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Using adult female morphological characters for differentiating *Tetrancyhus urticae* complex (Acari: Tetranychidae) from greenhouse tomato crops in UK

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Abstract

Variation in seven female morphological characters were examined for 18 populations of spider mites of the *Tetranychus urticae* and *T. cinnabarinus* complex from greenhouse tomatoes in various locations in the UK. *Tetranychus cinnabarinus* could be readily separated from *T. urticae* by variation in the number of setae on tibia I (10-13 setae, or addition of 1-3 solenidia normally present in males) in females and this difference was correlated with those of previously studied characters (shape of dorsal lobe in female and shape of aedeagus in male) and additional characters examined in this paper. Several populations of mites identified as *T. cinnabarinus* in this study were green in colour. Hyper-necrotic responses in tomato plants were present in most *T. cinnabarinus* populations with variation in the number of setae on tibia I but absent in *T. cinnabarinus* population without variation in the number of setae on tibia I (i.e. 13 setae).

Key words: Acari, spider mites, *Tetranychus urticae*, *Tetranychus cinnabarinus*, species complex, tomato, hyper-necrotic response.

Introduction

The differentiation between *Tetranychus urticae* Koch and *Tetranychus cinnabarinus* (Boisduval) using morphological characters is often difficult because they are both polymorphic and there is significant intraspecific variation among populations on different host plants and from different geographic locations (e.g. van de Bund & Helle 1960; Wang 1981). This has been extremely unfortunate because both species are economically very important throughout the world.

Boudreaux (1956) first revived *T. cinnabarinus* as a distinct species and separated it from *T. urticae* using breeding experiments as well as morphological characters: (A) the shape of dorsal integumentary lobes in the diamond-shaped area between the third and fourth dorsal central setae on female opisthosoma, (B) the shape of male aedeagus and (C) the colour of live summer females and newly laid eggs. The separation of the two species was supported by some subsequent studies (e.g. Parr & Hussey 1960; van de Bund & Helle 1960; Jordaan 1977; Brandenburg & Kennedy 1981) but rejected by others (e.g. Dupont 1979; Mollet & Sevacherian 1984). Meyer (1987), after reviewing studies known to her by then, followed Dupont (1979) in considering *T. cinnabarinus* as a synonym of *T. urticae*. This was generally accepted in several subsequent books by specialists of the Tetranychidae (e.g. Ehara 1993; Baker & Tuttle 1994; Bolland *et al.* 1998). A recent study using morpho-

logical, biological and molecular data (Kuang & Cheng 1990) showed distinctions between *T. urticae* (populations from UK and China) and *T. cinnabarinus* (from China). Unfortunately this important study was not noticed/considered by subsequent authors (e.g. Bolland *et al.* 1998).

Kuang and Cheng (1990) confirmed the usefulness of the three morphological characters A-C mentioned above in separating *T. urticae* and *T. cinnabarinus* in their study. In addition, they showed that *T. urticae* females have 10 setae on tibia I but *T. cinnabarinus* has 10-13 setae (addition of up to three solenidia) on tibia I. The difference in this fourth character was also noted earlier by Keh (1952), Boudreaux (1956) and van de Bund and Helle (1960), but ignored by subsequent authors (e.g. Dupont 1979; Mollet & Sevacherian 1984; Meyer 1987) who questioned and debated the value of characters A-C for separating the two species. The value of leg setation in the classification of the Tetranychidae has been underestimated in the past. After a very detailed comparative study, Lindquist (1985) revealed a wealth of information in leg setation in this family and called for more study on leg setation.

In the last few years, interesting populations of spider mites of the *T. urticae* complex have been found on greenhouse tomatoes in the UK. Spider mite feeding damage on tomato leaves normally consists of fine necrotic speckling that is economically unimportant until the mite population is quite large. However, populations of green and red forms of spider mites have been found causing more severe damage symptoms at various locations. Foster and Barker (1978) first reported the symptoms, which consist of premature chlorosis of infested leaflets which subsequently wither and die. This hyper-necrotic response by tomato plants to spider mite feeding was reported sporadically in southern UK during the 1980s and became increasingly common during the early 1990s (G. Hayman, personal communication, 1997). More recent studies indicate that the symptoms are only expressed under certain environmental conditions and are possibly associated with water stress (Jacobson, unpublished data).

