Grower Summary

CP 197

Rapid DNA-based identification of brown marmorated stink bug, *Halyomorpha halys*

Final 2020

Project title:	**Rapid DNA-based identification of brown marmorated stink bug, *Halyomorpha halys***
Project number:	CP 197
Project leader:	Glen Powell, NIAB EMR
Report:	Final report, February 2020
Previous report:	None
Key staff:	NIAB EMR Glen Powell, Celine Silva QMUL Elizabeth Clare, Joseph Trafford, Rosie Drinkwater
Location of project:	Queen Mary University of London (QMUL) NIAB EMR
Industry Representative:	N/A
Date project commenced:	1 November 2019

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The results and conclusions in this report are based on an investigation conducted over a 4-four-month 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.

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GROWER SUMMARY

Headline

A highly sensitive and specific DNA-based test has been developed and validated for detection of the invasive pest insect, brown marmorated stink bug (*Halyomorpha halys*), enabling us to rapidly diagnose any material (e.g. dried and fragmentary eggs) which may be found in agricultural settings.

Background

The UK faces the emergence of a new, invasive crop pest recently confirmed to be present in South-East England, in the wild. The brown marmorated stink bug (BMSB) (Pentatomidae: *Halyomorpha halys*) is a serious and generalist pest native to Asia but spreading globally with serious impacts on agricultural productivity. Modeling based on regional climate data and the insect life cycle suggests that South-East England is the most suitable region of the UK for establishment. Adult BMSB were found in Hampshire in 2018/2019, but other life stages (indicating breeding populations) have not yet been reported. The insect poses a likely imminent threat to UK horticulture as it can feed on and damage a wide range of plant species, including soft fruit, ornamentals, field vegetables and tree fruit. The adults and immature stages of this new invasive pest are very similar in appearance to those of native UK shield bug species making it difficult to differentiate between them. Indeed, when egg masses are found in crops it is currently impossible to identify them to species.

This project was designed with four objectives:

1) Develop a high-throughput, rapid DNA-based method for forensic detection of BMSB.

2) Evaluate the reliability of this test for species-level identification of adults, nymphs and egg masses.

3) Investigate the feasibility of combining the new test with monitoring (e.g. using pheromone-baited traps) as part of an early BMSB surveillance programme.

4) Evaluate this test for secondary detection of parasitoids which may contribute to natural biocontrol.

A key deliverable of the project was to make the test available to UK growers for the 2020 season in the event that BMSB is suspected or detected and mass screening required.

Summary

In this project, we assembled an extensive database of DNA sequences from BMSB and other shield bug species that are commonly confused with this pest. This reference database will be made available as a resource internationally to aid other researchers in DNA-based identification of BMSB. The database has enabled the design and testing of new PCR primers as part of this project. Two new sets of primers (named 8XT and 13XT) have a high affinity for BMSB DNA. The 8XT primers show particularly high sensitivity for amplification of BMSB DNA and work even with trace material at extremely low DNA concentrations (with effective detection following 10,000 times dilution of the original DNA) and degraded samples (e.g. empty egg cases following hatching which were dried and not otherwise preserved). In combination with high-throughput sequencing this provides a robust and rapid (diagnosis within a few days) tool for BMSB identification of degraded fragmentation trace material.

A secondary goal of the project was to test existing 'general' primers for insects, with the potential to amplify DNA from BMSB but also from other insect species in mixed samples. Such general primers will aid the development of a less sensitive assay for BMSB detection, but one with the additional advantage of simultaneously detecting other insect species that are economically important (e.g. parasitoids and other natural enemies of BMSB, or other shield bug pest species that pose a risk to crops). The project has therefore also identified and tested two additional sets of PCR primers (Beth and Zeale) with potential for application to more complex insect species mixes. One of these primers (Beth) shows wide success with other insect species and some potential for detection of egg parasitoids of BMSB and other shield bug pests (subfamily Scelioninae), although some redesign of one of the primer pairs would be necessary to allow effective and reliable species differentiation of parasitoids.

In summary, this project provides a robust DNA-based test for BMSB and the basis for commercialisation of a diagnostic tool for BMSB and other insect species relevant to this invasive pest. Samples can already be processed at QMUL on a contract basis (http://research.sbcs.qmul.ac.uk/e.clare/Elizabeth_Beth_Clare_BMSB.html). The company that carried out all sequencing analysis as part of the project (NatureMetrics, UK) are currently in discussion with us regarding full commercialisation of the technology to provide a rapid diagnostic test for UK growers.

Financial benefits

Brown marmorated stink bug has expanded its global range in recent years and become a major agricultural pest of a wide range of crops. As a generalist insect, able to feed on more

than 100 different host-plant species, outbreaks often result in substantial economic damage to multiple crops. Adult and immature (nymph) stages of the pest inflict damage when they insert their stylet mouthparts into plant tissue for feeding and injection of toxic saliva. The insects particularly target flower buds and fruiting bodies on a variety of crops, resulting in the marketable produce becoming scarred, discoloured and deformed. The pest causes substantial losses in arable field crops (e.g. sweetcorn) in addition to vegetables (e.g. tomatoes, peppers, beans), tree fruit (e.g. apples and pears) and soft fruit (e.g. raspberries). UK apples and pears are at a particularly high risk of damage. In 2010, BMSB damage to apple crops alone in the Mid-Atlantic region of the USA was estimated to result in losses of 37 million US Dollars while stone fruit growers in the same region and period lost more than 90% of their crop. In one region of Italy (Emilia-Romagna), combined losses to pear, peach and nectarine crops have been estimated to exceed 350 million Euros in one year (2018 figures).

Effective control of BMSB requires action to be taken at an early stage following colonisation of new areas. Attract-and-kill approaches, combining applications of aggregation pheromone and a contact insecticide to selected trees, are showing promise for control in USA orchards. However, any effective mitigation strategies are reliant on rapid and accurate identification of the pest. The detection methodology developed during this project can facilitate significant advances in the speed, accuracy and sensitivity of DNA-based identification of this invasive pest species, with particularly useful application to material which is not morphologically identifiable (e.g. eggs).

Action points

- Crops at potential risk of BMSB damage following its establishment in the UK include top fruit (particularly apples), soft fruit and field vegetables (including sweetcorn and legumes). Growers should be vigilant for signs of shield bug life stages (adults, nymphs and eggs) in and around crops. To aid identification and check whether samples are BMSB, a diagnostic test is now available via the Clare Lab at QMUL on a contract basis (http://research.sbcs.qmul.ac.uk/e.clare/Elizabeth_Beth_Clare_BMSB.html). Further discussions with commercial partners are ongoing to establish the analysis as a permanent commercially-available diagnostic test for growers.
- The development of this test required construction of an extensive database of DNA sequences (BMSB and closely-related / potentially-confused species). This will be made available to other researchers via the BOLD website (http://www.boldsystems.org/) as a web-accessible public project. It will be included

as a downloadable supplement to any academic publications arising from the project.

- New PCR primers have been designed. One primer set has extremely high affinity for BMSB DNA and was therefore suitable for the development of a specific detection test targeting the invasive pest. A set of generalist primers could be more suitable for simultaneous detection of parasitoid DNA, although some modification of the primer design would be required to achieve this.
- Further research is required to determine false positive rates. This is currently being undertaken with a second round of DNA sequencing which includes a number of samples of insect DNA but excludes BMSB DNA. These will be analysed blind and we will look for BMSB identifications where none should be possible (no BMSB DNA added, therefore false positives). Results are anticipated in October 2020.