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The results and conclusions in this report are based on a series of experiments. The conditions under which the studies were carried out and the results have been reported with detail and 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 the 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 this report represents a true and accurate record of the results obtained.

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Headlines

There are three different types of spider mites on UK tomato crops, which have different rates of population growth on different cultivars and are capable of producing different types of damage. The three types of spider mite are green *Tetranychus urticae* (two-spotted spider mite), red *Tetranychus cinnabarinus* (carmine mite) and green *T. cinnabarinus*. Increasing type VI trichome density on tomato leaves reduces population growth of all three types of spider mites. In terms of speed of population growth and rate of dispersal, the threat posed by the three types of spider mites may be ranked as follows: red *T. cinnabarinus* > *T. urticae* > green *T. cinnabarinus*. The differences are sufficiently great to warrant different control strategies. Both types of *T. cinnabarinus* can cause the more severe form of damage (hyper-necrosis) to tomato plants at low population densities. This increases the importance of green *T. cinnabarinus* relative to *T. urticae*. Crop managers and IPM consultants must be aware that the status of spider mite populations could be constantly changing and a control strategy that is successful one season may subsequently fail.

Background and expected deliverables

The British Tomato Growers' Association (TGA) has stated that spider mites present one of the greatest challenges in their quest to produce pesticide-free crops. Previous attempts to improve biological control with predators have had limited success because the biology and behaviour of the spider mites, and their interactions with host plants and natural enemies were so poorly understood.

There are different forms of spider mites on UK tomato crops, which have different rates of population growth on different cultivars and are capable of producing different damage symptoms. The overall aim of this project was to quantify some of the most important differences between the mites and to predict the impact that they could have on the biological control system. The TGA recognised that this study in itself would not solve spider mite problems for tomato growers but would provide another important piece in a complicated puzzle that has confounded IPM practitioners for many years.

The project drew on the complementary expertise of invertebrate biologists, taxonomists, biological control specialists, plant breeders, agronomists and statisticians from research and industry backgrounds. The study focused on biological and behavioural parameters that were considered to be key factors in the growth and spread of spider mite populations; *i.e.* adult longevity and fecundity, offspring development time, and mobility. All these factors were considered in relation to the physical structure of the host plant. In addition, cross breeding studies investigated the ability of the various types of mites to cross-breed and the relative fitness of the offspring from those crosses.

Summary of the project and main conclusions

<u>Spider mites in UK tomato crops</u>

In recent Defra funded studies, the authors collected populations of spider mites from many sources in the UK and investigated their morphology (physical features). The mites were placed in three statistically distinct "groups"; the first were green with the morphological features of *T. urticae*, the second were red with *T. cinnabarinus* features, and the third were green with *T. cinnabarinus* features. The latter were previously unknown.

In some situations, the two *T. cinnabarinus* groups are known to cause a severe form of damage to tomato plants. This occurs at low population densities and can result in leaf death (“hyper-necrosis”). *Tetranychus urticae* has not been associated with this type of damage.

<u>Effect of trichome density of tomato plants on spider mite</u>

The surface of tomato plants has up to seven different types of glandular and non-glandular hair like structures called trichomes. One of the more common glandular trichomes (type VI) provides the plant with a natural mechanical and chemical defence against pests. The density of these trichomes varies on different tomato cultivars and at different times of the year. Increasing type VI trichome density on tomato plants was shown to significantly reduce fecundity and longevity of adult females of all three types of spider mites. The physical structure of the tomato plant is therefore a potentially useful, but so far unexploited, component of the biological system.

Movement and spread of different types of spider mites on tomatoes

Adult female red *T. cinnabarinus* laid more eggs and lived longer than either *T. urticae* or the green form of *T. cinnabarinus*:

- Longevity = 12.8, 10 and 9.7 days for red *T. cinnabarinus*, *T. urticae* and green *T. cinnabarinus* respectively.
- Fecundity = 39.8, 21.8 and 17.2 eggs for red *T. cinnabarinus*, *T. urticae* and green *T. cinnabarinus* respectively.

The combined results of these biological studies indicate that the population growth of red *T. cinnabarinus* will be considerably more rapid than the other two types of mites.

The development time of the offspring of the three types of mites was not significantly different.

The mobility of adult female red *T. cinnabarinus* and *T. urticae* was similar, but both were more mobile than green *T. cinnabarinus*. These results suggest that red *T. cinnabarinus* and *T. urticae* will disperse throughout tomato crops more rapidly than green *T. cinnabarinus*.

Cross breeding studies of the different types of spider mite

Cross breeding studies between the three types of spider mites showed that:

- Green *T. cinnabarinus* and *T. urticae* were compatible and always produced viable green offspring.
- Crosses of either green *T. cinnabarinus* or *T. urticae* with red *T. cinnabarinus* had varying success depending on the direction of the cross:
    - When the red *T. cinnabarinus* was female, the F1 were red and females were sterile or of very low fecundity.
    - When the red *T. cinnabarinus* was male, the F1 were red or green; green females being fecund and red females being sterile or of very low fecundity.
    - F2 from the same crosses saw only the production of viable green offspring.

- The sex ratio of spider mites is usually female biased (3:1). However, if females are unmated, or if mating is unsuccessful, only male offspring are produced. The sex ratio of offspring from crosses between pure lines was always female biased.
  - Crosses between green *T. cinnabarinus* and *T. urticae* were female biased.
  - Crosses between *T. urticae* and red *T. cinnabarinus* were more male biased.
  - The outcome of crosses between green and red *T. cinnabarinus* depended on the direction of the cross; when the red *T. cinnabarinus* was female the FI were male biased but when it was male the F1 were female.

The combined results of the crossbreeding studies and subsequent morphological examinations of the offspring proved difficult to interpret. The morphology of the mites indicated that green *T. cinnabarinus* are more closely related to red *T. cinnabarinus* than *T. urticae*. However, breeding compatibility and measurements of the fitness of the offspring imply the opposite. Although it seems probable that green *T. cinnabarinus* is an existing hybrid of *T. urticae* and red *T. cinnabarinus*, laboratory crosses between *T. urticae* and red *T. cinnabarinus* have never produced such a hybrid.

Where mixtures of the various types of spider mites exist in a single crop, the development of the overall population over time will depend on whether they breed only with conspecific individuals (*i.e.* same species / type) or cross breed. If they breed only with conspecific individuals, then red *T. cinnabarinus* will predominate due to their greater population growth. However, if they cross breed, our results suggest that the red form could gradually disappear from the overall population due to the poorer fecundity and male bias of the offspring.

