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<b>Project leader:</b>	Rosemary Collier, University of Warwick
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<b>Key staff:</b>	Marian Elliott
<b>Location of project:</b>	Warwick Crop Centre, University of Warwick, Wellesbourne
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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

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.

[Name]

[Position]

[Organisation]

Signature ..... Date .....

[Name]

[Position]

[Organisation]

Signature ..... Date .....

### Report authorised by:

[Name]

[Position]

[Organisation]

Signature ..... Date .....

[Name]

[Position]

[Organisation]

Signature ..... Date .....

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# GROWER SUMMARY

## Headline

The pest mite causing damage to outdoor spinach crops was confirmed as *Tyrophagus similis*. This species feeds mainly on material and organisms associated with organic matter in the soil and may damage spinach only as a consequence of its need to avoid adverse soil conditions.

## Background

During late summer-autumn 2010, outbreaks of mites caused damage to outdoor spinach crops in southern England. These outbreaks occurred at several locations. The problem has occurred previously but it is sporadic in nature. The aims of this project were to:

1. Confirm the identity of the mites causing damage to spinach crops.
2. Develop a laboratory culturing technique to provide mites for experiments on control
3. Collate and summarise information on the biology of the mites that would be relevant to predicting and controlling infestations
4. Identify potential control methods and test them on a small scale

## Summary

Despite the very wet weather in summer 2012, small samples of mites were obtained from field-grown spinach (provided by growers and field-collected by Warwick Crop Centre) on three occasions and these were sent to Fera for identification. All samples contained *Tyrophagus similis*, confirming the identity of this pest.

Attempts to establish a culture and undertake tests on control methods were unsuccessful due to the small number of mites recovered in 2012.

### *Literature review*

A literature review was undertaken and key points are as follows:

*Tyrophagus similis* appears to be quite widely distributed world-wide and the main studies on it have been undertaken in Japan and the Yemen. In Japan, mites damage spinach grown in greenhouses in particular. The mites penetrate spinach shoots and feed on young leaves. The shoots are readily accessible because they are close to the soil surface. As the plants grow, the damaged leaves show small holes and/or deformation.

*Tyrophagus similis* is one of a number of species of small arthropods that have an important role in mineral turnover, vegetation succession and decomposition of organic matter. It has been observed to feed mainly on organic fertilizers, plant detritus, small organisms and the dead bodies of soil arthropods. The development of *T. similis* populations on organic wastes and immature composts may be due to their feeding on fungi that occur in these materials. Generally, the growth of fungi is greater on organic wastes and immature composts than on mature ones, because mature composts have already been decomposed by several forms of microorganisms. *Tyrophagus similis* lives in, and on, soil at depths of 0-5 cm and numbers decrease with increasing depth.

The low temperature threshold for development for *T. similis* is 7°C and females can lay several hundred eggs in their lifetime. Egg viability of *T. similis* declined at temperatures above 30 °C, female survival was reduced at temperatures above 35°C, and of *T. similis* maintained at 10, 15, 20 and 25 °C, those maintained at 10 °C had the greatest fecundity during their lifetime.

In Japanese research on spinach crops grown in greenhouses, the *T. similis* population in the soil remained at low levels during the hot season from May to September, increased rapidly in October and November, remained at a high level during the cool season from December to February, and further increased in April. The mites, which were in the soil, infested the spinach plants mostly in late autumn and early spring. The high temperatures in the greenhouses from spring to early autumn were considered to be one of the main causes of population decrease. It was hypothesised that the mites initially increase in number on, or in, cultivated soil that is rich in organic matter and then invade the spinach plants. It seemed likely that the mites use the spinach plants as a shelter from harsh physical conditions in the surface soil (e.g. high temperature and dryness) in the warm season, because few mites inhabited spinach plants during the cool season even when mite density in the soil was relatively high. If this is the case, then the movement of the mites to the

spinach plants might not be primarily to obtain food. It seemed to the Japanese researchers that *T. similis* was more closely associated with the soil than with spinach plants, because it was feeding on and living in various types of organic matter in the soil. If the mites live mainly in the soil, attempts to control the mites by spraying the plants with pesticide might be expected to have a limited effect. In Japan, attempts have been made to control *Tyrophagus similis* with agrochemicals. Such agrochemicals have been used generally after an increase in damage is observed, but in many cases, their effects have been unsatisfactory. This suggests that the chemicals had little direct contact with the mites in both the soil and spinach plants.

