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Cuticular lobes in the *Tetranychus urticae* complex (Acari : Tetranychidae) : a reliable taxonomic character?

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ABSTRACT. Taxonomic work on spider mites has mostly been based on morphological characters. The shape of lobes on dorsal integumentary ridges of summer females was used in the past as an additional morphological character to discriminate between the green and the red forms of the two-spotted spider mite *Tetranychus urticae*. However, we showed that lobes were not always present or were not completely formed in some females, presumably because of micro-environmental conditions. No clear-cut differences were put forward between green and red forms concerning that ridge structure. This underlines the care needed when this criterion is used. The possible role of lobes in adaptation to water balance is discussed.

KEY WORDS : Acari; spider mites, *Tetranychus urticae*; cuticular lobes, taxonomy.

INTRODUCTION

Descriptions of species of Tetranychidae (Acari) are mostly based on morphological characters. However, in the *Tetranychus* genus, some species are very difficult to distinguish as the identification is based on small differences in characters that express a range of variations. For instance, KOCH (1836) gave the first denomination *Tetranychus urticae* in his description in 1836. The mite described by KOCH was collected in Germany on *Urtica dioica*. Nevertheless, two forms are recorded, a green and a carmine or red form. Both forms are morphologically very similar and have a worldwide distribution. However, the green form is generally found in cold and temperate climates, while the red form occurs over much of the warmer temperate zone and subtropics (DUPONT, 1979). In the literature, important confusion exists due to the unclear form or species status resulting in the existence of numerous synonyms (VAN IMPE, 1991). BOUDREAUX (1956) raised the green and red forms to species status on the basis of breeding experiments and morphological characters such as the shape of cuticular lobes of adult summer females. BOUDREAUX indicated that red summer females (renamed by him *Tetranychus cinnabarinus*) bear narrow pointed cuticular lobes, while green summer females (*Tetranychus urticae)* bear broader and more rounded cuticular lobes. Recognition of these species depends thus almost entirely upon observations made on the cuticle, since colour was not considered as a reliable character in the revision of the spider mite family Tetranychidae by PRITCHARD & BAKER (1955). The value of the shape of dorsal lobes in females as a morphological character is subject to criticism. Indeed, the variation is difficult to interpret because of overlapping characters in both forms (VAN DE BUND & HELLE, 1960); lobes must be viewed in the same position (DOSSE & BOUDREAUX, 1962); dorsal strial lobe densities change with temperature and humidity (MOLLET & SEVACHERIAN, 1984); green mites were even identified as *Tetranychus cinnabarinus* when shape of lobes and number of setae on tibia I were used as morphological characters (ZHANG & JACOBSON, 2000). However, lobes were also used to distinguish between strains of two-spotted spider mites (HANCE *et al.*, 1998), using a biometrical approach.

Little information is available on the relation between integumental structures and their physiological functions for *Tetranychus urticae*. The definition of the lobes and their description are vague : the cuticle of the spider mite is supplied with numerous parallel ridges, and lobes are the result of the presence of incisions in these cuticular ridges (BOUDREAUX & DOSSE, 1963). The lack of information on cuticular structures provokes contradictory observations. Indeed, according to Boudreaux, males do not bear lobes on the cuticular folds (BOUDREAUX, 1956). However, in the description of cuticle structures, MOTHES-WAGNER & SEITZ (1984) indicated lobes on the male cuticle. The lack of information about nymphal stages and males is linked to the higher interest of taxonomical characters of females.

In this paper, we examine if the shape of lobes on the mediodorsal hysterosoma (between the third and fourth pair of dorsocentral hysterosomal setae) represents a useful tool to separate summer females within and between colour forms.

MATERIAL AND METHODS

Origins of mites and culture

Mites were collected on several nettle plants (*Urtica dioica*) in Southern France and in Belgium. Two samples of *T. urticae* were collected in July 2000 in France : the first sample came from Anduze (44˚03' N; 3˚59' E) and was composed of red mites, while the second sample

came from the vicinity of Montpellier in Lattes (43˚36'N; 3˚53'E) and was composed of green mites. One sample was collected in August 2000 in Belgium, in Rixensart (50˚42'N; 4˚32'E) and was composed of green mites.

Mites were reared for about one month in a conditioned temperature-humidity room held at 20˚C ± 3˚C, relative humidity 80%, and photoperiod 16/8 L/D. As these conditions were rather unfavourable (some females showed a winter orange colour, did not feed and did not lay eggs), three weeks before preparation of mites for observations, conditions were changed to : temperature 24˚C ± 0.5˚C, relative humidity 50%, photoperiod unchanged. This caused all females to attain summer colour, to start feeding and laying eggs. Then, for each population, ovipositing females were placed on isolated detached bean leaves in boxes filled with moist cotton wool. After 24 hours, all adult females were removed and the eggs were left until their development into adults. These adults were the observed mites.