Financial benefits to growers

The TGA recognised that this project was a necessary and logical step in the progression towards reliable non-chemical control of spider mites and that actual financial benefits would not become apparent until a later stage.

The results have improved our understanding of the biology and behaviour of the various types of spider mites found in UK tomato crops and have strengthened the foundation of knowledge upon which more robust biological control strategies may be built. Ultimately, this will contribute to the development of biological control programmes that are specifically tailored to the needs of individual growers.

Action points for growers

The results of the experiments reported in this project further illustrate the complexity of the spider mite / tomato plant relationship. For example, there is considerable inherent variability in the mite populations and it has proved very difficult to simplify this by placing the mites into groups with similar biology and behaviour. Furthermore, the variation in physical structure of different cultivars of tomatoes at different times of the year impacts on their suitability as hosts for the mites and further complicates the pest / plant interactions. Despite this, the experiments have improved many aspects of our knowledge. The key points are:

- Increasing type VI trichome density on tomato plants reduces population growth of all three types of spider mites. The TGA should encourage plant breeders to determine the type VI trichome density that is optimum for biological control and to investigate the possibilty of incorporating this into commercial cultivars.

- The fecundity of red *T. cinnabarinus* on tomato is approximately twice that of green *T. cinnabarinus* and *T. urticae*, which will lead to more rapid population growth. Red *T. cinnabarinus* will therefore be the most difficult of the three types to control with predators and will require a different strategy. This will probably involve the earlier release of substantially more *Phytoseiulus persimilis*. Growers should also consider incorporating a control measure specifically directed towards eggs, *eg.* an IPM compatible chemical ovicide. The inclusion of additional biological agents that are more targeted towards eggs (perhaps *Amblyseius californicus*) should also be considered.

- Red *T. cinnabarinus* and *T. urticae* are both more mobile than green *T. cinnabarinus*. When releasing biological control agents against red *T. cinnabarinus* and *T. urticae*, it would therefore be advantageous to treat the surrounding uninfested plants to help restrict dispersal from the infested area. This will be less important with green *T.*

cinnabarinus, for which greater benefit will be gained from concentrating the biological control material within the foci of infestation. The combined effect of fecundity and mobility should mean that green *T. cinnabarinus* is the easiest of the three types of spider mites to control.

- The full implications of the crossbreeding and taxonomic studies remain difficult to predict. However, crop managers and IPM consultants must be aware that the status of spider mite populations could be constantly changing and a control strategy that is successful one season may subsequently fail.

- It is important to consider whether growers need to know whether they are dealing with *T. urticae* or green *T. cinnabarinus*, as this will require sending specimens to a specialist for identification.
  - The most serious implication would have been if green *T. cinnabarinus* had the same population growth rate as red *T. cinnabarinus* but could not be easily distinguished from *T. urticae*. In fact, this was not the case.
  - In terms of biological control, it is probably not important to distinguish between them because green *T. cinnabarinus* should always be easier to control than *T. urticae.*
  - However, green *T. cinnabarinus* are associated with hyper-necrotic damage and it would be useful to know whether the crop was at risk from this type of damage.

SCIENCE SECTION

General Introduction:

The Tomato Growers'Association (TGA) has stated that spider mites present one of the greatest challenges in their quest to produce pesticide-free crops. Previous attempts to improve biological control with predators have had limited success because the biology and behaviour of the spider mites, and their interactions with the host plants and natural enemies, were so poorly understood. This project investigates biological and behavioural variation among spider mite populations found in UK tomato crops and aims to improve the foundation of knowledge upon which more robust control strategies may be constructed. The studies form a single step in a sequence that will eventually enable biological control programmes to be tailored to the specific needs of individual tomato growers.

A MAFF funded project in the late 1990's (Jacobson & Croft, 2000), demonstrated a strong negative relationship between the density of type VI trichomes (hair-like structures with glandular heads) on fully expanded tomato leaves and both the fecundity and longevity of female *Tetranychus urticae* (two-spotted spider mite). As a consequence, population growth of *T. urticae* was slower as numbers of these trichomes increased. However, the link between tomato cultivar and resistance to *T. urticae* was complicated because the density of trichomes on each cultivar varied with leaf age, light intensity, temperature and photoperiod (Croft, unpublished; Nihoul, 1993).

The work had been commissioned by MAFF to investigate the potential role of the tomato plant in biological control systems and therefore also explored the effect of trichome density on the predator, *Phytoseiulus persimilis*, which is extensively used by British tomato growers to control spider mites. Unfortunately, type VI trichomes were also shown to impede the performance of this predator. Funding ceased before the optimal trichome density, that would be most beneficial to the biological control system, could be determined.

Most of those studies were done on a single strain of *T. urticae* that had been maintained in culture at Stockbridge House for many years. However, towards the end of the MAFF project, interest grew in other strains of spider mites that were associated with the more severe (hyper-necrotic) form of damage to tomato plants. Similar, though less comprehensive measurements were made of the population growth of a strain of the *Tetranychus cinnabarinus* (carmine mite) at different trichome densities. While the relationship showed a similar trend to *T. urticae*, the overall population growth of *T. cinnabarinus* was always significantly greater. This would clearly have an impact on the success of biological control. However, MAFF funding ceased before this could be further investigated.

There remains uncertainty over the identity of the spider mites that attack protected crops. Some taxonomists believe *T. urticae* and *T. cinnabarinus* to be distinct species (Kuang & Cheng, 1990), while others consider them to be variants within a single species (*eg.* Bolland *et al*., 1998). Towards the end of the MAFF funded project, the identity of spider mites collected from UK tomato crops was investigated by morphological characterisation, including some characters previously unused by classical taxonomists. This was done primarily to determine which "types" of spider mites were associated with hyper-necrotic damage. However, the study made an interesting discovery that indicated the existence of a third form of spider mites on UK tomato crops (Zhang & Jacobson, 2000). The 24 populations examined were placed in three statistically distinct "groups"; those in the first group were green with the morphological features of *T. urticae*, the second were red with *T. cinnabarinus* features but the third were green with *T. cinnabarinus* features. Green *T. cinnabarinus* were previously unknown. Once again, MAFF funding ceased before the implications of this discovery could be properly investigated.

Both colour forms of *T. cinnabarinus* were linked to hyper-necrotic damage but the examined populations of *T. urticae* were only associated with normal damage.

Biological control of spider mites with predators on tomatoes has been inconsistent and remains one of the most important barriers to the TGA achieving its goal of pesticide-free crop production. The inherent variation in the performance of the spider mites has almost certainly contributed to control failures and to the frequent need to resort to the application of chemical acaricides.

