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On the perception of leaf morphology and visible light by *Tetranychus urticae* Koch (Acariformes, Tetranychidae)

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Original research

ABSTRACT

The two-spotted spider mite *Tetranychus urticae* is one of the most polyphagous and most damaging pests worldwide. The behaviour of the mite differs on its various host plants and this could influence crop defence strategies. We investigated the perception of leaf morphology by *T. urticae* in relation to the effects of visible light on the selection of sites on which females prefer to live and feed. Mite females chose the shaded surface when smooth and glabrous lemon leaves were offered as a surface for living and feeding, showing a photophobic behaviour. However, they clearly preferred the abaxial surface of bean leaves regardless of the presence or absence of visible light. As no differences were found regarding the food quality between the adaxial and abaxial leaf surfaces, mite behaviour seems to be related firstly to the perception of leaf morphology and secondly to visible light. As regards the first factor, *T. urticae* prefers the abaxial surface of leaves with a complicated architecture, i.e. veins in relief, on which they can easily build their dense web, and this factor has priority in comparison to the negative effect of the visible radiations. The second factor is important on smooth and glabrous leaves, these being unsuitable for building the complicated characteristic webs of *T. urticae*.

Keywords *Tetranychus urticae*; leaf architecture; visible light; bean leaf; behaviour; lemon leaf

Introduction


The behaviour of phytophagous mites on plants has important practical implications for the management of their populations in the field. Various authors have investigated the influence of environmental factors (temperature, relative humidity, photoperiod, UV wavelengths etc.) on these microarthropods (Force 1967; Rodriguez *et al.* 1967; Barcelo 1981; Nihoul 1993) and others have focused on the influence of plant chemistry (Juniper and Southwood 1986), of leaf surface morphology or of some features of the external foliar architecture on phytophagous mites (Grostal & O'Dowd 1994; Walter & O'Dowd 1995; Karban *et al.* 1995; Park & Lee 2002), as well as on the phytoseiid mites and predator-prey interactions (Duso 1992; Duso & Pasqualetto 1993; Norton *et al.* 2001; Seelmann *et al.* 2007; Schmidt 2014). Leaf architecture, including microarchitecture, provides microhabitats that strongly affect the presence and performance of mites on host plants (Walter 1992; Karban *et al.* 1995; Kreiter *et al.* 2002; Ferreira *et al.* 2011; Schausberger 2021). Foliar tomentum for example, positively influences the abundance of many species of mites in all trophic levels, both in natural and agricultural ecosystems, while glabrous leaves are less preferred by the mites (Duso & Pasqualetto 1993; Walter & O'Dowd 1995; Seelmann *et al.* 2007). Leaves represent for micro-arthropods not only a food source but also the microhabitats on which they live (Southwood 1986; Walter

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1996). The adaxial (upper) and abaxial (under) leaf surfaces, have different microclimates due mainly to transpiration (Willmer 1986), and this can be decisive for the choice of living place for the tiny mites. Moreover, epicuticular wax deposits are more evident on the adaxial leaf surface (Baker *et al.* 1975; Eigenbrode & Espelie 1995) and this could be an insurmountable limit for various phytophagous mite species with very short and tiny stylets, leading them to prefer the abaxial leaf surface.

Tetranychus urticae Koch, for example, clearly prefers the abaxial leaf surface, although its stylet lengths allow it to penetrate foliar tissues and get food from both leaf surfaces (Sances *et al.* 1977; Park & Lee 2002), leading to the hypothesis that other factors may be important in abaxial/adaxial surface choice. Barcelo (1981) demonstrated the detrimental effects of UV-B on various biological parameters of *T. urticae* females and Ohtsuka and Osakabe (2009) argued that the preference for the lower leaf surfaces in *T. urticae* is a behavioural adaptation to enable avoidance of UV-B radiation, helped by their ability to perceive and avoid 320–340 nm wavelengths (Sakai & Osakabe 2010). However, new UV-blocking films used in greenhouses as protection from different pests and diseases (Espí & Salmerón 2002), are able to neutralize the UV negative effect, allowing the species to infest also the upper surfaces of the leaves as well. Using visible light source, Mori (1962) showed a positive photokinesis of *T. urticae* females at temperatures ranging from 15°C to 30°C, but no data are available on the perception of leaf architecture by *T. urticae* in relation to the effects of visible light.

