was reported to be a highly effective attractant of *N. viridula*. Practical evaluation of pheromone baited traps began in this project in 2013, in and around glasshouses in the north London area, and later at infested sites near the Instituto Superior de Agronomia, Lisbon.

### Summary

The pheromone lures were placed within plastic cages which were then placed in Delta traps. The inner floor of the Delta trap was covered with a removable sticky plate. In a preliminary study, the 'glue' used on the sticky plate had been shown to be capable of retaining both adult and nymphal stages of healthy *N. viridula*. The traps were always

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placed in pairs 5-10m apart and similarly orientated. One trap of each pair would contain a pheromone lure while the other would be a control without an attractant. Pheromone lures were replaced at 5-6 week intervals.

The traps were first tested between weeks 33 and 39 in 2013 within a vegetable 'allotment' immediately outside a commercial glasshouse which had suffered a serious infestation of *N. viridula* during the previous growing season. The allotment contained the usual range of vegetable crops that are grown in UK gardens including several types of podding legumes. There was also a fig tree within the allotment which is known to be a favoured host of *N. viridula* in Mediterranean countries. No *N. viridula* were caught in any of the traps. Extensive surveys, which involved beating foliage of numerous species of shrubs and herbaceous plants in the immediate vicinity, also failed to detect any *N. viridula* during 2013.

Due to the lack of *N. viridula* at the UK site in 2013, work was transferred to parkland at the Instituto Superior de Agronomia, Lisbon which was known to be infested with the pest. Traps were placed in early September 2013 which was much later in the year than would have been the case had it been the intention to work at that site from the outset. No *N. viridula* were caught in any of the pheromone-baited or unbaited traps but it was impossible to say whether this was because the pheromone was ineffective, or because the insects' behaviour had changed towards the end of the season; *i.e.* they were no longer seeking a mate. There was, however, an unexpected development in that a significant number of large tachinid flies were captured in each baited trap while none were present in the unbaited traps. The tachinids were subsequently identified by a specialist taxonomist as *Trichopoda pennipes*; a parasitoid that attacks true bugs and specifically members of the Pentatomidae and Coreidae. The fly is sometimes referred to as the "feather-legged fly" because of the prominent fringe of feather-like bristles on its legs.

*Nezara viridula* was not reported at any UK sites until the end of the 2014 growing season which was too late to continue any monitoring or control studies. However, more comprehensive trapping exercises were carried out at two infested sites in Portugal between June and October 2014. The results were comparable to 2013. No *N. viridula* were found in any pheromone-baited traps nor in any unbaited traps. However, an average of 7.3 *T. pennipes* was caught per pheromone-baited trap.

Russell IPM has been assured that the pheromone has been extremely attractive to *N. viridula* in Japan. It is therefore possible that the European populations are of a different genotype to those found in Japan and respond to a different combination / ratio of chemical components in the pheromone. The fact that the parasite does respond to the pheromone indicates that the differences are only marginal.

*Trichopoda pennipes* is not specific to *N. viridula* and this would probably eliminate it as a released biocontrol agent unless it is already in the UK. It has not yet been recorded as indigenous to the UK but this may be because no one has specifically looked for it or stumbled across it by chance. We know it is present in Europe and information from North America indicates that it is capable of surviving at least as far north as Ontario and Massachusetts where winter conditions are far more severe than in southern England. The pheromone tested in this project could provide an opportunity to accelerate a search for *T. pennipes* in the UK.

### **Financial Benefits**

Current control measures against *N. viridula* in the UK are based on broad spectrum insecticides which are extremely disruptive to IPM. This can lead to secondary problems with other pests which must also be controlled with chemical insecticides. The disruption and termination of the IPM programme would result in UK growers losing an important marketing advantage over their overseas competitors. Initial observations suggest that losses due to direct damage, secondary pest problems and the loss of goodwill with retail customers could be substantial. While this project has not yet found a solution to this problem, the results do indicate a way forward for further research.

### **Action Points**

A formulated pheromone has been shown to be attractive to the parasitic tachinid, *T. pennipes*. If *T. pennipes* were present in the local ecosystem, then the pheromone could be used to draw that parasitoid into crops infested by *N. viridula*.