The overall aim of this HDC funded project was to quantify important differences between the three types of spider mites found on UK tomato crops and to predict the impact that those differences could have on the biological control system. The TGA recognised that this study in itself would not solve spider mite problems for tomato growers but would provide another important piece in a complicated puzzle that had confounded IPM practitioners for many years.

The study focussed on biological and behavioural parameters that were considered to be key factors in the growth and spread of spider mite populations; *i.e*. adult longevity and fecundity, offspring development time, and mobility. All these factors were considered in relation to the density of type VI trichomes on the host plant. In addition, cross breeding studies investigated the ability of the three types of mites to cross-breed successfully and the relative fitness of the offspring from those crosses.

Section A. The biology of three types of spider mites

Objective

To quantify and compare adult longevity and fecundity, and offspring development time, in three “types” of spider mites on tomato plants.

Introduction

A previous project (Jacobson & Croft, 2000) demonstrated a strong negative relationship between the density of type VI trichomes on fully expanded tomato leaves and important components of population growth of *Tetranychus urticae* (two-spotted spider mite). A similar trend was seen with a red form of *T. cinnabarinus* (carmine mite), although its population growth was predicted to be significantly greater than *T. urticae*. These factors could have important implications for the design and management of biological control programmes based on invertebrate natural enemies. A third type of spider mite, which was a green form of *T. cinnabarinus*, was also identified in that project. However, its biology was not studied and its status as a pest, relative to other two types of spider mites, was not known.

The following experiment quantified, for each of the three types of spider mite, biological parameters that were considered to be key factors in their population growth; *i.e.* adult longevity and fecundity, and offspring development time. The factors were assessed in relation to trichome density to determine whether the relative performance of the three types of spider mites could vary on different tomato cultivars.

Materials and methods

Production of synchronised cultures of spider mites

Stock cultures of each of the three types of spider mites were maintained on tomato plants (cv Spectra) in an experimental glasshouse maintained at minimum 21°C (vent 24°C) and 16h day length with supplementary lighting when necessary.

Ten batches of 20 adult females were removed from each culture and each batch was placed on the abaxial surface of an excised tomato (cv Spectra) leaflet in a Petri dish. The females were removed after a 48h egg laying period. When the emerging offspring reached the protonymph stage, they were transferred to large tomato plants (separate plants for each spider mite type) in an experimental glasshouse and allowed to continue their development. They were monitored regularly and individuals were selected for the experiment when the adult females began to lay eggs.

Tomato plants

Three tomato cultivars were selected that were known from preliminary studies to have a wide range of trichome densities on leaves; *i.e.* Jocker, Spectra and Criterium. They were grown under conditions consistent with commercial tomato production and the youngest fully expanded leaves collected for the experiment as required.

Experimental Procedure

For each tomato cultivar, the numbers of type VI trichomes were recorded per unit area (*i.e.* microscope field of view of 44.2mm^2) of a leaflet taken from the middle of the first fully expanded leaf. There were 10 replicates per cultivar per assessment.

Newly ovipositing females from the three synchronised cultures were individually placed on the abaxial surface of an excised tomato leaflet in a ventilated Petri dish. There were nine treatments, each being a different combination of cultivar and spider mite type, and 10 replicates per treatment.

After seven days, each female was transferred to a new leaf to prevent leaf deterioration effecting fecundity and to avoid later confusion of the original adults and offspring adults.

The longevity of the adult and the total number of eggs produced were recorded. The development time of the offspring was also recorded.

The experiment was done twice; *i.e.* in May and July.

Results and discussion

Trichome densities

Ten replicate counts of trichome densities were made on each cultivar in May and July. The means and standard deviations are summarised in Table 1.

Table 1. Mean (standard deviation) of type VI trichome densities on leaves of three tomato cultivars on two assessment dates.

	May		July	
	mean	s.d.	mean	s.d.
Jocker	84.2	(20.48)	72.4	(13.35)
Spectra	67.6	(19.16)	48.2	(8.43)
Criterium	26.8	(15.21)	40.4	(7.59)

There were marked differences between cultivars, but also differences in the trichome density at the two assessment dates. In particular, there was an obvious interaction, the range of means in July being somewhat smaller than in May.

Analysis of variance showed a highly significant effect of cultivars ($P<0.001$) but also a very significant interaction between cultivar and time ($P=0.002$) where Jocker and Spectra density decreased over time and Criterium density increased.

These observations are broadly consistent with the results of previous studies in which the density of trichomes has been shown to vary at different times of year and at different light intensities (Croft, unpublished; Nihoul, 1993). The most important consideration here was that the selected cultivars provided a wide range of trichome densities upon which to base these experiments.

Analysis of longevity and fecundity

Table 2 shows the summary statistics for longevity of the adult spider mites on the three cultivars in the two experiments. A survey of Table 2 shows a marked difference in longevity between cultivars ($P<0.001$) with overall mean longevity on Jocker being 7.5 days, Spectra 11.0 days and Criterium 14.0 days (approximate s.e.d. 0.81 d). For all three spider mite types longevity is reduced with increasing trichome density.

It is also clear that red *T. cinnabarinus* shows greater longevity than the other mites; green *T. cinnabarinus* being 9.7d, *T. urticae* 10.0d and red *T. cinnabarinus* 12.8d (s.e.d.=0.81d) and this is confirmed by ANOVA that shows a significant ($P<0.001$) effect for spider mite types.

Table 3 shows the corresponding fecundity data to that presented for longevity in Table 2. As always, there was considerable inherent variability between individual females and therefore the egg numbers were transformed (n to $\log_{10}(n+1)$) before being analysed. The ANOVA confirmed a strong impact of spider mite type on fecundity ($P<0.001$). Log-transformed means for the spider mite types are green *T. cinnabarinus* 2.217, *T. urticae* 2.641 and red *T. cinnabarinus* 3.301 (s.e.d.= 0.1972) corresponding to non-transformed means of 17.2, 21.8 and 39.8 respectively.

The ANOVA confirmed a strong impact of cultivar ($P<0.001$) on fecundity. Overall log-transformed means for cultivars are: Jocker 1.826, Spectra 3.050 and Criterium 3.283 (s.e.d. = 0.1972) corresponding to arithmetic means of 10.4, 31.1 and 37.2 respectively.

Table 2: Mean longevity of three types of spider mites on three tomato cultivars on two assessment dates.