In the present study we used leaves of two plant species, the common bean *Phaseolus vulgaris* L. and lemon, *Citrus limon* (L.) Osbeck, both being suitable hosts for *T. urticae* but showing strong differences in their leaf surface morphology. We aimed to investigate on the effects of visible light and leaf architecture on the selection of sites on which *T. urticae* females prefer to live and feed.

Material and methods

Mite cultures

The two-spotted spider mite *T. urticae* was collected from weeds and *Solanum melongena* L. in an organic farm in Balestrate (Palermo, Italy) (38° 1'41.49"N 13° 1'55.61"E) and reared on potted bean plants (*P. vulgaris*) in a conditioned greenhouse (22–32°C, 55–74% RH and natural photoperiod), for eight months before the beginning of the experiments. In order to adapt the population to the *Citrus* plants, about 700 females and males of *T. urticae* were transferred onto lemon fruits, kept in a conditioned room at 26±2°C and 75±5% RH, one month before the beginning of tests.

To obtain the young females for tests, about 50 females from each rearing, were transferred with a fine paintbrush (4/0) onto a clean bean leaf, or onto a clean lemon fruit, and removed after 24 hours. About 200 eggs were laid in this time interval. After completion of the postembryonic development, young females (max 24h old) were individually paired with a male on a leaf disc (bean or lemon leaf), for 24 hours to ensure fertilization and afterwards used for the experiments.

Experimental design

Light source and experimental conditions

The light source used in the experiments produces visible light, $\lambda \approx 550$ nm, supplied by two NL-T8 36W/865 cool daylight fluorescent lamps with a luminous flux of 3,250 lm and 6,500 K of colour temperature. Light sources were placed about 70 cm above the experimental area (1 m²). Temperature and relative humidity measured in the experimental area did not differ from that registered at the centre of the conditioned room (25±1°C and 70±5% of R.H.). The photoperiod was of 16:8h (Light:Dark).

No-choice experiment

In order to verify the influence of food quality obtained by the mites from both the adaxial and abaxial surface of each type of leaves, we carried out a no-choice test using each leaf surface for both bean and lemon leaves. The outcomes for comparisons were the survival and the oviposition rate of mated young females. Bean or lemon leaf-discs (3cm diameter) were used for these tests. Discs were individually placed on wet cotton wool in a Petri dish (9cm diameter, 1.4cm height). For each leaf type, two sets of tests were carried out: i) Adaxial surface upward (AdSU); ii) Abaxial surface upward (AbSU). One fertilized young female was introduced on each leaf disc. Each test was replicated 30 times and lasted 5 days. Every 24 hours, the number of alive females and the number of eggs laid were recorded; eggs were removed daily.

Choice experiment

To verify the influence of the leaf surface morphology and visible light on mite distribution, whole bean or lemon leaves were used. Available leaf areas were $22.1 \pm 3.11 \text{ cm}^2$ and $28.5 \pm 3.15 \text{ cm}^2$ (mean \pm SE per side) for bean and lemon leaves respectively. Females allowed to move between the adaxial and abaxial surfaces of natural- or reversed-orientated leaves of bean or lemon. Illumination was supplied at the top of leaves (Figure 1). The choice tests performed, are reported in table 1. Whole petiolate leaves were used in all choice tests. The petiole was wrapped with cotton wool and inserted into a plastic test tube (10 x 1.4 cm) filled with water, in order to keep the leaf turgid (Figure 1). Test tubes with leaves were placed in a 12-place tube-holder (5 tubes per each tube-holder). One young *T. urticae* female was put on each leaf and each test was replicated 30 times.