In addition to the direct effect on the plants, hyper-necrosis is also important because it disrupts biological control of spider mites. Control of spider mites in UK tomato crops is usually based on the combined use of the predatory mite, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae), and the selective acaricide, Torq (50% w/w fenbutatin oxide). Normal spider mite damage symptoms develop relatively slowly and populations of *P. persimilis* can usually be established on the plants before the damage becomes economically important. However, hyper-necrosis develops rapidly at small spider mite population densities and there is insufficient time to achieve control with *P. persimilis.* Torq must therefore be applied at an early stage of the infestation. This has placed too much dependence on the acaricide and there is evidence that some spider mite populations are becoming resistant to it (Jacobson *et al.* 1999).

In this paper, we examine if we can use morphological characters, especially the setation on tibia I of females used by Kuang and Cheng (1990), to separate *T. urticae* and *T. cinnabarinus* from greenhouse tomato crops in UK. We also examine the correlation of this charcter with other characters (including colour forms) and damage symptoms on host plants.

Materials and methods

During 1997 to 1998, spider mites were collected from 18 populations from leaves of greenhouse tomatoes in various locations in the UK (Table 1). A sample of twenty adults (females and males) from each sample were mounted in Hoyer's medium on slides for microscopic examination. The number of setae on tibia I and body colour were first used to distinguish *T. urticae* and *T. cinnanabirus* according to Kuang and Chen (1990) and Boudreaux (1956). In addition, lobes on dorsal hys-

terosomal striae in females and shape of aedeagus in males were examined to confirm the identification based on the number of setae on tibia I and body colour.

To compare variation among samples, seven characters were measured/counted for five female specimens from each sample. These characters are:

1. Number of setae on tibia I;
2. Length of tibia I;
3. Distance between bases of solenidia in the two duplex setae: ω"-ω'(N1);
4. Ratio between the length of setae v_2 and the distance to setae sc_1: $v_2/(v_2\text{-}sc_1)$;
5. Length of setae sc_1;
6. Distance between base of genital setae: $g_1\text{-}g_1$;
7. Ratio between length of subcapitular setae (*m*) and distance between their bases: *m*/(*m*-*m*).

Terminology and abbreviation for body and leg setae follow Lindquist (1985). Measurements were made in micrometers. Specimens examined are deposited in Department of Entomology, The Natural History Museum (London, UK), Horticultural Research International (Cawood, Selby, North Yorkshire, UK) and Landcare Research (Auckland, New Zealand).

Variation in seven characters listed above was examined with regard to determined species and colour forms. ANOVA was then performed to reveal the significance of variation in the seven characters between two species (or colour forms) and among populations within each species (or colour forms).

Results

Identification of mites based on key characters

Hundreds of specimens of *T. urticae* complex from greenhouse tomato crops were examined and they conform to the definition of *T. urticae* complex by Meyer (1987: 137). Briefly, females are characterized by a diamond-shaped striae between the third and fourth pair of dorsal central hysterosomal setae and triangular, semi-circular and oblong lobes on striae in this area, whereas males are characterized by having a small knob of aedeagus with similar or slightly different anterior and posterior projections and its dorsal surface broadly rounded or angulated.

Females in populations 1, 2, and 21 had no variation in the number of setae on tibia I (9 normal setae and one solenidion) and were green in colour (Table 1). They are *T. urticae* according to Kuang and Cheng (1990) and Boudreaux (1956). Further examinations of additional properly mounted specimens (e.g. of population 21) showed that (1) the lobes on dorsal hysterosomal striae in the diamond-shaped area in females are mostly semi-circular to oblong, wider than tall, with only a few triangular lobes and (2) the knob of aedeagus in males produces more or less similar anterior and posterior projections and its dorsal surface broadly rounded. These additional observations match characterization of *T. urticae* by Boudreaux (1956) and Kuang and Cheng (1990), and endorse the above determinations using setae on tibia I.