*Trichopoda pennipes* has not yet been recorded in the UK. The pheromone could be a useful tool in surveying a range of habitats in the UK for its presence. The possibility of its use as a biocontrol agent would be greatly increased if it were found in the wild.

Other monitoring and IPM compatible control systems for *N. viridula* could not be tested in this project due to the lack of populations of the pest in UK commercial crops until the very end of the 2014 season. There are still several options for both monitoring and control of *N. viridula* which have realistic potential and should be tested in crops as soon as the opportunity arises.

## SCIENCE SECTION

#### Introduction

*Nezara viridula* is believed to be native to Ethiopia but is now widely distributed across tropical and subtropical regions of the world. It feeds on a wide range of plants and is a serious pest of many important food crops. It has been imported into the UK on fruit and vegetable products for many years but was not found in the wild until 2003. It is mainly confined to the south-east of England which is thought to be the northern limit of its outdoor range. It is now considered to be established in London and the surrounding area. Breeding populations have most commonly been found in man-made habitats such as parks, gardens and allotments, where it is seems particularly fond of leguminous plants. The adults are strong fliers and are capable of long-distance natural dispersal during warm weather.

*Nezara viridula* has found a favourable niche in heated glasshouses in the north London area and it seems highly likely that it will eventually be transported to other parts of the country on produce and / or packing materials. It also seems likely that the pest would survive year round any where in the UK if it were within a glasshouse with frost protection between crops.

In 2012, the HDC commissioned the production of a factsheet to provide an introduction to the biology and recognition of the pest and to help growers to distinguish it from less damaging native species of plant bugs (Jacobson, 2012). This document was intended to help prevent unnecessary insecticidal treatments due to misidentification of the target organism. Also in 2012, HDC obtained an EAMU (Number 1994/12) enabling growers to use lambda-cyhalothrin (Hallmark with Zeon Technology) against *N. viridula* on pepper and aubergine crops. However, this product is extremely harmful to the biological control agents used against other pests in the IPM programmes for these crops. Most notably, applications of lambda-cyhalothrin could lead to secondary problems with *Frankliniella occidentalis* (western flower thrips) and associated infection with tomato spotted wilt virus (Jacobson, 2009; Jacobson, 2010; O'Neill, 2009). It is vitally important that UK growers have access to IPM compatible control measures against *N. viridula* as soon as possible.

In 2013, HDC commissioned Project PE 014, in which Dr Jacobson and Associates collated information about monitoring and control measures used against *N. viridula* in other parts of the world (Jacobson *et al.*, 2013). The study gleaned information via literature searches, insecticide databases and direct from suppliers of biological, chemical and other IPM-

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related products, as well as from professional contacts in the International Organisation for Biological Control and the International Biocontrol Manufacturers' Association who had first hand experience of *N. viridula* in other countries and other crops. The *N. viridula*-related results of PE 014 were submitted to HDC as an interim report in April 2013 and have since been summarised for growers in a Technical Briefing Note (Jacobson, 2013). The desk study identified several monitoring and control measures that could be exploited by UK growers in the short, medium and longer-term. Based on these results, it should be possible to develop monitoring methods that can be used to accurately time IPM compatible treatments that are based on biological, physical and chemical techniques.

Sexually mature males of *N. viridula* have been shown to release a pheromone which is said to be attractive in the field to females, males, and late-stage larvae of the same species (Aldrich *et al.*, 1987; Miklas *et al.*, 2003; Tillman *et al.*, 2010; Shimizu and Tsutsumi, 2011; DoBae *et al.*, 2012). In parallel to the HDC desk study, partners in PE 014 accessed the chemical components of the pheromone so that they could be tested in traps in the 'field'. Practical evaluation of these traps began in this project in 2013 in and around glasshouses in the north London area and later at infested sites near the Instituto Superior de Agronomia, Lisbon.

Other possible methods of detecting and monitoring populations of *N. viridula* include light traps (Combs, 2000). However, trials involving these traps require secure sites where the traps are not vulnerable to interference or vandalism. Such sites were not available to this project.