For each replicate, the position of the females was recorded at one-hour interval for the first five hours and afterwards at a 24-hour interval for five days. The female's survivorship and oviposition rate were recorded as mentioned above.

Statistical analysis

To analyse survivorship in the no-choice tests, as the response is live or death, the Binary Logistic Regression (BLR) was adopted, using the time (5 days) and the four treatments (adaxial and abaxial surface of bean and lemon leaves) as factors. For the oviposition rate, the General Linear Model (GLM) was adopted. The assumption of normality was verified on the basis of the analysis of residuals and the comparison between treatments was evaluated using the Bonferroni simultaneous tests for differences between means, with family-wise error rate equal to 5%.

In the choice tests, the response was the position of the female at the different time intervals: *up* (illuminated surface) and *down* (shaded surface). Being a binary response, a BLR analysis

Table 1 Choice tests carried out with young fertilized females of *Tetranychus urticae*

| Test | Acronyms | Type of leaf | Orientation of leaf | Illuminated surface | Initial position of female |
|--------|------------|--------------|---------------------|---------------------|--------------------------------------|
| (i) | BNI | Bean | Natural | Adaxial | Illuminated side (adaxial) (Fig 1.1) |
| (ii) | LNI | Lemon | | | |
| (iii) | BNS | Bean | Natural | Adaxial | Shaded side (abaxial) (Fig 1.2) |
| (iv) | LNS | Lemon | | | |
| (v) | BRI | Bean | Reversed | Abaxial | Illuminated side (abaxial) (Fig 1.3) |
| (vi) | LRI | Lemon | | | |
| (vii) | BRS | Bean | Reversed | Abaxial | Shaded side (adaxial) (Fig 1.4) |
| (viii) | LRS | Lemon | | | |