Scanning Electron microscopy

Sister adult females were taken between three and four days after emergence. Individual females were taken with a small brush and glued onto metal plates. For scanning electron microscopy (SEM Philips XL20), specimens were flash frozen (-212° C) in liquid nitrogen under vacuum for cryo-SEM (Oxford CT1500 cryo-system), transferred to the preparation chamber, and then to the SEM chamber where, when the cuticle was moist, the frozen samples were sublimated (-80° C) to remove ice particles on the summits of the lobes. Specimens were viewed under 2--5 KV at 170 to 190° C.

Observations were made on the standard mediodorsal hysterosoma region. The longitudinal striae between the third and fourth pair of dorsocentral setae form a diamond-shaped figure between these setae (Tuttle & Baker, 1968). Observations were made in this area, near to the third dorsocentral hysterosomal setae. Eight red females from Anduze, five green females from Lattes and 12 green females from Rixensart were observed.

RESULTS

Pinpointing of the diamond-shaped figure on the hysterosoma is easy and is indicated in Fig. 1. Each female bears a different shape of this diamond-shaped figure. The size of the figure is quite similar but the outline varies particularly at the meeting points of the sides. This diamond-shaped figure is only present in adult females. In this area, the lobe size of our females was very variable. Indeed, some green and red individuals were characterised by typical lobes while others did not possess lobes or only less-developed lobes. The sampling location, mite colour and observation results are shown in Table 1. Figures 2 and 3 show the typical lobes for the red and green females. The lobes of green females were mostly semi-circular to oblong and wide, while sometimes triangular lobes were observed. The lobes of red females were mostly triangular to semi-circular and taller than on the green form; the lobes were generally more separated at their base. Figures 4 and 5 show less-developed and smooth lobes. In this case, it is impossible to distinguish between the green and the red forms. The variability in lobe development was high although the mites were related and reared in the same environmental conditions, and all observed females showed the colour of summer females : green or red according to the population.

TABLE 1

Presence and absence of lobes for the observed mites.

sampling location	Mite colour	Number of observed females	Number of females with typical lobes	Number of females without obes or with less developed lobes
France (Anduze)	Red	8	6	2
France (Lattes)	Green	5	3	2
Belgium (Rixensart)	Green	12	12	0

DISCUSSION

As shown in many studies (Van de Bund & Helle, 1960; Dosse & Boudreaux, 1962; Boudreaux & Dosse, 1963; Hatzinikolis, 1970), both green and red forms of the two-spotted spider mite *Tetranychus urticae* exhibit typical lobes as described by Boudreaux (1956). However, shape variability is found and some mites bear less-developed or smooth lobes. This variability weakens the reliability of this character for distinguishing between the forms and for comparing mites within a colour form. We found that separation between forms is not as clear as has been reported in the past (Boudreaux, 1956; Dosse & Boudreaux, 1962). Nevertheless, individual variability has already been pointed out (Van de Bund & Helle, 1960). Dosse & Boudreaux (1962) had criticized this last study by reporting that mites were improperly observed, however, at the same time they explained the variability by the occurrence of hybrid populations. Hybrids were also observed by Dupont (1979). Variability in dorsal strial lobe densities was also noticed, and linked to temperature and humidity (Mollet & Sevacherian, 1984).

Twenty-five females were analysed in this study. However, variability of lobe development was unexpectedly high, even for females coming from the same location. Indeed, if females bear less-developed or smooth lobes, it is impossible to relate the mite colour to lobe observations. Variability interpretation is difficult because this variability was noted while observed females were related and reared, after field collection, under the same environmental conditions. The differences we observed did not depend upon age as females were almost of the same age. A part of the variability recorded in the literature could be the result of individual preparation and, for instance, the way of mounting individuals for microscopy. In our case this bias is drastically reduced, as individuals were not dried to a critical point but handled directly in the microscope using liquid nitrogen. Critical point drying needs, indeed, successive baths in acetone or ethanol solutions that could dissolve or damage some structures. Our method allowed the preservation of the whole cuticle structure.