Plants which are more attractive to *N. viridula* than the crop have been used as traps in and around valuable broad acre crops in the USA, Australia and New Zealand (see Jacobson *et al.*, 2013, pages 25-26). Unpublished reports from allotments in the London area indicate that podding beans are highly attractive to the pest. The size and growth habit of dwarf French beans could make them ideal candidates for use as trap plants under the main crop canopy in glasshouse grown sweet peppers and aubergines. Alternatively, pymetrozine (Chess) has been used to control related pests in other crops (Jacobson, 2002). This chemical insecticide with anti-feedant properties should be properly evaluated both as a high volume spray and applied via the irrigation system in a commercial crop situation. Both of these approaches to the control of *N. viridula* require an infested glasshouse crop in which to conduct trials. Unfortunately no such crops were available to the team during this project.

## **Materials and Methods**

Formulated pheromone was obtained by Russell IPM from a source in Japan where it was reported to be a highly effective attractant of *N. viridula*. The pheromone was maufactured and incorporated into lures in August 2013 (Batch No S/1756) with a guaranteed shelf life until August 2015 if stored in sealed foil packages at temperatures below 10°C.

The pheromone lures were placed within plastic cages which were then placed in Delta traps (Figure 1). The inner floor of the Delta trap was covered with a removable sticky plate. In a preliminary study, the 'glue' used on the sticky plate had been shown to be capable of retaining both adult and nymphal stages of healthy *N. viridula*. The pheromone lures were replaced at 5-6 week intervals.



Figure 1. The assembled Delta trap (left) and the plastic cage housing the pheromone lure (right) which was incorporated within the trap.

The traps were always placed in pairs 5-10m apart and similarly orientated. One trap of each pair would contain a pheromone lure while the other would be a control without an attractant. They were tested on four occasions in various habitats in the following situations:

In week 33 2013, two pairs of traps were placed in a vegetable 'allotment' immediately outside a commercial glasshouse in Roydon Essex, which had suffered a serious infestation of *N. viridula* during the previous growing season. The allotment contained the usual range of vegetable crops that are grown in UK gardens including several types of podding legumes. There was also a fig tree within the allotment which is known to be a favoured host of *N. viridula* in Mediterranean countries. This whole site was closely monitored between April and September 2013. This incuded extensive beating of foliage to collect insects from numerous species of shrubs and herbaceous plants. The traps remained in place until week 39 2013.

#### Test 2

In week 36 2013, six pairs of traps were placed within the Tapada da Ajuda; a public environmental and botanical parkland of about 100 ha which is home to the Instituto Superior de Agronomia, Lisbon. This parkland was a known habitat for *N. viridula* and the pests could readily be found on a wide range of vegetation. The traps were maintained and regularly examined by an MSc student at the University who worked under the supervision of Dr Jacobson. They remained in place until week 46 2013.

#### Test 3

In week 26 2014, two pairs of traps were placed in a citrus orchard and a further two pairs of traps were placed in an olive grove, all in the grounds of the Instituto Superior de Agronomia, Lisbon. Populations of *N. viridula* were confirmed by visual inspection of plants in both situations. The traps remained in place until week 31.

#### Test 4

In week 27 2014, nine pairs of traps were placed in public parkland at Ferreira do Zêzere in the Santarém District of Portugal which is approximately 80 miles north east of Lisbon. The vegetation was varied, ranging from *Pinus pinaster* woodland to citrus and olive groves, as well as small vegetable gardens growing a range of cucurbitaceous and solanaceous 'crops'. *Nezara viridula* could be found throughout the horticultural area and was also a nuisance in domestic buildings. Care was taken to place the pairs of traps in as many different habitats as possible (*e.g.* Figure 2) but they were never closer than 100m to another pair. They remained in place until week 40 2014.



Figure 2. Two examples of the position of pheromone baited traps at Ferreira do Zêzere, Portugal, during 2104.

### **Results and Discussion**

No *N. viridula* were caught in any of the traps that were set at the UK site in 2013 (*i.e.* Test 1). Glasshouse crops at this site had been infested with the pest during the previous year but they had been sprayed with a synthetic pyrethroid insecticide at the end of that growing season. Extensive surveys, which involved beating foliage of numerous species of shrubs and herbaceous plants in the immediate vicinity, failed to detect any *N. viridula* during 2103.