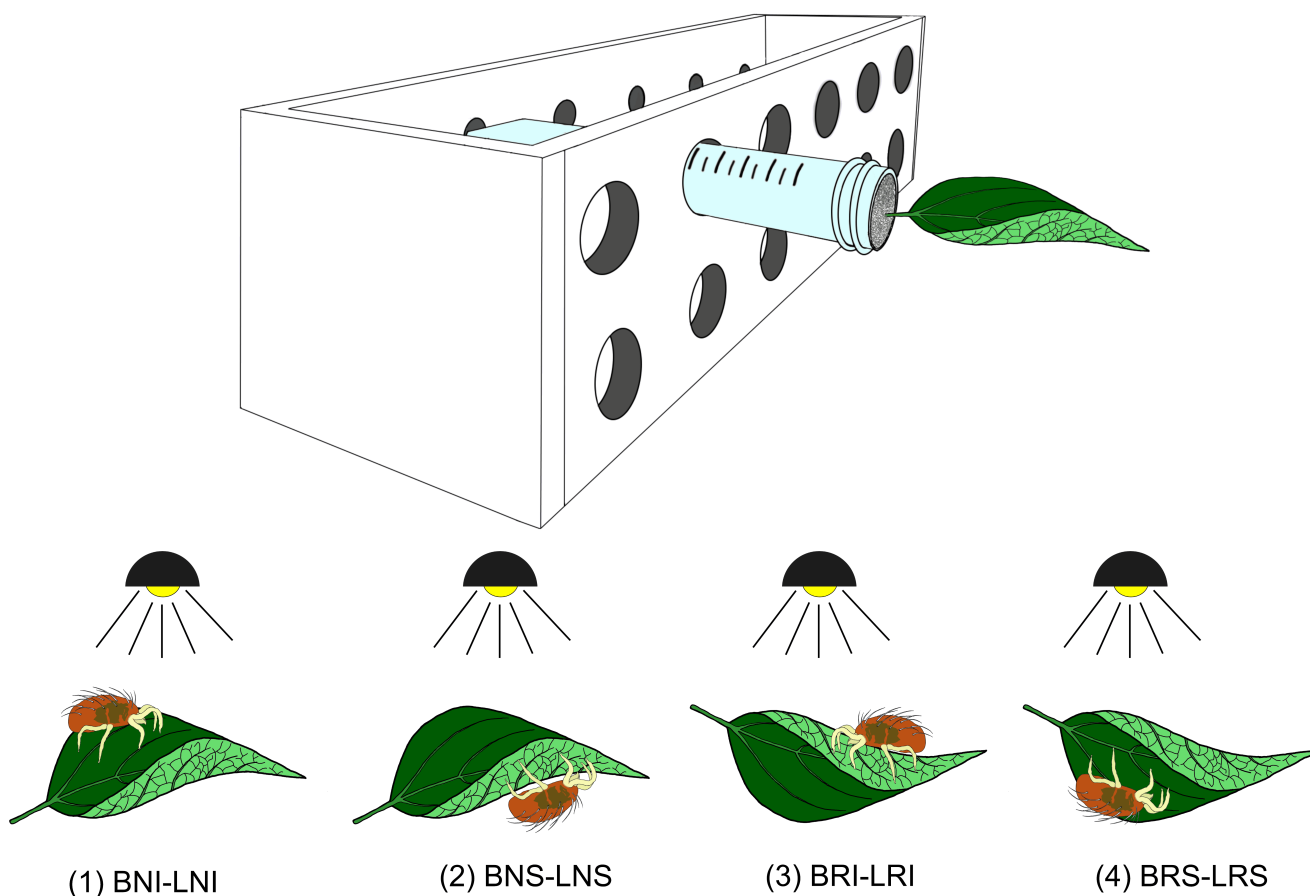


Figure 1 A diagram of experimental device used to hold the host plant leaves in the choice experiment, and the setup of four treatments for each host plant types: a mated female was placed on: (1) the illuminated-surface of a natural-orientated leaf of bean (BNI) or lemon (LNI), (2) the shaded-surface of a natural-orientated leaf of bean (BNS) or lemon (LNS), (3) the illuminated-surface of a reverse-orientated leaf of bean (BRI) or lemon (LRI), and (4) the shaded-surface of a reverse-orientated leaf of bean (BRS) or lemon (LRS).

was used to check the significance of the following factors: i) type of leaf (bean and lemon), the initial placement of the female (on the illuminated or on the shaded surface), ii) the orientation of the leaf (“natural” or “reversed”) and iii) time. The Bonferroni simultaneous tests were used to test the hypothesis $H_0: P=0.5$, i.e. the chance of finding the female on the illuminated (up) or on the shaded (down) surface of the leaf, is the same (family error equal to $\alpha=0.05$ and $k=8$).

To analyse the oviposition rate, the general linear model (GLM) was used, as for the no-choice tests. The interactions between leaf type, initial placement of mite, and orientation of leaf were taken into account and were analysed conjointly, using the Bonferroni Pairwise Comparisons (family-wise error rate equal to 5%). Analyses were carried out with Minitab® 17.1.0 software.

Results

No-choice experiment

Statistical analysis showed that, regarding survivorship, both time ($\chi^2 = 26.21$; $df = 4$; $P < 0.001$) and leaf type ($\chi^2 = 131.45$; $df = 3$; $P < 0.001$) were highly significant. The survival

rate of *T. urticae* at the end of the tests was high on bean leaves, up to 90%, and no significant differences were found between the two leaf surfaces (*Odds ratio* = 2.5684; *95% C.I.*: 0.49–13.52). On the contrary, differences were registered between the two lemon leaf surfaces: 33.3% vs. 76.7% of survival was registered at the end of the tests for the adaxial and abaxial surfaces respectively (*Odds ratio* = 0.2842; *95% C.I.*: 0.17–0.49) (Figure 2). Both of these survival rates were significantly lower than those on bean leaves. As regards the oviposition rate, no significant differences were noted between the adaxial and abaxial surfaces of either leaf type ($t = -0.33$; $df = 509$; $P = 0.745$), while the oviposition on both surfaces of bean leaves was significantly higher than that on both surfaces of lemon leaves ($t = -40.88$; $df = 509$; $P < 0.001$) (Figure 2): the mean oviposition rate in the test period was 10.5 and 10.4 eggs/female/day on the adaxial and abaxial surface of bean leaves respectively and 1.1 and 1.2 eggs/female/day on the adaxial and abaxial surface of lemon leaves respectively.