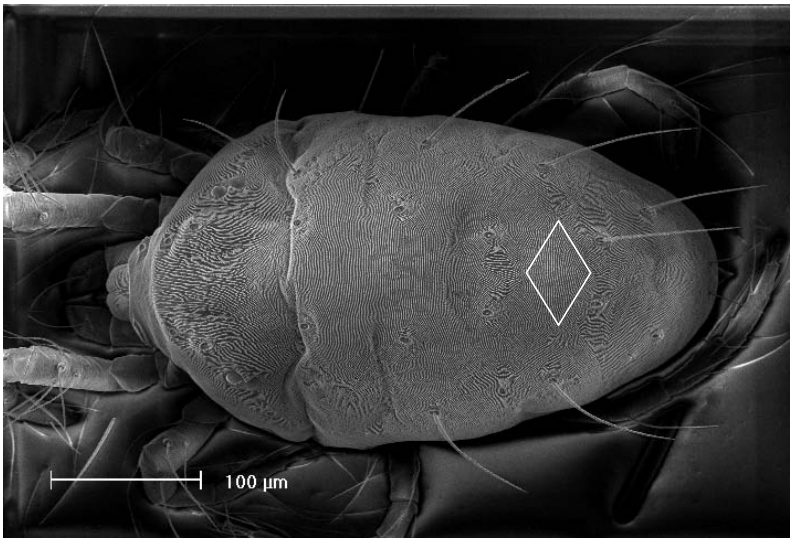
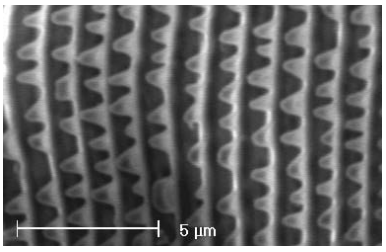


Fig.1



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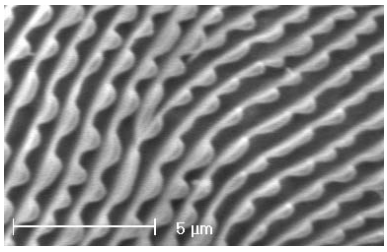

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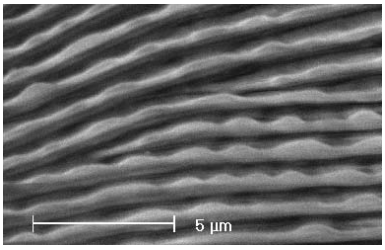

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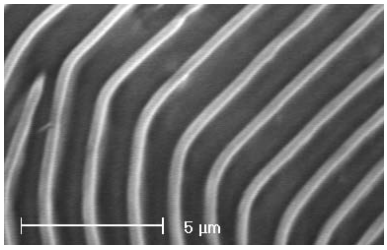

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Fig. 1. – A female *T. urticae*. The white diamond indicates the observed region of the dorsal cuticle.
Fig. 2. – Typical triangular or semicircular lobes of feeding red females. This female was sampled in Anduze (France).
Fig. 3. – Typical semi-oblong lobes of feeding green females. This female was collected in Lattes (France).
Fig. 4. – Less-developed lobes of a feeding green female. This female was collected in Lattes (France).
Fig. 5. – Smooth lobes of a feeding red female. This female was collected in Anduze (France).

Concerning the variability we observed in lobe development, an explanation could be found in the period of observation and in environmental conditions. Indeed, mites were firstly maintained in the laboratory in less than favourable conditions, during which time some females presented diapausing symptoms (orange, non-feeding and non-ovipositing). Subsequent modifications to the temperature and humidity conditions induced formation of the next generations of active individuals (green or red feeding mites according to the population). The observed feeding mites should show lobes, but it is possible that they were still affected by environmental conditions and by the environmental conditions experienced by their parents. Moreover, despite these controlled conditions, we cannot exclude completely the existence of small variations resulting from position of mites within the boxes. According to MOLLET & SEVACHERIAN (1984), humidity and temperature affect the dorsal strial lobe densities. It could partly explain the absence of lobes in some active females, but more experiments and information are needed to enable clear conclusions to be drawn. Our results suggest that care must be taken in the use of this morphological character in taxonomical work.

Concerning the role of lobes, BOUDREAUX (1958) hypothesised that changes in cuticular morphology may be due to differences between summer and diapause forms. Indeed, diapausing females lack lobes over much or all of their body. In summer active females, lobes may serve as an evaporative structure; the absence of that structure in diapausing (non-feeding) females would help them to conserve water. MILLER suggested another possible role by a personal communication to HENNEBERRY et al. (1965); the cuticular extensions could aid mite camouflage by giving the cuticle a relatively lustreless appearance.

DE BOER (1985) assumed some adaptive significance of the pigmentation associated with climate. Indeed, red and green forms display a worldwide distribution, but the green one is mostly present in cold and temperate climates while the red form occurs over much of the warmer temperate zone and subtropics (DUPONT, 1979). If in spite of variability, the shape of lobes differs according to colour forms, this could also indicate that shape corresponds to an adaptation to climate. The variability noticed in our study could perhaps be induced by very limited changes in abiotic conditions at the individual level. Moreover, observations were made after several generations from the collected mites; the variability could reflect a plasticity of the character.