Apart from temperature, dryness of the soil surface and tillage after cultivation might reduce mite numbers temporarily. Solar heating and hot water treatment may be effective for controlling *T. similis* in the soil in greenhouses. To effectively control the mites, it may be necessary to keep the temperature above 35 °C for several hours. Cultural approaches to reduce damage to spinach could be by: (1) Reducing the use of organic fertilizers during the mite season, (2) removing plant residues on the soil before cultivation, and (3) reducing the use of immature organic materials, because they increase fungi that are suitable food for *T. similis*. Other mites are certainly likely to be predators of *T. similis*, but an effective biological control system has not been developed.

It is likely that the target for control should be mite populations in the soil rather than the mites in the spinach and there may be many mites in the soil at times when there are none on the spinach plants. Thus, surveying mite numbers in the soil may be of value. This can be done using Tullgren funnels to extract mites from soil samples, although this method is labour-intensive. A Japanese group developed a relatively simple 'trap' for monitoring mites in soil. The trap is a piece of folded moistened filter paper containing a small amount of dry yeast. Rather than deploy these traps in the field, the researchers took samples of soil from the field, placed them in polythene bags and then the traps were placed on the surface of the soil in each sample bag. The sample bags were sealed and the traps were checked at intervals and the mites identified and counted. When this approach was compared with the use of Tullgren funnel samples, higher numbers of mites were obtained.

#### *Potential control methods*

Potential control methods highlighted by the literature review include:

- Control with pesticides

- This depends on a suitable active ingredient being available and approved for spinach.
  - The review indicates that the mites may be a difficult target and that soil treatment might be more effective than treatment of the plants (which may be too late). Soil treatment requires an effective method of application.
- Biological control
    - There might be a suitable control agent available commercially.
    - However, this would be a very expensive, and potentially labour-intensive, way of controlling a sporadic pest.
    - Efficacy has not been demonstrated.
  - Physical control
    - Cultivation may reduce mite populations for one or more reasons.
    - Heating the soil (with black polythene?) may be effective.
    - Irrigation may make the soil environment less hostile at certain times and prevent the mites infesting the spinach plants.
  - Cultural control
    - Management of organic material in the soil may be key.
  - Monitoring
    - Use of the filter paper trap approach may highlight large mite populations.

## **Financial Benefits**

The results of this project will contribute to an understanding of this damaging, but sporadic, pest and identify possible methods of predicting and controlling infestations. The proposed project is complementary to the LINK project submitted by a consortium led by the HDC: Sustainable Crop and Environment Protection - Targeted Research for Edibles (SCEPTRE).

## **Action Points**

- Growers should aim to minimize the amount of partly-degraded organic matter in the soil prior to sowing a spinach crop.



## SCIENCE SECTION

### Introduction

During late summer-autumn 2010, outbreaks of mites caused damage to outdoor spinach crops in southern England (Figure 1). These outbreaks occurred at several locations. The problem has occurred previously (Chris Wallwork UAP, personal communication) but it is sporadic in nature. Samples of infested spinach were sent to Wellesbourne and attempts were made to:

- Make a basic identification
- Culture the mites in the laboratory

It was thought that the mites were a species of *Tyrophagus*, possibly *T. similis* but there was a need for confirmation of this identification. *Tyrophagus similis* is a pest of spinach in Japan and there are a number of papers on its biology and control which could provide useful background information. For example, Kasuga and Amano (2000b) found that egg hatch by *T. similis* declined sharply at temperatures above 30 °C, and that female survival was limited at temperatures above 35 °C. They also found that of *T. similis* maintained at 10, 15, 20 and 25 °C, those maintained at 10 °C had the greatest lifetime fecundity and that the developmental threshold of *T. similis*, i.e., the temperature at which development ceases, was 7 °C. In a later paper (Kasuga & Amano, 2003), they suggested that the mites survive in the soil feeding on detritus (so were not obligate plant feeders) and travel into the spinach buds when conditions at the soil surface become unfavourable (i.e. too hot or dry).

The aims of this project were to:

1. Confirm the identity of the mites causing damage to spinach crops.
2. Develop a laboratory culturing technique to provide mites for experiments on control
3. Collate and summarise information on the biology of the mites that would be relevant to predicting and controlling infestations
4. Identify potential control methods and test them on a small scale

The very wet weather conditions in the summer of 2012 meant that it was impossible to achieve objectives 2 and 4 within the timescale of the project and so an extension to the project was requested.