Choice experiment

The position of the mites one hour after the start of the tests, was found to be related to the type of leaf, the orientation of the leaf and the initial position of mite (Table 2). The most influential factor was the leaf type ($\chi^2 = 46.29$; $df = 1$; $P < 0.001$), followed by the interaction between the type of leaf and its orientation ($\chi^2 = 20.46$; $df = 1$; $P < 0.001$), the orientation of the leaf ($\chi^2 = 15.13$; $df = 1$; $P < 0.001$) and finally by the initial position of the mite ($\chi^2 = 13.53$; $df = 1$; $P < 0.001$). This result led us to analyse each test separately because both factors and interactions were significant. To analyse the position of *T. urticae* females at the different time intervals, the position of the mite in the previous time interval was added into the model. In these new models, the type of leaf remained significant, but the position of the mite at the previous time interval (1st hour), became the most significant factor ($\chi^2 = 158.91$; $df = 1$; $P < 0.001$). Similar trend was registered in all the following time intervals (Table 3).

On natural oriented bean leaves (BNI) (Figure 3A1), *T. urticae* females showed a strong preference for the shaded surface of the leaf, but the initial position of the mite also slightly influenced their choice. Indeed, when tetranychid females were initially placed on the illuminated surface (adaxial), 30% of them (9 females) stayed there and 70% (21 females) migrated onto the abaxial one, remaining there until the end of the tests ($p = 0.30$, *95% C.I.*: 0.13–0.56) (Table 2). However, the family error significance level of 0.05 was not reached in the first hour, because the individual level for the Bonferroni procedure has to be constrained at a very low level (0.00625). On the contrary, when the initial position of mites was on the shaded surface (abaxial) (Figure 3A2 and Table 2) (BNS tests), only 10% of females migrated onto the upper surface of the leaf (adaxial) ($p = 0.10$, *95% C.I.*: 0.02–0.38). In both tests, the

Table 2 Fitted probability on the position of mites on the illuminated surface one hour after the initial placement. H_0 : $P = 0.5$, i.e. the chance to find the mite on the two leaf surfaces is the same (Bonferroni test with family error equal to $\alpha = 0.05$ and $k = 8$). Probability value is not significant (n.s.) when the individual confidence interval includes 0.5. (1) Type of leaf; (2) Orientation of leaf; (3) Surface of initial position of female

| (1) | Tests | | Fitted probability±S.E. | Individual Confidence Interval (99.37%) |
|-------|----------|-------------|-------------------------|---|
| | (2) | (3) | | |
| Bean | Natural | Illuminated | 0.30±0.083 | 0.13–0.56 n.s. |
| Bean | Natural | Shaded | 0.10±0.054 | 0.02–0.38 |
| Bean | Reversed | Illuminated | 0.90±0.054 | 0.63–0.98 |
| Bean | Reversed | Shaded | 0.57±0.091 | 0.32–0.78 n.s. |
| Lemon | Natural | Illuminated | 0.20±0.073 | 0.07–0.46 |
| Lemon | Natural | Shaded | 0.20±0.073 | 0.07–0.46 |
| Lemon | Reversed | Illuminated | 0.26±0.081 | 0.11–0.53 n.s. |
| Lemon | Reversed | Shaded | 0.07±0.045 | 0.01–0.35 |