Usefulness of shape of lobes as a taxonomical character is always subject to discussion, and at present it does not help us distinguish between mites as long as its precise role remains unknown. Recently, green mites were identified as *T. cinnabarinus* using shape of lobes and other morphological characters (ZHANG & JACOBSON, 2000). Up to now, green mites have never been reported as *T. cinnabarinus*. This shows the complexity in mite identity. Moreover, biological information such as breeding experiments sometimes show reproductive compatibility, partial compatibility or reproductive incompatibility (HELLE & PIETERSE, 1965).

In the future, studies on the physiological processes of the cuticle and its structure at each stage could help us understand the roles and the variability during the mite life according to environmental conditions. Moreover, molecular analyses may help to define more precisely the status of the different populations and to determine if the recorded variability could be due to a phenotypic plasticity or a genetic variability.

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REFERENCES

BOUDREAUX, H.B. (1956). Revision of the two-spotted spider mite (Acarina, Tetranychidae) Complex, *Tetranychus telarius* (Linnaeus). *Ann. Entomol. Soc. America*, 49 : 43-49.

BOUDREAUX, H.B. (1958). The effect of relative humidity on egg-laying, hatching, and survival in various spider mites. *J. Insect Physiol*., 2 : 65-72.

BOUDREAUX, H.B. & G. DOSSE (1963). The usefulness of new taxonomic characters in females of the genus *Tetranychus* Dufour (Acari : Tetranychidae). *Acarologia*, 1 : 13-33.

DE BOER, R. (1985). Reproductive Barriers. In : *World Crop Pests. Spider Mites. Their Biology, Natural Enemies and Control. Volume1A.* HELLE, W. & M.W. SABELIS (Ed.). Elsevier, Amsterdam, Oxford, New York and Tokyo : 196.

DOSSE, G. & H.B. BOUDREAUX (1962). Some problems of spider mite taxonomy involving genetics and morphology. *Advances in Acarology*, 1 : 343-349.

DUPONT, L.M. (1979). On gene flow between *Tetranychus urticae* Koch, 1836 and *Tetranychus cinnabarinus* (Boisduval) Boudreaux, 1956 (Acari : Tetranychidae) : synonymy between the two species. *Entomol. Exp. Appl*., 25 : 297-303.

HANCE, TH., NEUBERG, P. & CH. NOÈL-LASTELLE (1998). The use of fecundity, lobe bimetry and the RAPD-PCR technique in order to compare strains of *Tetranychus sp. Exp. Appl. Acarol*., 22 : 649-666.

HATZINIKOLIS, E.N. (1970). Contribution à l'étude de l'espèce *Tetranychus telarius* (linnaeus, 1758) (complex) (Acarina : Tetranychidae). *Annls Inst. phytopath. Benaki N.S*., 9 : 207-218.

HELLE, W. & A.H. PIETERSE (1965). Genetic affinities between adjacent populations of spider mites. *Entomol. Exp. Appl.*, 8 : 305-308.

HENNEBERRY, T.J., J.R. ADAMS & G.E. CANTWELL (1965). Fine structure of the Integument of the Two-Spotted Spider Mite, *Tetranychus telarius* (Acarina : Tetranychidae*). Ann. Entomol. Soc. America,* 58 : 532-535.

KOCH, C.L. (1836). *Tetranychus urticae. Deutsche Crustacea, Myriapoda*, 1 : 10.

MOLLET, J.A. & V. SEVACHERIAN (1984). Effect of temperature and humidity on dorsal strial lobe densities in *Tetranychus* (Acari : Tetranychidae). *Internat. J. Acarol.*, 10 : 159-161.

MOTHES-WAGNER, U. & K.A. SEITZ (1984). Fine structure of the cuticle and structural changes occurring during moulting in the mite *Tetranychus urticae* I. Fine structure of the cuticle. *Acarologia*, 25(3) : 253-258.

PRITCHARD, A.E. & E.W. BAKER (1955). *A revision of the spider mite family Tetranychidae.* Memoirs Series volume 2. Pacific Coast Entomol. Soc., San Francisco.

TUTTLE, D.M. & E.W. BAKER (1968). *Spider mites of southwestern United States and a revision of the family Tetranychidae.* University of Arizona Press, Tucson, Arizona.

VAN DE BUND, C.F. & W. HELLE (1960). Investigations on the *Tetranychus urticae* complex in Northwest Europe (Acari : Tetranychidae*). Entomol. Exp. Appl.*, 3 : 142-156.

VAN IMPE, G. (1991). De la difficulté de désigner correctement un organisme vivant. Le cas du Tétranyque tisserand, *Tetranychus urticae* Koch, 1836 (Acari : Tetranychidae). *Bull. Annls. Soc. r. Belg. Ent*., 127 : 71-76.

ZHANG, Z-Q & R.J. JACOBSON (2000). Using adult female morphological characters for differentiating *Tetranychus urticae* complex (Acari : Tetranychidae) from greenhouse tomato crops in UK. *Syst. & Appl. Acarology*, 5 : 69-76.