Females in populations 3, 4, 5, 6, 9, 19, 24 and 27 showed variation in the number of setae on tibia I (9 normal setae and 1 solenidion, plus up to 3 solenidia that are normally present on tibia I of male) and they were red in colour (Table 1); they are *T. cinnabarinus* according to Boudreaux (1956) and Kuang and Cheng (1990). Further examinations of additional properly mounted specimens (e.g. of population 3) showed that (1) the lobes on dorsal hysterosomal striae in the diamond- shaped area in females are mostly triangular to semi-circular (rarely oblong), often taller than wide and (2) the knob of aedeagus in males produces acute anterior projections and a slightly rounded posterior pro-

jection (not obvious in some specimens) and its dorsal surface obtusely angulate. These additional observations are similar to the characterization of *T. cinnabarinus* by Kuang and Cheng (1990) and Boudreaux (1956), and endorse the above determinations using setae on tibia I.

TABLE 1. *Tetranychus urticae* and *T. cinnabarinus* of 18 populations from greenhouses in UK and their colour form, damage symptom and variation in the number of setae on tibia I.

Mite Species	Mite colour	Reference number	Locality and host of collection*	Date of collection	Damage symptom	Number of setae on Ti I
T. u.	green	1	Cawood, North Yorkshire	17/02/1998	n/a	10
T. u.	green	2	Cawood, North Yorkshire	17/02/1998	normal	10
T. u.	green	21	La Lande, Guernsey	27/07/1998	normal	10
T. c.	red	3	Cawood, North Yorkshire	26/09/1997	necrotic	10-13
T. c.	red	4	Kyminge, Kent	06/09/1997	normal	10-13
T. c.	red	5	Hernhill, Kent	06/09/1997	necrotic	10-13
T. c.	red	6	Yeovil, Somerset	06/09/1997	chlorotic	13
T. c.	red	9	Hernhill, Kent	11/03/1998	necrotic	10-13
T. c.	red	19	Camblesforth, North Yorkshire	24/07/1998	normal	12-13
T. c.	red	24	Cottingham, East Yorkshire	27/07/1998	slight chlorotic	10-13
T. c.	red	27	Cardiff, Gwent	30/07/1998	normal to slight chlorotic	13
T. c.	green	7	Arreton, Isle of Wight	29/07/1997	necrotic	10-13
T. c.	green	8	Leconfield, East Yorkshire	29/10/1997	normal	13
T. c.	green	10	Southport, Lancashire	26/05/1998	necrotic	10-13
T. c.	green	18	Arreton, Isle of Wight	08/06/1998	necrotic	10-13
T. c.	green	20	La Roque, Jersey	27/07/1998	normal	10-13
T. c.	green	22	Taunton, Somerset	28/07/1998	slight chlorotic	10-13
T. c.	green	25	Cottingham, East Yorkshire	27/07/1998	normal	10-13

* Greenhouse tomato crops except population 1 one which was from a laboratory culture on bean plants and population 2 which was from a laboratory culture on tomato plants.

Females in populations 7, 8, 10, 18, 20, 22 and 25 were green in colour but showed variation in the number of setae on tibia I (Table 1). The two characters used above contradict each other and do not allow species determination. Further examinations of additional properly mounted specimens (e.g. of population 10) showed that lobes on dorsal hysterosomal striae in females and shape of aedeagus in males are similar to those of *T. cinnabarinus* rather than *T. urticae*. Thus, they are considered as *T. cinnabarinus*.

Correlated differences in other characters

Analysis of variance of seven characters between the two species while accounting for variation among populations within species showed significant differences between *T. urticae* and *T. cinnabarinus* in five charcters and intraspecific variation was less significant than interspecific variation for these characters (Tables 2, 3). Similar analysis between green and red forms without regard to species association showed significant differences between red and green mites in only three char-

acters and variation among population within each colour form was more significant than variation between colour forms for these characters (Table 4).