Figure 1. Spinach damaged by an infestation of mites (image provided by Chris Wallwork, UAP)

## Materials and methods

### 1. Confirm the identity of the mites causing damage to spinach crops.

This was done by asking growers for samples from infested crops and sending them for verification of identity by mite taxonomists at Fera.

### 2. Develop a laboratory culturing technique to provide mites for experiments on control

This followed from the initial attempt to culture the mites prior to the start of the project. In 2012 attempts were made to do this on pot-grown spinach in controlled environment rooms in the Insect Rearing Unit at Warwick Crop Centre. There may be other approaches that could be used, based on information in the literature.

### 3. Collate and summarise information on the biology of the mites that would be relevant to predicting and controlling infestations

A literature survey was undertaken and all relevant information was summarized, highlighting any information that might help growers to predict or avoid infestations.

#### **4. Identify potential control methods and test them on a small scale**

The aim is to use the literature, conversations with agrochemical companies and other experts to identify potential control methods, chemical or otherwise. It is worth considering the pesticides already approved for use on spinach and others that might be approved through a SOLA before looking more widely. Tests will be done on a small scale in the laboratory or in small outdoor plots using mites from the culture.

## **Results**

#### **1. Confirm the identity of the mites causing damage to spinach crops.**

The summer of 2012 was extremely challenging because of the prolonged period of rainfall and very wet conditions in the field. Small samples of mites were obtained from field-grown spinach (provided by growers and field-collected by Warwick Crop Centre) on three occasions and these were sent to Fera for identification. All samples contained *Tyrophagus similis*, confirming the identity of this pest.

#### **2. Develop a laboratory culturing technique to provide mites for experiments on control**

The use of pot-grown spinach plants to culture *T. similis* was not effective, possibly because the numbers of mites used to infest the plants were very low.

A large amount of plant material was obtained from commercial crops but the infestation was very low and it would have taken many days to extract a very small number of mites. As an alternative approach, small plots of spinach were sown in one of the Dutch Lights at Warwick Crop Centre and the material (sorted and the remaining unsorted material) was spread between the plants. These plots were overwintered and further plots have been sown in 2013.

### **3. Collate and summarise information on the biology of the mites that would be relevant to predicting and controlling infestations**

Information on *T. similis* from the literature is summarised below:

#### **Distribution**

*Tyrophagus similis* appears to be quite widely distributed world-wide and the main studies on it have been undertaken in Japan (papers by Kasuga and colleagues) and the Yemen (Al-Safadi, 1991) (who also worked at the University of Birmingham).

#### **Damage**

In Japan, mites of the genus *Tyrophagus* damage spinach grown in greenhouses in particular (Nakao 1989). The mites penetrate spinach shoots and feed on young leaves. The shoots are readily accessible because they are close to the soil surface. As the plants grow, the damaged leaves show small holes and/or deformation. Mites of this genus have spread throughout the commercial spinach growing areas of Japan and the problem has increased over time (Kasuga and Amano 2000a). They can also damage spinach seed during germination (Kasuga and Amano, 2006).

*Tyrophagus similis* Volgin is the mite species that causes the most damage to spinach in Japan (Kasuga and Amano 2000a). Damage by *T. similis* is mostly observed in greenhouses in early spring and late autumn when greenhouse temperatures are generally moderate (Kasuga & Amano, 2003). *Tyrophagus similis* can also damage melon, cucumber, pumpkin and maize (Nakao and Kurosa, 1988). Whilst *T. similis* is abundant in the Yemen, its pest status there is less clear (Al-Safedi, 1987).

#### **Biology**

*Tyrophagus similis* is one of a number of species of small arthropods that has an important role in mineral turnover, vegetation succession and is a decomposer of organic matter in combination with microflora (Al-Safedi, 1987). This species has been found in grassland, soil, plants, old hay, chaff, duck and bee nests (Hughes, 1976) and greenhouses. In greenhouses, *T. similis* has been observed to feed mainly on organic fertilizer, plant detritus, bryophytes and the dead bodies of soil arthropods (Kasuga & Amano, 2003). More recently, Kasuga and Honda (2006) fed *Tyrophagus similis* on three organic wastes (cattle

faeces, sawdust, and rice straw), an organic fertilizer (rapeseed meal), three composts (made from cattle faeces, rice husks, and rice straw), three fungi (*Fusarium oxysporum*, *Pythium aphanidermatum*, and *Rhizoctonia solani*) and eight vegetables (lettuce, cucumber, komatsuna, spinach, pak choi, garland chrysanthemum, Welsh onion, and tomato), to determine their suitability as food, using the fecundity of females as an indicator. Rapeseed meal, fungi, and most vegetables were suitable food sources. The organic wastes and composts were unsuitable. *Tyrophagus similis* lives in, and on, soil at depths of 0-5 cm and numbers decrease with increasing depth (Kasuga & Amano, 2005).