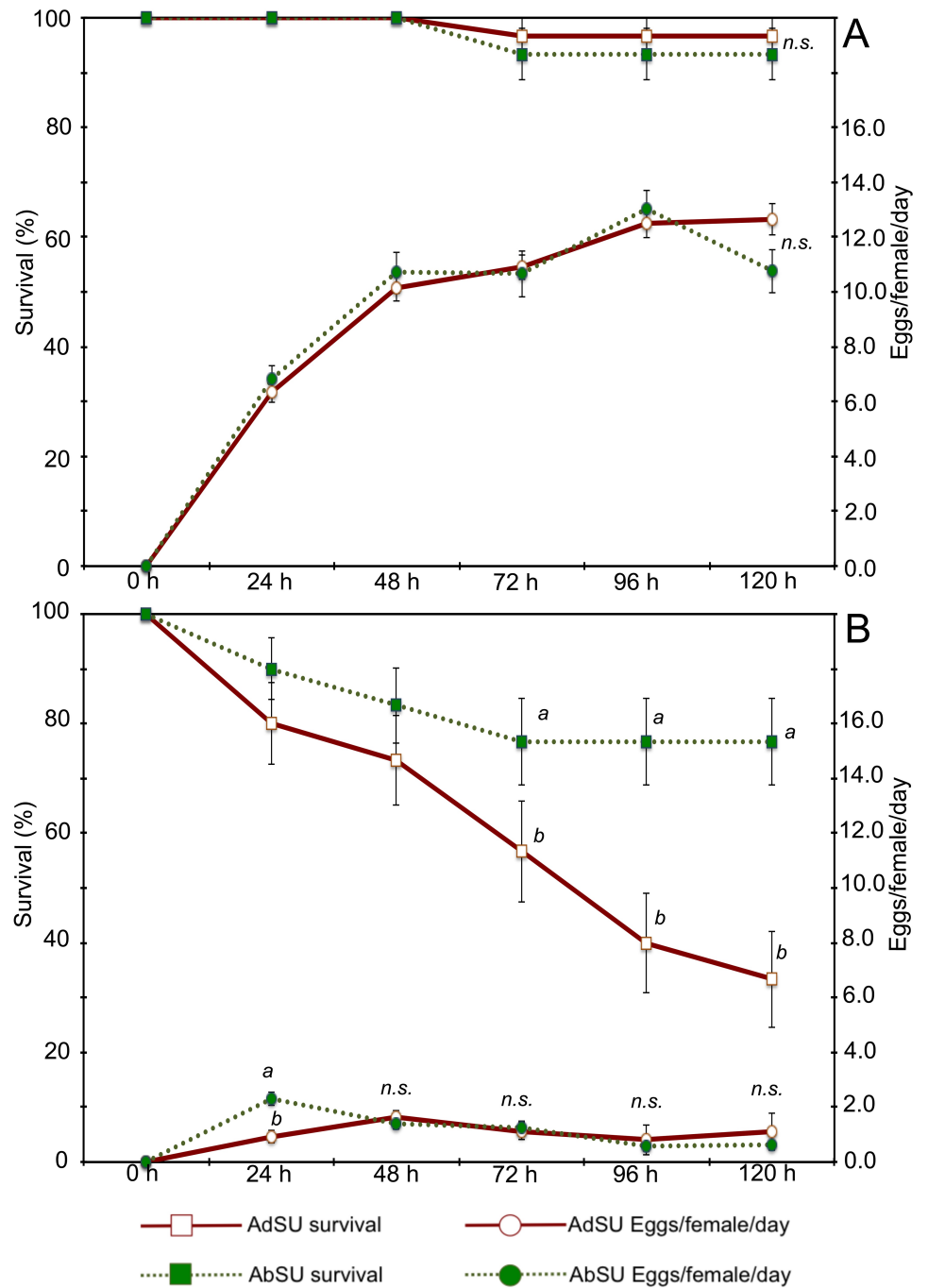


Figure 2 Survival (squares) and oviposition rate (circles) of *Tetranychus urticae* females fed on adaxial (AdSU-open squares and circles) or abaxial (AbSU-closed squares and circles) surface of bean (A) and lemon (B) leaves. Different letters denote significant differences ($P < 0.05$)

movements of females between the two leaf surfaces mostly occurred during the first hour. For example, in BNI tests 21 females moved towards the shaded surface of the leaf (abaxial) within the 1st hour, one female moved after 2 hours, two females after 3 hours, one female after 24 hours and one after 2 days. Only four females did not change their initial position, remaining on the adaxial illuminated surface. In BNS tests, only one female moved from the abaxial (shaded) surface to the adaxial one (illuminated) and stayed there for the entire test period. Another

two females moved towards the illuminated surface during the first 1–3 hours, but afterwards returned to the shaded one, staying there until the end of the tests.