TABLE 2. Variation in seven characters in *Tetranychus urticae* and *T. cinnabarinus* from 18 populations in greenhouses in UK. Measurements in micrometers and in the format: mean±s.e.

Mite Species	Mite colour	Reference Number	No. setae on Ti I	Length of Ti I	Distance ω"-ω'(N1)	Ratio $v_2/(v_2\text{-}sc_1)$	Length of sc_1	Distance $g_1\text{-}g_1$	Ratio $m/(m\text{-}m)$
T. u.	green	1	10	63.0±2.3	19.0±0.5	3.10±0.06	144.0±3.5	40.8±1.4	0.90±0.01
T. u.	green	2	10	62.2±0.8	18.4±0.5	2.86±0.05	143.8±1.7	38.2±1.4	0.91±0.01
T. u.	green	21	10	56.8±1.5	15.0	3.21±0.10	135.8±2.9	35.2±2.1	0.97±0.01
T. c.	red	3	10.6±0.6	60.6±1.2	18.0±0.8	3.07±0.16	147.6±3.4	29.0±1.3	0.90±0.02
T. c.	red	4	10.6±0.6	64.2±1.9	18.0	3.06±0.11	144.4±1.6	32.4±1.2	0.89±0.02
T. c.	red	5	12.1±0.6	60.0±2.1	18.8±0.7	3.25±0.04	140.2±2.5	28.6±1.4	0.88±0.02
T. c.	red	6	13	63.6±0.9	19.0±0.7	3.13±0.08	143.0±1.7	31.8±1.4	0.84±0.01
T. c.	red	9	12.0±1.0	61.3±3.6	18.7±0.3	3.29±0.17	141.3±1.8	31.0±1.5	0.91±0.05
T. c.	red	19	12.8±0.2	61.0±0.6	18.4±0.4	3.40±0.09	137.4±2.3	31.8±0.7	0.90±0.03
T. c.	red	24	11.2±0.7	57.8±1.4	16.0±0.6	3.15±0.11	138.0±1.3	27.6±1.6	0.84±0.02
T. c.	red	27	13	58.8±0.7	17.4±0.6	3.16±0.08	139.4±2.4	34.4±1.0	0.91±0.03
T. c.	green	7	10.6±0.6	62.5±1.0	17.6±0.3	3.08±0.77	143.8±2.2	34.0±2.2	0.88±0.02
T. c.	green	8	13	61.2±0.7	18.0	3.07±0.10	143.8±1.7	33.6±1.0	0.88±0.03
T. c.	green	10	11.2±0.7	61.0±0.8	18.4±0.5	3.30±0.11	136.6±2.2	28.8±0.7	0.91±0.03
T. c.	green	18	10.5±0.5	59.5±1.2	18.3±0.3	3.09±0.06	138.0±1.5	32.3±1.0	0.89±0.03
T. c.	green	20	11.2±0.7	58.5±1.5	16.6±0.7	3.26±0.07	139.8±2.0	31.2±1.2	0.88±0.02
T. c.	green	22	11.8±0.7	58.8±1.8	14.4±1.2	3.32±0.09	145.2±3.4	33.4±1.6	0.80±0.08
T. c.	green	25	11.8±0.7	55.8±1.5	15.6±1.0	3.02±0.10	145.6±1.5	31.2±0.7	0.95±0.03

TABLE 3. Statistical analysis of interspecific and intraspecific variation in seven characters (see material and methods for detailed descriptions) in *Tetranychus urticae* and *T. cinnabarinus* from 18 populations in greenhouses in UK. Measurements in micrometers and in the format: mean±s.e.