The two main research groups working on this species have studied its basic biology and responses to temperature. Al-Safedi (1987) found that female mites laid an average of 18 eggs, singly or in small clusters and that at 21°C the complete life-cycle took 10-12 days. It is difficult to reconcile some of the information from this study with the more detailed work undertaken in Japan (Kasuga and Amano, 2000b). Kasuga and Amano found that the low temperature threshold for development ( $t_0$ ) for *T. similis* is 7°C and that females can lay several hundred eggs in their lifetime (Table 1), laying as many eggs at 10 and 15°C as at 20 and 25°C (Kasuga & Ammano, 2000b). The numbers of eggs laid per day during the oviposition period were in the range of 8-11 (Table 1). A temperature of 30°C was not suitable for development of the immature stages, which in this case agrees with the conclusions of Al-Safedi (1991). Mites survived better at relative humidities of 76, 87 and 100% than at 53 and 66% (Kasuga & Ammano, 2000b).

**Table 1.** Developmental parameters for *T. similis* (from Kasuga and Amano, 2000b).

	Total longevity of adult (days)	Lifetime fecundity (days)	Mean generation time (days)	Mean number of eggs laid per day during oviposition period
10	124	663	102	8
15	83	547	61	11
20	42	335	38	10
25	22	149	22	10

In the Japanese research in greenhouses, the *T. similis* population in the soil remained at low levels during the hot season from May to September, increased rapidly in October and November, remained at a high level during the cool season from December to February,

and further increased in April (Kasuga and Amano, 2003). The mites, which were in the soil, infested the spinach plants mostly in late autumn and early spring. The high temperatures in the greenhouses from spring to early autumn were considered to be one of the main causes of population decrease, even though the soil must buffer the mites from direct sunlight. These findings were consistent with the previous laboratory observations (Kasuga and Amano, 2000b) where they found that egg viability of *T. similis* declined at temperatures above 30 °C, that female survival was reduced at temperatures above 35°C, and that of the *T. similis* maintained at 10, 15, 20 and 25 °C, those maintained at 10 °C had the greatest fecundity during their lifetime.

Kasuga and Amano (2003) hypothesised that the mites initially increase in number on, or in, cultivated soil rich in organic matter and then invade the spinach plants. It seemed likely to them that the mites used the spinach plants as a shelter from harsh physical conditions in the soil surface (e.g. high temperature and dryness) in the warm season, because few mites inhabited spinach plants during the cool season even when mite density in the soil was relatively high (approximately 30 mites per 100 cm<sup>3</sup> of soil). If this is the case, then the movement of the mites to the spinach plants might not be primarily to obtain food and it seemed that *T. similis* was more closely associated with the soil than with spinach plants because it was feeding on, and living in, various types of organic matter in the soil. Thus, if the mites mainly live in the soil, attempts to control the mites by spraying the plants with pesticide would be expected to have a limited effect.

Following their study on the best diets for *T. similis*, Kasuga & Honda (2006) altered their view slightly in terms of organic wastes and hypothesised that the increase of *T. similis* on organic wastes and immature composts may be due to their feeding on fungi that occur in these materials. Generally, the growth of fungi is greater on organic wastes and immature composts than on mature ones, because mature composts have already been decomposed by several forms of microorganisms.

## **Control**

In Japan, attempts have been made to control *Tyrophagus similis* with agrochemicals. For example, Dichlorvos emulsifiable concentrate and DCIP (bis(2-chloro-1-methylethyl) ether) granules have been approved for use to control this species in Japan. Such agrochemicals have been used generally after an increase in damage is observed, but in many cases, their effects have been unsatisfactory. This suggests that the chemicals in both the soil and spinach plants had little direct contact with the mites (Kasuga and Amano, 2002; 2003).