Due to the lack of *N. viridula* at the UK site in 2013, work was transferred to parkland at the Instituto Superior de Agronomia, Lisbon which was known to be infested with the pest (*i.e.* Test 2). Traps were placed in early September which was much later in the year than would have been the case had it been the intention to work at that site from the outset. No *N. viridula* were caught in any of the pheromone-baited or unbaited traps but it was impossible to say whether this was because the pheromone was ineffective or because the insects' behaviour had changed towards the end of the season; *eg.* perhaps they were now focused on finding a 'hibernation' site rather than a mate? There was, however, an unexpected development in that a significant number of large tachinid flies were captured in each baited trap (Figure 3) while none were present in the unbaited traps. The tachinids were subsequently identified by a specialist taxonomist as *Trichopda pennipes* (Fabricus) (Dr Dominiqe Collins, Fera, personal communication, January 2014).

*Trichopoda pennipes* (Figure 4) is a parasitoid that attacks true bugs, specifically members of the Pentatomidae and Coreidae. It is native to North and South America but was apparently accidentally introduced into southern Europe about 20 years ago. Fauna Europeae (a database of the scientific names and distribution of all living multicellular European land and fresh-water animals) lists France, Italy and Spain as countries from which *T. pennipes* has been recorded but an on-line reference also lists Slovenia. In the United States it has been used in biological control programmes with *N. viridula* one of its prime targets (Jacobson *et al.*, 2013). The fly is sometimes referred to as the "feather-legged fly" because of the prominent fringe of feather-like bristles on its meta-tibiae.



Figure 3. *Trichopda pennipes* as it appears on the sticky base of a pheromone trap



Figure 4. *Trichopda pennipes* photographed live (Courtesy of Stephen Cresswell, 'AmericanInsects.net')

The results of trapping in Test 3 were comparable to Test 2. No *N. viridula* were caught despite the pheromone traps being in position from June. No *T. pennipes* were caught in the citrus orchard but an average of 1.5 *T. pennipes* were caught per pheromone-baited trap per week in the olive grove.

The most comprehensive of all the trapping exercises was completed in Test 4. Nine pairs of traps were in position over a 14 week period from June to October 2014. *Nezara viridula* could be found on a wide range of vegetation throughout the whole period. Nevertheless, no *N. viridula* were found in any pheromone-baited nor on any unbaited traps. However, an average of 7.3 *T. pennipes* was caught per pheromone-baited trap while none were caught in the unbaited traps.

Russell IPM has been assured that the pheromone has been extremely attractive to *N. viridula* in Japan although there is no published information to support the claims. It is therefore possible that the European populations are of a different genotype to those found in Japan and respond to a different combination / ratio of chemical components in the pheromone. The fact that the parasite does respond to the pheromone indicates that the differences are only marginal.

*Trichopoda pennipes* is not specific to *N. viridula* and this would probably eliminate it as a released biocontrol agent unless it is already in the UK. It has not yet been recorded as indigenous to the UK but this may be because no one has looked for it or stumbled across it by chance. We know it is present in mainland Europe and information from North America indicates that it is capable of surviving at least as far north as Ontario and Massachusetts (O'Hara and Woods, 2004) where winter conditions are far more severe than in southern England. The pheromone tested in this project could provide an opportunity to accelerate a search for *T. pennipes* in the UK.

## Conclusions

It would appear that the pheromone sold for use in monitoring traps to catch *N. viridula* in Japan is not effective against the populations found in Western Europe. This may be because the populations in Europe and Japan are different genotypes.

The pheromone has been shown to be attractive to the parasitic tachinid, *T. pennipes*. If *T. pennipes* were present in the local ecosystem, then it seems possible that the pheromone could be useful in drawing that parasite into crops infested by *N. viridula*.

As yet, *T. pennipes* has not been recorded in the UK. The pheromone could be an extremely useful tool in surveying a range of habitats in the UK for the presence of *T. pennipes*. The possibility of its use as a biocontrol agent would be greatly increased if it were found in the wild.

Other monitoring and IPM compatible control systems for *N. viridula* could not be tested in this project due to the lack of populations of the pest in commercial crops until the very end of the 2014 season. There are still several options for both monitoring and control of *N. viridula* which have realistic potential and should be tested in commercial crops as soon as the opportunities arise.

## Knowledge and Technology Transfer

The Pepper Technology Group has been regularly updated on both progress and constraints to progress throughout this project.

A review of the existing Technical Briefing Note (Jacobson, 2013), or a possible article about *N. viridula* for HDC News, are subject to further discussion before the project ends.

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