Cv		Mean number of days lived by adult female spider mite from different groups			
		Spider mite	Days	se	No. of mites
Criterium	May	Green *T. cinnabarinus*	13.38	2.828	8
		Red *T. cinnabarinus*	18.43	1.757	7
		T. urticae	15.00	2.062	8
	July	Green *T. cinnabarinus*	11.40	0.933	10
		Red *T. cinnabarinus*	15.44	1.668	9
		T. urticae	10.33	1.167	9
Jocker	May	Green *T. cinnabarinus*	7.33	0.882	9
		Red *T. cinnabarinus*	8.43	1.462	7
		T. urticae	7.87	1.540	8
	July	Green *T. cinnabarinus*	5.78	1.103	9
		Red *T. cinnabarinus*	9.50	1.165	8
		T. urticae	6.33	0.850	9
Spectra	May	Green *T. cinnabarinus*	10.67	1.683	9
		Red *T. cinnabarinus*	12.62	2.705	8
		T. urticae	10.44	0.530	9
	July	Green *T. cinnabarinus*	9.60	1.231	10
		Red *T. cinnabarinus*	12.62	1.085	8
		T. urticae	10.33	1.374	9

Table 3: Mean fecundity of three types of spider mites on three tomato cultivars on two assessment dates.

		Mean fecundity of spider mites (during life) from different groups			
Cv		Spider mite	number of eggs	se	No. of spider mites
Criterium	May	Green *T. cinnabarinus*	32.25	11.008	8
		Red *T. cinnabarinus*	58.43	6.121	7
		T. urticae	23.62	5.679	8
	July	Green *T. cinnabarinus*	22.20	3.162	10
		Red *T. cinnabarinus*	54.11	10.946	9
		T. urticae	32.78	7.088	9
Jocker	May	Green *T. cinnabarinus*	5.22	3.435	9
		Red *T. cinnabarinus*	15.29	5.339	7
		T. urticae	8.12	2.997	8
	July	Green *T. cinnabarinus*	7.56	3.083	9
		Red *T. cinnabarinus*	15.50	5.635	8
		T. urticae	11.00	3.682	9
Spectra	May	Green *T. cinnabarinus*	12.89	2.922	9
		Red *T. cinnabarinus*	38.50	9.608	8
		T. urticae	17.89	2.690	9
	July	Green *T. cinnabarinus*	23.20	4.335	10
		Red *T. cinnabarinus*	56.88	6.862	8
		T. urticae	37.44	7.946	9

Offspring Development Time

The overall mean development period is about 17 days. Analysis of variance (unbalanced) showed that all main effects (except mite species) and all interactions were highly significant when measured against the pooled error within samples. The main effect of "time of year" made the largest contribution to the analysis, though it resulted in only a 1.65 day mean difference in development. Since all the interactions of "time of year" with the other main factors are significant, the overall result seems to have little biological meaning.

Conclusions

- Increasing trichome density on tomato plants significantly reduced fecundity and longevity of adult females of all three types of spider mites.

- Adult female red *T. cinnabarinus* were more fecund and lived longer than either *T. urticae* or the green form of *T. cinnabarinus.*

    - Longevity = 12.8, 10 and 9.7 days for red *T. cinnabarinus*, *T. urticae* and green *T. cinnabarinus* respectively.
    - Fecundity = 39.8, 21.8 and 17.2 eggs for red *T. cinnabarinus*, *T. urticae* and green *T. cinnabarinus* respectively.

- Development time of offspring of the three types of mites was not significantly different.

Section B. Cross breeding Studies

Objective

To determine the ability of the three types of spider mites to crossbreed and to compare the fitness of their offspring.

Introduction

The following studies were designed to measure the ability of the three types of spider mites to crossbreed and to examine the fitness of the offspring resulting from those crosses.

By establishing the presence or absence of reproductive barriers, the crossbreeding studies will provide a good indication of the degree of genetic isolation between the three spider mite types. Complete compatibility in crossbreeding would suggest that there is a strong degree of relatedness. Conversely, the less compatible the crossbreeding, the less closely related the spider mite types.

Where crossbreeding occurs, it often results in offspring that have poor fitness and do not compete well with purer genetic lines. This will clearly have implications for the speed of establishment of certain populations and therefore for the approach to biological control. Two important measurements of fitness of spider mite offspring are sex ratio and fecundity. Sex ratio is normally female biased but can become more male biased when compatibility is low because unmated females produce male only offspring. The following experiment recorded these fitness factors as secondary indicators of the compatibility of the crosses.

Materials and methods

Production of Parents (Figure 1)

Batches of ten adult mated female spider mites were selected from either *T. urticae*, green *T. cinnabarinus* or red *T. cinnabarinus* cultures and placed in a Petri dish containing fresh excised tomato leaves (cv Spectra). The dishes were kept in a controlled environment room (16L:8D, 21 ±2^{o}C) and the females removed after a 24 hour egg laying period.

The offspring were retained in the original dishes and allowed to continue their development. Approximately 15 female nymphs were then transferred from each of these dishes to new dishes. Thus the original and new dishes respectively contained mixed sex nymphs and female nymphs only.