Mite species and statistics	No. setae on Ti I	Length of Ti I	Distance ω"-ω'(N1)	Ratio $v_2/(v_2\text{-}sc_1)$	Length of sc_1	Distance $g_1\text{-}g_1$	Ratio $m/(m\text{-}m)$
T. urticae	10	60.7±1.1	17.5±0.5	3.06±0.06	141.2±1.8	38.1±0.07	0.93±0.01
T. cinnabarinus	11.6±0.2	60.2±0.4	17.50±0.2	3.18±0.02	141.6±0.6	31.5±0.4	0.88±0.01
Interspecific variation[1]	25.92***	0.344^{NS}	0.044^{NS}	4.676*	0.074^{NS}	50.62***	4.964*
Intraspecific variaton[2]	2.864**	3.309***	5.581***	1.762^{NS}	2.388**	2.328**	1.391^{NS}

[1] *F* -values from ANOVA; ^{NS} for not significant at P=0.05; * for P<0.05; ** for P<0.01; *** for P<0.001.

[2] *F* -values from ANOVA for variation among populations within each species.

TABLE 4. Statistical analysis of variation between and within two colour forms in seven characters (see material and methods for detailed descriptions) in *Tetranychus urticae* and *T. cinnabarinus* from 18 populations in greenhouses in UK. Measurements in micrometers and in the format: mean±s.e.

Mite colour forms (CF) and statistics	No. setae on Ti I	Length of Ti I	Distance ω"-ω'(N1)	Ratio $v_2/(v_2\text{-}sc_1)$	Length of sc_1	Distance $g_1\text{-}g_1$	Ratio $m/(m\text{-}m)$
Green mites	11.9±0.2	60.9±0.6	18.0±0.2	3.20±0.04	141.4±0.9	30.8±0.5	0.88±0.01
Red mites	11.0±0.2	59.8±0.5	17.2±0.3	3.13±0.03	141.7±0.8	33.9±0.6	0.89±0.01
Between CF variation[1]	12.25***	3.275^{NS}	9.317**	2.465^{NS}	0.037^{NS}	18.479***	0.828^{NS}
Within CF variation[2]	3.557***	3.171***	5.581***	1.866*	2.3891**	4.217**	1.654^{NS}

[1] *F* -values from ANOVA; ^{NS} for not significant at P=0.05; * for P<0.05; ** for P<0.01; *** for P<0.001.
[2] *F* -values from ANOVA for variation among populations within green forms or red forms.

Variation in the number of setae on tibia I and necrotic response in plants

It is evident from Table 1 that hyper-necrotic responses in tomato plants were not caused by *T. urticae* with 10 setae only on tibia I in females. It interesting to note that hyper-necrotic responses were associated with only some *T. cinnabarinus* populations with variation in the number of setae on tibia I but absent in *T. cinnabarinus* populations without variation in the number of setae on tibia I (i.e. 13 setae only).

Discussion

An important result from this study is that *T. cinnabarinus* populations on greenhouse tomato plants that we studied in the UK could be either red or green (about 50% for each). The colour of the mite therefore can not be reliably used to separate *T. urticae* and *T. cinnabarinus* in the UK because it is poorly correlated with other morphological differences between two species. Meyer (1987) also questioned the use of colour in separating species of this complex. It is unfortunate therefore that the green form of this complex is commonly referred to as *T. urticae* and the red form as *T. cinnabarinus* in the literature on Tetranychidae (e.g. Baker & Tuttle 1994). However, it should be noted that in most other parts of the world, *T. cinnabarinus* is red in colour and the green form has never been reported (e.g. Meyer 1987; Kuang & Cheng 1990; Baker 1994 & Tuttle).

The green mites identified by Dillion (1958: 446) as British *T. telarius* (L.) may be related to the green *T. cinnabarinus* that we studied as he noted six or seven setae proximad of basal set of duplex setae on tarsus I in females. In *T. cinnabarinus*, the addition of up to three solenidia on tibia I of females is correlated with the addition of two solenidia proximal to the proximal set of duplex setae (Boudreaux 1956). Our study of British *T. cinnabarinus* in this study confirmed this pattern. Tibia I in female spider mites usually have four normal setae proximal to proximal set of duplex setae. Thus Dillion (1958) noted 2-3 additional setae, two which should be the two added solenidia and the extra one could be the posteriorly displaced normal solenidion ω3(N1).