## Cultural control

Apart from temperature, dryness of the soil surface and tillage after cultivation might reduce mite numbers temporarily. Solar heating and hot water treatment may be effective for controlling *T. similis* in the soil in greenhouses. To effectively control the mites, it may be necessary to keep the temperature above 35 °C for 5 hours (Kasuga and Amano 2000b). The study by Kasuga & Honda (2006) on the 'best' diet for *T. similis* suggested ways to reduce damage to spinach by: (1) Reducing the use of organic fertilizers (such as rapeseed meal in their case) during the mite season, (2) removing plant residues on the soil before spinach cultivation, and (3) reducing the use of immature organic materials, because they increase fungi that are suitable for *T. similis*.

## Biological control

Other mites are certainly likely to be predators of *T. similis* (Al-Safedi, 1987). For example, *Hypoaspis aculeifer* was investigated as a predator (Kasuga et al., 2006). It can eat a whole range of small animals and in laboratory tests it was possible to rear it on *T. similis*, although *T. similis* was not the 'best' host. In addition, its optimum temperature range is higher than that of *T. similis* (Kasuga et al., 2006), so it might not be a suitable predator in the field in the UK. The same thing was true for another predatory mite investigated in Japan, *Hypoaspis yamauchii*, which was a predator of *T. similis* at 20-25°C but not at 15 or 30°C (Saito, 2012).

## Timing of treatments

The results of a study on control with agrochemicals suggested to Kasuga & Amano (2003) that the mites could be controlled successfully by application of agrochemicals in the autumn. They considered that an effective method of chemical control would be spraying the soil or mixing granules in the soil. However, it would be difficult to determine the threshold at which control measures should begin because there is a time lag between mite feeding and the subsequent spinach damage and certainly if control measures are not begun until spinach damage is apparent, it may be too late.

It is likely that the target should be populations in the soil rather than the mites in the spinach, as the latter are probably relatively inaccessible to insecticides. In addition, there may be many mites in the soil at times when there are none on the spinach plants. Thus, surveying mite numbers in the soil is of value because mite outbreaks in the soil often cause spinach damage. Kasuga & Amano (2003) used Tullgren funnels to extract mites from soil samples, but this method is labour-intensive. Kasuga *et al.* (2005) developed a relatively simple 'trap' for monitoring mites in soil. The trap is a piece of folded moistened

filter paper containing a small amount of dry yeast. Rather than deploy these traps in the field, Kasuga *et al* took samples of soil from the field, placed them in polythene bags and then the traps were placed on the surface of the soil in each sample bag. The sample bags were sealed and the traps were checked at intervals and the mites identified and counted. When this approach was compared with the use of Tullgren funnel samples, higher numbers were obtained.

#### **4. Identify potential control methods and test them on a small scale**

Potential control methods highlighted by the literature review include:

- Control with pesticides
  - i. This depends on a suitable active ingredient being available and approved for spinach.
  - ii. The review indicates that the mites may be a difficult target and that soil treatment might be more effective than treatment the plants (which may be too late). Soil treatment requires an effective method of application.
- Biological control
  - i. There might be a suitable control agent available commercially.
  - ii. However, this would be a very expensive and potentially labour-intensive way of controlling a sporadic pest.
  - iii. Efficacy has not been demonstrated.
- Physical control
  - i. Cultivation may reduce mite populations for one or more reasons.
  - ii. Heating the soil (with black polythene?) may be effective
  - iii. Irrigation may make the soil environment less hostile at certain times and prevent the mites infesting the spinach plants
- Cultural control
  - i. Management of organic material in the soil may be key.
- Monitoring
  - i. Use of the filter paper trap approach may highlight large mite populations.

## **Discussion**

Although weather conditions did not favour research on this pest in summer 2012, sufficient numbers were obtained to confirm its identity and the literature review revealed some useful



background information. It looks as if pesticidal control may not be a viable option for a number of reasons including a possible lack of suitable actives, and the difficulties of targeting the mites in space and time. Cultural approaches may be a better option, but would require some forward planning.

## Conclusions

The pest mite causing damaging to outdoor spinach crops was confirmed as *Tyrophagus similis*. This species feeds mainly on material and organisms associated with organic matter in the soil and may damage spinach as a consequence of its need to avoid adverse soil conditions.

## Knowledge and Technology Transfer

There have been no knowledge transfer activities to date.

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