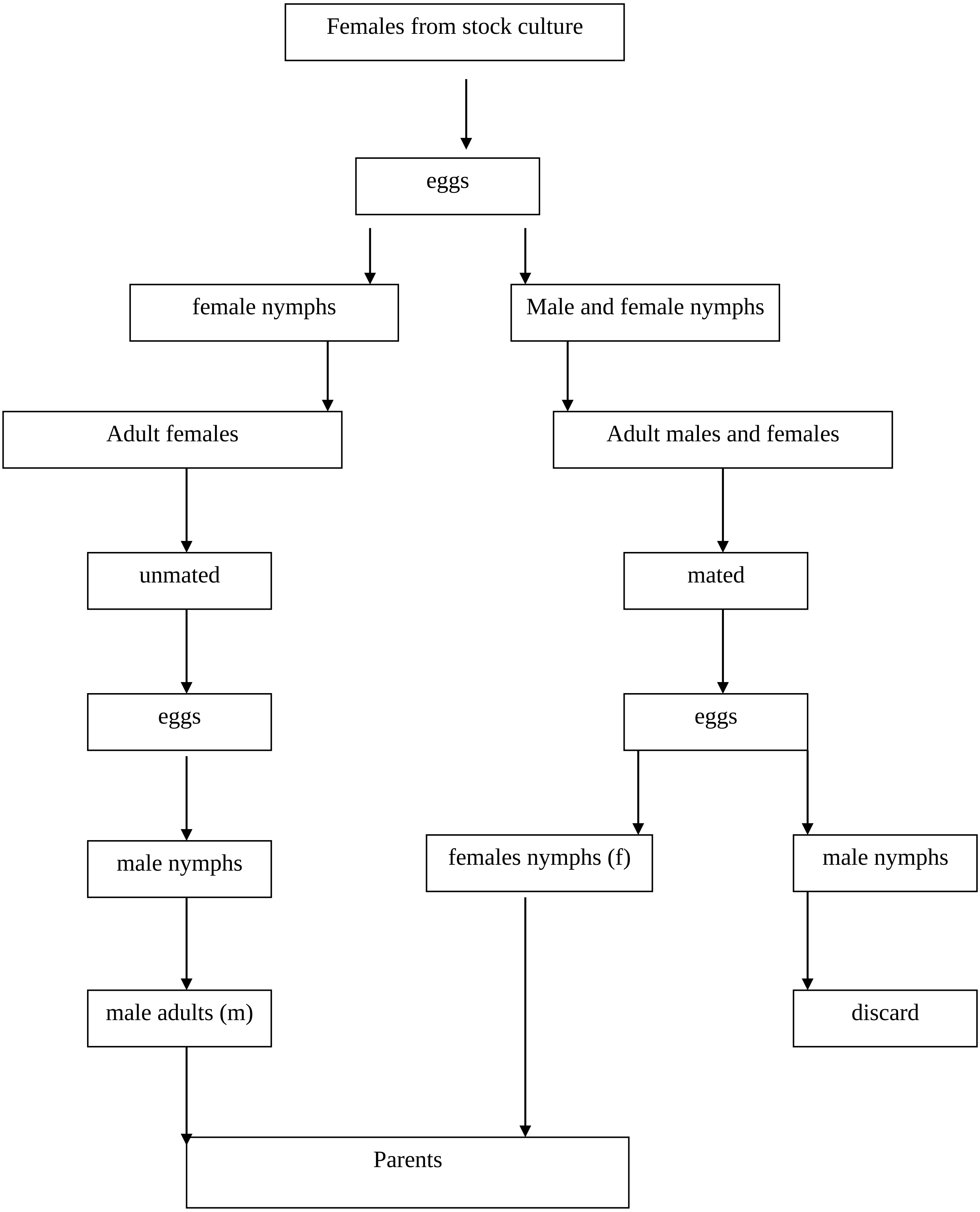
The dishes containing female mites only remained unmated and subsequently their offspring provided the male parents for the crosses. The female mites in the mixed sex dishes were mated and therefore produced male and female offspring. The female nymphs from these dishes provided the female parents for the crosses.

Parent crosses

The following parent crosses were set up:

Cross	Female parent	Male parent
1	Red *T. cinnabarinus*	Green *T. cinnabarinus*
2	Green *T. cinnabarinus*	Red *T. cinnabarinus*
3	Red *T. cinnabarinus*	*T. urticae*
4	*T. urticae*	Red *T. cinnabarinus*
5	Green *T. cinnabarinus*	*T. urticae*
6	*T. urticae*	Green *T. cinnabarinus*
7	Red *T. cinnabarinus*	Red *T. cinnabarinus*
8	Green *T. cinnabarinus*	Green *T. cinnabarinus*
9	*T. urticae*	*T. urticae*

Figure 1. Production of parents.



For each cross there were five Petri dishes (replicates) containing small populations of spider mites. The adults were removed when approximately 30 eggs had been produced in each dish, and males and females were stored separately in 10% lactic acid for taxonomic identification (see Section C).

The eggs were kept in the same Petri dishes (at 16L:8D, 21 ±2^{o}C) and developed into the F1 generation. When they became adults, the sex ratio, colour and fecundity (over 2-3 days) were recorded. When F2 nymphs were present, the F1 adults were removed and stored in 10% lactic acid for identification.

When the F2 reached the adult stage, the colour, sex ratio and egg production were recorded and then they were stored in 10% lactic acid for identification.

Results and discussion

Fecundity in F1's

A Poisson regression of egg numbers *versus* numbers of females was initially done for the three types and the slopes compared. Poisson modelling was used, based on the following argument:

Suppose that any individual lays λ eggs, i.e. $x_i \sim P(\lambda)$, where x_i is the number laid by individual i. Since we are not measuring the eggs laid per individual, but the collective number of k females, let us have y_k be the total number of eggs from those k females, so that $y_k \sim P(k\lambda)$. If this is written in a 'pseudo'-glm notation as

$$E(y_k) = k\mathrm{e}^{\lambda} = \mathrm{e}^{\log(k)+\lambda}$$

so that using $\log_e(k)$ as an offset and fitting a constant only (equivalent to λ) provides a formal fit to the model. Differences between the slopes of the different Poisson regressions can be compared by adding the factor for types / crosses.

The analyses showed that there are overall differences between the F1's, in that red *T. cinnabarinus* is more fecund than the other two types of spider mite (Table 4).

Table 4. The mean fecundity (eggs / female / day) of red or green F1 spider mites from parent crosses between the three spider mite types (* denotes no offspring produced).

	Parent Crosses		Fecundity	
	Female	Male	F1 Red	F1 Green
1	Red *T. cinnabarinus*	green *T. cinnabarinus*	0.00	*
2	green *T. cinnabarinus*	red *T. cinnabarinus*	0.00	6.89
3	red *T. cinnabarinus*	*T. urticae*	0.07	*
4	*T. urticae*	red *T. cinnabarinus*	1.98	9.35
5	green *T. cinnabarinus*	*T. urticae*	*	5.47
6	*T. urticae*	green *T. cinnabarinus*	*	6.20
7	red *T. cinnabarinus*	red *T. cinnabarinus*	10.30	*
8	green *T. cinnabarinus*	green *T. cinnabarinus*	*	8.37
9	*T. urticae*	*T. urticae*	*	8.06

In terms of fecundity, crosses between *T. urticae* and green *T. cinnabarinus* (both ways) were shown to be very compatible.

Crosses of red *T. cinnabarinus* females with males from the other two spider mite types produced predominantly sterile red adult female offspring suggesting low compatibility in the parents due to hybrid sterility.

However, the opposite crosses, *i.e.* red *T. cinnabarinus* males with females from the other two spider mite types, were more successful, producing fecund green adult female offspring from green *T. cinnabarinus* females and low-fecund red females and fecund green females from *T. urticae* females. These results suggest that the reproductive barrier is within the red *T. cinnabarinus* females.