In his comparative study of chaetotaxy in Tetranychidae, Lindquist (1985) showed that tibia I of adult spiders mites normally has 9(+1ϕ), and 2-4 solenidia are usually added to tibia I of the adult male. Wauthy *et al.* (1998) also noted this in *T. urticae* and also the addition of two solenidia proximal to the proximal pair of duplex setae in males. Boudreaux (1956) noted that females of *T. cinnabarinus* had the addition of the 5 male solenidia when he synonymized *Tetranychus multisetis* McGregor and *Tetranychus dianthica* Dosse. Our studies of *T. cinnabarinus* in the UK confirmed

that the position of these solenidia are the same as those in males of *T. urticae, T. cinnabarinus* and many other spider mites. The addition of these male solenidia in *T. cinnabarinus* females is therefore apomorphic as it is not present in females of *T. urticae* and other related spider mites.

It is unfortunate that the number of male solenidia added to females of *T. cinnabarinus* is variable. Boudreaux (1956) did use this character in his key to species of the *T. urticae* complex as some populations of *T. cinnabarinus* had no male solenidia at all. Kuang and Cheng (1990) showed that the Chinese *T. cinnabarinus* showed variation in the number of solenidion on tibia I in females. Our study showed that some populations had 3 solenidia on tibia I in females while other populations showed variation. We did not observe any populations of *T. cinnabarinus* without the addition of 3 (rarely 1 and sometimes 2) male solenidia on tibia I of female. This character is therefore useful for separating *T. urticae* and *T. cinnabarinus* in the UK and provides a simple, practical method for applied acarologists. The advantage of using this character is that it is easy to observe using several females which are common in greenhouse samples. The use of female dorsal lobes is also useful but a very good microscope or SEM is needed and the mounting position of the specimens in the slide may affect the results (Meyer 1987). The shape of male aedeagus is also useful but males are sometimes rare in greenhouse samples and specimens need to be mounted in a perfect lateral position to reveal the shape of aedeagus; the variation in shape among populations (e.g. Wang 1981) caused added difficulty for applied acarologists using this character.

Debates on the specific status of *T. urticae* and *T. cinnabarius* have never ended since the first revision of the Tetranychidae by Prichard and Baker (1955) who listed 43 synonyms under *T. telarius* (L) (= *T. urticae).* More evidence seems to suggest that *T. cinnabarinus* is a species, or at least a subspecies, derived from *T. urticae* and it may be hypothesized that the speciation is still in progress. The extent of speciation can be seen in many breeding experiments demonstrating complete reproduction isolation between the two or reduced fertility of hybrid offsprings (Keh 1952; Boudreaux 1956; Dillion 1958; Parr & Hussey 1960; van de Bund & Helle 1960; Monrae 1963; Jordaan 1977; Kuang & Cheng 1990; P. Croft *et al.*, personal communication, 2000). The experiments showing the lack of these reproductive barriers are the exceptions (i.e. Dupont 1979) rather than the usual. Several morphological characters commonly used in the past (by Boudreaux 1956 and Kuang & Cheng 1990) can be used to separate the two species but may sometimes be unreliable. We also examined a few new female characters which showed significant variation between the two species. It is predictable that if we examine more characters, more specific differences may be found as we only randomly selected a few due to the lack of funded time for this project.

An interesting result from the study of variation in the number of setae on tibia I in *T. cinnabarinus* females is that hyper-necrotic responses in tomato plants were not caused by *T. urticae* which has 10 setae only on tibia I in females, nor by *T. cinnabarinus* populations without variation in the number of setae on tibia I (i.e. 13 setae only). It is interesting to note that hyper-necrotic responses were associated with only some *T. cinnabarinus* populations with variation in the number of setae on tibia I. Whether this association is coincidental is unknown at this stage.

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