Grower Summary

SF 124

Development and validation of a molecular diagnostic test for strawberry tarsonemid mite

Final 2013

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Use of pesticides

Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use, except where the crop or situation is the subject of an off-label extension of use.

Before using all pesticides check the approval status and conditions of use.

Read the label before use: use pesticides safely.

Further information

If you would like a copy of the full report, please email the HDC office (hdc@hdc.ahdb.org.uk), quoting your HDC number, alternatively contact the HDC at the address below.

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Project Number:	SF 124
Project Title:	Development and validation of a molecular diagnostic test for strawberry tarsonemid mite
Project Leader:	Professor Jerry Cross
Contractor:	East Malling Research
Industry Representative:	Richard Harnden, Berry Garden Growers Ltd
Report:	Final Report 2013
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Previous report/(s):	Annual Report 2012
Start Date:	01 April 2011
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Project Cost:	£44,014

Headline

- A laboratory based molecular test that specifically identifies strawberry tarsonemid mite (*Phytonemus pallidus* ssp. *fragariae*) (Ppf) has been successfully developed.

- In conjunction with a mite extraction step this test can be used to screen strawberry foliage and crowns for the presence of both adult and immature Ppf and their eggs.

Background and expected deliverables

Management in UK fruiting plantations

UK growers currently use a combination of approaches to control the strawberry tarsonemid mite. (1) They source clean certified planting material but experience shows that the material from the main Dutch and Spanish suppliers often has low levels of infestation. (2) Plantations are inspected frequently in spring and early summer for signs of damage and infestation and infested plants are grubbed and destroyed. This approach rapidly becomes costly and uneconomic. (3) *Amblyseius* predatory mites are introduced to prevent or suppress outbreaks but this approach is only partially effective and cannot contain outbreaks in hot weather conditions. (4). Spray applications of abamectin (Dynamec) or tebufenpyrad (Masai) when damaging infestations start to develop, give partial control so delaying the spread or infestation and damage. The number of applications of abamectin (Dynamec) and tebufenpyrad (Masai) are limited to 3 and 1 respectively and, in any event, sprays used during flowering and fruiting on everbearers are undesirable.

Need for a rapid, sensitive and reliable diagnostic test

Ensuring that planting material is free from the pest is clearly the best way of controlling strawberry tarsonemid mite. Testing for the presence or absence of the pest currently relies on visual searching of samples of growing points for the presence of mites under a stereo microscope. It is very time consuming and laborious to search large samples in this way and there is a high risk that small numbers of mites will be missed. There is an opportunity to develop a highly sensitive, rapid DNA-based molecular test for the pest which will enable growers to ensure that planting material is free from tarsonemid mite, or at least to be more aware of the degree of risk. Note that the evidence suggests that tarsonemid mite is not ubiquitous and that it is not present in some propagation crops (e.g. UK planting material is normally free of the pest). The problem is more a question of sample size as of sensitivity of the test. It will only be possible to sample a small sub-sample of the total number of plants in the propagation crop, e.g. 500-1000 growing points, each from a separate randomly chosen plant. A highly sensitivity test is needed to ensure the pest can be detected in such a

sample; no such test is currently available and searches for sequence data upon which such a test would be developed are also not available, suggesting that no other groups outside of the UK are in the process of such a development. A decision will need to be taken as to the tolerance level (risk of the pest being present) in the sampled crop. The risk of one or more young leaves being infested in a sample of n leaves for a given tolerance can be estimated from probability statistics using the binomial distribution.

Summary of the project and main conclusions

Using conventional methods the identification of strawberry tarsonemid mite, *Phytonemus (Tarsonemus) pallidus* ssp. *Fragariae* (Ppf), requires the separation of mites from leaf material, slide mounting of the mites and identification based on the appearance of key morphological characteristics. The objective of this project was to develop a molecular approach (based on DNA detection) to identify Ppf with the aim of reducing the time and expertise required to perform the test, which it is hoped will reduce the cost of the analysis. A molecular marker that could be used to discriminate Ppf from other species of mite commonly found on strawberries was identified and an assay designed based on real-time PCR. The result is a species-specific and sensitive method that can be used for rapid identification of all life stages of Ppf. The method does not discriminate live and dead mites, although visual assessment of the sample prior to the PCR test does give an indication of viability. The complete test ensures the detection of an infestation level of 0.5% with 95% confidence. The test can be undertaken by scientists at Fera.

Financial benefits

Strawberry tarsonemid mite can cause devastating crop losses in highly valuable protected strawberry crops, with losses exceeding £10,000 per ha per season being incurred in some instances. Ensuring that planting material is free from the pest is clearly the best way of controlling strawberry tarsonemid mite and avoiding such potential losses. The development of a highly specific PCR test for Ppf will make the current screening method more effective as it will, for the first time, allow the non-adult stages of this pest to be identified thus increasing the chances of detecting infested stocks prior to planting.

Action points for growers

To achieve good and consistent results from Ppf screening tests, it is important that the following guidelines are observed by growers when collecting and submitting samples to the Fera laboratory:

- Sample the most actively growing parts of the plant i.e. crowns and small newly emergent or partially unfurled leaves as they are the most likely places to find Ppf when present.

- Each sample should be no more than 250g in weight and consist of approximately 600 leaves or pieces of crown material of a similar size.

- Do not include roots and avoid taking material from senescent or rotting leaves and crowns as well as excessive extraneous material such as soil which can interfere with the test.

- Samples should be clearly labelled and sent in sealed plastic bags by prior arrangement with the laboratory to avoid delays in processing the material, which may result in samples deteriorating and becoming unsuitable for screening