For the analysis of these crosses, the non-fertile groups were excluded leaving five sets of crosses. A significant difference was found between the regression lines. The parameter estimates for the individual regressions are as follows:

	estimate	s.e.	t(13)	antilog of estimate
Green *T.cinnabarinus* f x red *T.cinnabarinus* m	1.642	0.230	7.14	5.167
T.u rticae f x red *T.cinnabarinus* m (red)	0.693	0.270	2.57	2.000
T.urticae f x red *T.cinnabarinus* m (green)	2.137	0.147	14.57	8.472
T.urticae m x green *T.cinnabarinus* f	1.6843	0.0684	24.61	5.388
Green *T.cinnabarinus* f x *T.urticae* m	1.656	0.129	12.82	5.240

A simple comparison of the basic estimates suggests that the two colour types of F1 offspring from the *T. urticae (*female) x red *T. cinnabarinus* (male) cross flank the other F1 offspring, with the green sub-type being far more fecund and red sub-type less fecund.

In general, the results in the F1 fecundity suggest that a cross between populations of green or red spider mites would produce green offspring. Although actual fecundity was not recorded in the F2, observations of whether eggs were produced or not are recorded in Table 5. These results further verify that crosses between populations of red and green mites will result in green offspring. However, as shown by this experiment and the biological studies (section A), the higher fecundity recorded for red *T. cinnabarinus* may be the dominating factor as to the outcome of mixed spider mite populations.

Table 5. Observations of colour and egg production in the F2 of three spider mite type crosses

Parent Crosses		Colour of F1 adults	Colour of F2 adults	
Female	Male		red	green
Red *T. cinnabarinus*	Green *T. cinnabarinus*	Red	*	*
Green *T. cinnabarinus*	Red *T. cinnabarinus*	Green	*	F
Red *T. cinnabarinus*	*T. urticae*	Red	*	*
T. urticae	Red *T. cinnabarinus*	Red	*	F
		Green	NF	F
Green *T. cinnabarinus*	*T. urticae*	Green	*	F
T. urticae	Green *T. cinnabarinus*	Green	*	F
Red *T. cinnabarinus*	Red *T. cinnabarinus*	Red	F	*
Green *T. cinnabarinus*	Green *T. cinnabarinus*	Green	*	F
T. urticae	*T. urticae*	Green	*	F

F = fecund

NF = not fecund

*= no offspring

Sex ratios of F1 and F2

Sex ratios of F1 and F2 crosses are shown in Table 6. Note that the s.e.'s are generally very small, primarily because the sample sizes are relatively large. This essentially means that nearly all the sex ratios are significantly different from one another. The 'normal' sex ratio for spider mites is female biased and can be considered to be approximately three females to one male (Helle & Pijnacker, 1985). The sex ratio data were recorded from groups of spider mites with the total numbers given in the column marked *n*. The results shows that from the pure crosses of *T. urticae* or red or green *T. cinnabarinus*, a standard sex ratio for offspring is highly female biased.

However, three of the original crosses produced F1 with more male biased sex ratios suggesting females were not being successfully mated and there were reproductive barriers. These crosses were red *T. cinnabarinus* (female) x green *T. cinnabarinus* (male) and both crosses of red *T. cinnabarinus* and *T. urticae*. All other crosses produced highly female biased sex ratios. These results indicate that reproductive barriers exist between red *T. cinnabarinus* and the other two spider mite types.

Table 6. Estimated male:female sex ratios in F1 and F2 generations from crosses between three spider mite types.

type	♀	♂	F1	(s.e.)	n	F2	(s.e.)	n
1	Red *T. cinnabarinus*	Green *T. cinnabarinus*	1.413	0.2745	111	*		
	Green *T. cinnabarinus*	Red *T. cinnabarinus*	0.238	0.0320	359	0.110	0.0206	323
2	Red *T. cinnabarinus*	*T.urticae*	0.439	0.0772	146	*		
	T.urticae	Red *T. cinnabarinus*	0.587	0.1008	154	0.280(r)	0.1235	32
						0.266(g)	0.0660	100
3	Green *T. cinnabarinus*	*T.urticae*	0.167	0.0219	476	0.259	0.0434	219
	T.urticae	Green *T. cinnabarinus*	0.182	0.0328	240	0.249	0.0400	246
4	Red *T. cinnabarinus*	Red *T. cinnabarinus*	0.230	0.0251	557	0.205	0.0334	270
5	Green *T. cinnabarinus*	Green *T. cinnabarinus*	0.174	0.0145	1160	0.098	0.0251	191
6	*T.urticae*	*T.urticae*	0.234	0.0343	306	0.166	0.0324	218

The sex ratios in the F2 are more comparable to the sex ratio of the 'normal' crosses. This is because the least compatible crosses were eliminated at the F1 stage and therefore contribute little to the F2.

Overall, crosses between the green *T. cinnabarinus* and *T. urticae* are seemingly without barriers. But crosses of these two spider mite types with red *T. cinnabarinus* did show some degree of incompatibility. In population terms crosses between red and the green spider mites would produce green hybrids that, although fecund, would not be as fecund as red *T. cinnabarinus*.

Conclusions

Cross breeding studies between the three types of spider mites showed that:

- Green *T. cinnabarinus* and *T. urticae* were compatible and always produced viable green offspring.

- Crosses of either green *T. cinnabarinus* or *T. urticae* with red *T. cinnabarinus* had varying success depending on the direction of the cross:
  - When the red *T. cinnabarinus* was female, the F1 were red and females were sterile or of very low fecundity.
  - When the red *T. cinnabarinus* was male, the F1 were red or green; green females being fecund and red females being sterile or of very low fecundity.
  - F2 from the same crosses saw only the production of viable green offspring.

- The sex ratio of spider mites is usually female biased (3:1). However, if females are unmated, or if mating is unsuccessful, only male offspring are produced. The sex ratio of offspring from crosses between pure lines was always female biased.
  - Crosses between green *T. cinnabarinus* and *T. urticae* were female biased.
  - Crosses between *T. urticae* and red *T. cinnabarinus* were more male biased.
  - The outcome of crosses between green and red *T. cinnabarinus* depended on the direction of the cross; when the red *T. cinnabarinus* was female the FI were male biased but when it was male the F1 were female biased.

C. Taxonomic features

Objective

To determine whether the offspring of crosses between the three types of spider mites found on UK tomato crops are hybrids of the parents.

Introduction

Despite many years of research in many countries, there remains uncertainty over the identity of the spider mites that attack protected crops. Some taxonomists believe *Tetranychus urticae* and *T. cinnabarinus* to be distinct species (Kuang & Cheng, 1990), while others consider them to be variants within a single species (*eg.* Bolland *et al*., 1998). The identity of spider mites collected from 24 sources in the UK was recently investigated by morphological characterisation (Zhang & Jacobson, 2000), including some characters previously unused by classical taxonomists (*eg* numbers of setae on tibia 1). The populations were placed in three statistically distinct "groups"; those in the first group were green with the morphological features of *T. urticae*, the second were red with *T. cinnabarinus* features but the third were green with *T. cinnabarinus* features. Although green *T. cinnabarinus* were previously unknown, the examination of other morphological features confirmed that this type was more similar to *T. cinnabarinus* than *T. urticae*. Therefore, the third type was tentatively described as green *T. cinnabarinus*.

The experiments described in Section B of this report showed that crossbreeding occurred between the three types of spider mites, indicating that speciation was not complete. However, the offspring of crosses between red and green forms often exhibited poor levels of fitness, which raised questions about their specific status. In the following study, the morphological features of the offspring were examined to reveal whether they could be hybrid forms of the parents.

Materials and methods

Adults from the three types of spider mites, and the adult offspring from their crosses, were preserved in 10% lactic acid and supplied to Dr Zhi Qiang Zhang for morphological characterisation. The study focused on an important morphological feature that had originally been used to separate the three types of spider mites (*i.e.* number of tibia I setae).

Results and discussion

The examination of parents verified that red and green *T. cinnabarinus* have 13 setae on tibia 1, whereas *T. urticae* has 10 setae on tibia I. The patterns were exactly as reported by Zhang & Jacobson (2000). The numbers of setae on tibia 1 of F1 and F2 crosses of these parents are shown in Table 7.

Table 7. The number of setae on tibia 1 recorded in the F1 and F2 offspring from three spider mite types and their crosses

Parent generation of spider mite cross		The number of setae			
		F1		F2	
Female	Male	Green	Red	Green	Red
Red. *T. cinnabarinus*	Red *T. cinnabarinus*	-	13	-	13
T. urticae	*T. urticae*	10	-	10	-
Green *T. cinnabarinus*	Green *T. cinnabarinus*	13	-	13	-
Red. *T. cinnabarinus*	Green *T. cinnabarinus*	-	13	-	-
Green *T. cinnabarinus*	Red. *T. cinnabarinus*	13	-	13	-
T. urticae	Green *T. cinnabarinus*	13	-	13	-
Green *T. cinnabarinus*	*T. urticae*	13	-	13	-
T. urticae	Red. *T. cinnabarinus*	10	10	10*	10#
Red. *T. cinnabarinus*	*T. urticae*		13	-	-

* from green and red F1, # from green F1

In summary, it can be seen that:

- The offspring of true crosses always retained the same number of setae as their parents.
- The offspring of crosses (either way) between red and green *T. cinnabarinus* always had 13 setae; *i.e.* the same as the parents.
- In crosses (either way) between *T. urticae* and green *T. cinnabarinus*, the 13 setae feature of green *T. cinnabarinus* dominated.
- The only results that varied with the direction of the cross were between *T. urticae* and red *T. cinnabarinus*. In these crosses, the character exhibited by the mother dominated in the offspring; *i.e.* when the female was *T. urticae*, the offspring had 10 setae, but when the female was red *T. cinnabarinus*, the offspring had 13 setae.

The combined results of the crossbreeding studies (Section B) and these morphological investigations are difficult to interpret. The morphology of the mites indicates that green *T. cinnabarinus* are more closely related to red *T. cinnabarinus* than *T. urticae*. However, breeding compatibility and measurements of the fitness of the offspring imply the opposite.

It seems possible that green *T. cinnabarinus* is an existing hybrid of *T. urticae* and red *T. cinnabarinus*. However, our laboratory crosses between *T. urticae* and red *T. cinnabarinus* have never produced such a hybrid.

It is now known that the population growth and dispersal of these three types of mites are potentially different and that this could influence the approach to biological control on tomato crops (Sections A and D). What is unclear is whether the status of mite populations on crops is stable or whether it is constantly changing as the different forms interbreed. If the latter is true, then biological control strategies will have to be very flexible.

D. Movement in three types of spider mite

Objective

To measure the rate of movement of three types of spider mites on tomato leaves with a wide range of trichome densities.

Introduction

The mobility of spider mites on tomato plants is clearly one of the most important factors in determining the rate at which the pest population spreads throughout the crop. It is also an important consideration when designing biological control strategies and must be taken into account when selecting appropriate biological control agents.

Previous work has indicated that mobility of other mites has varied between cultivars of tomatoes with different trichome densities (Jacobson & Croft, 2000). It was therefore important to quantify movement for all three types of spider mites found on tomato crops in the UK.

The following study recorded the movement of the mites on tomato leaves over a 24 hour period. The work was done in two stages. The first stage compared the mobility of different life cycle stages to determine which was likely to be most important to the dispersal of the population. The second stage compared the movement of that life cycle stage of the three types of spider mites taking into account population density and trichome density.

Materials and methods

Preliminary studies

Preliminary studies to establish which development stage of spider mites would be selected for the movement experiments showed that nymphs moved very little in comparison to the teneral (pre-egg laying) or young female (3-6 day old) adults. The teneral stage proved to be the least reliable to study because of the difficulty in separating it from the younger deutonymph stage and due to its relatively short duration (approximately one day). Young adult females were therefore selected for the main experiment.

<u>Main experiment</u>

Culturing mites and plants

Synchronised populations of mites and tomato plants were produced for the experiments as described in Section A. The youngest fully expanded leaves (consisting of three pairs of leaflets and a single terminal leaflet) were collected as required.

Experimental procedure

A tomato leaf was floated upside down on water in a white tray. A single central leaflet was infested with the required number (see below) of adult females of one of the three types of spider mites. The mites were prevented from moving initially by placing the lid from an epindorff tube over them for one hour. After this initial acclimatisation period, the barrier was removed and the tray kept in a controlled environment room (16L:8D, 21 ± 2^{o}C) over another water-filled tray for 24 hours. After this time, the distance each mite had moved along the length of the leaf from the release spot was recorded.

There were a total of 27 combinations of spider mite type (red *T. cinnabarinus*, green *T. cinnabarinus* or *T. urticae*), spider mite density (*i.e.* 1, 5 and 10 per introduction point) and tomato cultivar (Criterium, Spectra and Jocker) and each was replicated ten times.

Results and discussion

Data were analysed by analysis of variance. For single mites the analysis was based on a simple factorial with ten replicates, the replication (or sample) variance making up the residual error term. For groups of mites, the error was split into a replication variance (as above) and a within group variance, *i.e*. the difference between mites in the same community.

Overall green *T. cinnabarinus* were shown to move significantly less than either red *T. cinnabarinus* or *T. urticae* ($P=0.011$) (Table 8).

Table 8. The mean distance moved over 24 hours by young adult female spider mites. (21 ± 2°C, 16L:8D)

Spider mite type	Distance moved (cm)
Red *T. cinnabarinus*	6.297
T. urticae	6.306
Green *T. cinnabarinus*	4.2
(SED, 24 df)	(0.758)

The results for all combinations of treatments are shown in Table 9. For single mites there was a significant effect of mite-type ($P<0.05$), with green *T. cinnabarinus* mites moving considerably less than the other two types. For groups of 5 and ten mites this significance was at the 10% probability level.

Density of spider mites did not affect distance moved. For groups of five there is a significant difference between cultivars ($P=0.028$) and the suggestion of an interaction ($P=0.088$), while for groups of ten there is a small mite effect ($P=0.089$).

Table 9: The effects of spider mite type, density and cultivar on the movement (cm) of spider mites

no	mite	Jocker	Criterium	Spectra	mean		sed (81df)
1	Red *T. cinnabarinus*	9.00	5.18	7.39	7.19	mite	1.435
	T. urticae	5.74	9.15	6.70	7.20	cv	1.435
	Green *T. cinnabarinus*	2.61	2.79	4.60	3.33	mite.cv	2.485
	mean	5.79	5.71	6.23	5.91		
5	Red *T. cinnabarinus*	5.58	6.79	6.34	6.24	mite	0.914
	T. urticae	5.05	8.40	2.43	5.29	cv	0.914
	Green *T. cinnabarinus*	5.17	4.70	3.63	4.50	mite.cv	1.583
	mean	5.27	6.63	4.13	5.34		
10	Red *T. cinnabarinus*	5.63	5.05	5.25	5.31	mite	0.786
	T. urticae	7.40	6.06	6.45	6.64	cv	0.786
	Green *T. cinnabarinus*	4.27	5.64	5.03	4.98	mite.cv	1.362
	mean	5.77	5.58	5.58	5.64		

Conclusion

The mobility of adult female red *T. cinnabarinus* and *T. urticae* was similar, but both were more mobile than green *T. cinnabarinus*. These results suggest that green *T. cinnabarinus* will disperse more slowly than the other two types.

E. Possible impact of findings on biological control strategies

In many respects, the results of the experiments reported in this project further illustrate the complexity of the spider mite / tomato plant relationship and help to explain why biological control of this pest has been so inconsistent over the years. In particular:

- There is considerable inherent variability in the mite populations and it is proving very difficult to simplify this by placing the mites into groups with similar biology and behaviour.
- The variation in morphology of different cultivars of tomatoes at different times of the year impacts on their suitability as hosts for both herbivores and natural enemies. This is becoming a more important consideration as growers are encouraged to supply their customers with a wider range of tomato products.
- In some situations, some types of spider mites cause a severe form of damage called hyper-necrosis. This requires a very different approach to pest management, yet its occurrence remains difficult to predict with any reliability.

Despite these difficulties, the present experiments have improved many aspects of our knowledge of the pest and its relationship with the plant, and have strengthened the foundation upon which to base further studies towards robust pesticide-free control strategies. The key points to be drawn from this project are:

- Increasing type VI trichome density on tomato plants significantly reduces fecundity and longevity of adult females of all three types of spider mites. The morphology of the tomato plant is therefore a potentially useful, though so far unexploited, component of the three-way biological system (*i.e.* host plant / herbivore / predator).
    - The TGA should encourage plant breeders to determine the type VI trichome density that is optimum for biological control and to investigate the possiblity of incorporating this into commercial cultivars.

- The fecundity of red *T. cinnabarinus* on tomato is approximately twice that of green *T. cinnabarinus* and *T. urticae*, which will lead to more rapid population growth.

    - Red *T. cinnabarinus* will be the most difficult of the three types to control on tomato with predators.
    - It will require a different approach than for the two green forms, probably involving the earlier release of substantially more *Phytoseiulus persimilis*.
    - It would also be beneficial to incorporate into the control programme for red *T. cinnabarinus* a control measure specifically directed towards eggs. This could be an IPM compatible chemical ovicide or an additional biological agent (perhaps *Amblyseius californicus*).

- The mobility of adult female red *T. cinnabarinus* and *T. urticae* is similar, but both are more mobile than green *T. cinnabarinus*. The combined effect of fecundity and mobility should mean that green *T. cinnabarinus* is the easiest of the three types of spider mites to control.
    - When releasing biological control agents against red *T. cinnabarinus* and *T. urticae*, it would be advantageous to include the surrounding uninfested plants to help restrict dispersal from the infestated area. This will be less important with green *T. cinnabarinus*, for which greater benefit will probably be gained from concentrating the biological control material within the foci of infestation.

- Where mixtures of the various types of spider mites exist in a single crop, the development of the overall population over time will depend on whether they breed only with conspecific individuals (*i.e.* same species / type) or cross breed.
    - If they breed only with conspecific individuals, then red *T. cinnabarinus* will predominate due to their greater population growth. This seems to be consistent with the trends observed in commercial crops over the last decade.
    - If they cross breed, our results suggest that the red form could gradually disappear from the overall population due to the poorer fecundity and male bias of the offspring.
    - However, our experiments only considered breeding between individuals of the same generation. In a normal crop situation, there would be overlapping generations and back crosses, which could bypass reproductive barriers and lead to a different scenario.

- The full implications of the crossbreeding and taxonomic studies remain difficult to predict. However, crop managers and IPM consultants must be aware that the status of spider mite populations could be constantly changing and a control strategy that is used successfully one season may subsequently fail.

- It is important to consider whether growers need to know whether they are dealing with *T. urticae* or green *T. cinnabarinus,* as this will require sending specimens to a specialist for identification.
    - The most serious implication would have been if green *T. cinnabarinus* had the same population growth rate as red *T. cinnabarinus* but could not be easily distinguished from *T. urticae*. In fact, this was not the case.
    - In terms of biological control, it is probably not important to distinguish between them because green *T. cinnabarinus* should always be easier to control than *T. urticae.*
    - However, green *T. cinnabarinus* are associated with hyper-necrotic damage and it would be useful to know whether the crop was vulnerable to this type of damage if conditions were favourable.

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