This situation was dramatically different in tests with “reversed” bean leaves. When *T. urticae* females were placed on the illuminated surface of the leaf (in this case the abaxial one) (Figure 3A3 - BRI tests and table 2), 87% of mites (26 females) stayed there and only 7% migrated towards the shaded surface (adaxial) maintaining their position until the end of the tests ($p = 0.90$, 95% C.I.: 0.63–0.98). On the other hand, when tetranychid females were initially placed on the shaded surface of the leaf (adaxial) (Figure 3A4 - BRS tests), more than 50% of them migrated to the illuminated surface (abaxial) after 1 hour ($p = 0.57$, 95% C.I.: 0.32–0.78) (Table 2).

In BRI tests, only two females changed their initial position moving towards the shaded adaxial surface, while 26 females maintained their initial position; two females changed more times their position moving between the illuminated and shaded surface of the leaf. On the contrary, BRS tests greatly upset the females’ behaviour. Nine females remained in the initial position and 12 of them moved towards the illuminated abaxial surface within the first hour, remaining there until the end of the tests. The remaining 9 females changed their position between the two leaf surfaces several times during the test period. Behaviour of females in all abovementioned tests did not change significantly after 24 hours in comparison with that recorded after one hour, and also after the last position of females after 5 hours. Similarly, female behaviour was mainly conditioned by the position recorded at 24 hours, at all the following times: 48, 72, 96 and 120 hours (Table 3).

The behaviour of females on lemon leaves, differed significantly from that described above on bean leaves, considering the four tests overall, but some similarities were recorded in individual tests. For example, in both tests with “natural” orientation of lemon leaves (Figure 4A1–2 - LNI and LNS tests respectively), *T. urticae* females showed a clear preference for the shaded surface of the leaf after the first hour ($p=0.20$, C.I. 0.07–0.47, in both the LNI and LNS tests) (Table 2), but they were particularly agitated afterwards. In LNI tests two females remained in the initial position and 11 females moved towards the shaded abaxial surface within the first hour, remaining there until the end of the tests, but 17 females (56%) changed their position multiple times during the remaining test period. The number of females that maintained their initial position for the whole test period in LNS tests, was higher (18 females) in comparison to the previous tests, even if not significantly different, while 12 females changed their position multiple times. No one female remained on the illuminated adaxial surface for the entire test period.

In LRI tests (Figure 4A3 and Table 2), the females’ behaviour was similar to that observed in the above mentioned tests, although the family error significance level of 0.05 was not

Table 3 Statistics for the position of females during the test period in choice tests. At each time, the model adopted included the position of any single female in all previous time intervals: at the 2nd hour the position at the 1st one, at the 3rd hour the position at the 2nd and 1st hour, and so on. By means of a stepwise regression procedure, only the significant factors were selected after the 1st hour in the choice tests.

| Time (h) | c^2 | df | P | Goodness of fit test (Pearson) |
|----------|--------|----|-------|--------------------------------|
| 2 | 158.91 | 1 | 0.000 | 0.184 |
| 3 | 69.57 | 1 | 0.000 | 0.545 |
| 4 | 98.03 | 1 | 0.000 | 0.908 |
| 5 | 69.85 | 1 | 0.000 | 0.322 |
| 24 | 123.67 | 1 | 0.000 | 0.414 |
| 48 | 82.04 | 1 | 0.000 | 0.415 |
| 72 | 54.19 | 1 | 0.000 | 0.512 |
| 96 | 10.63 | 1 | 0.001 | 0.083 |
| 120 | 8.62 | 1 | 0.003 | 0.074 |

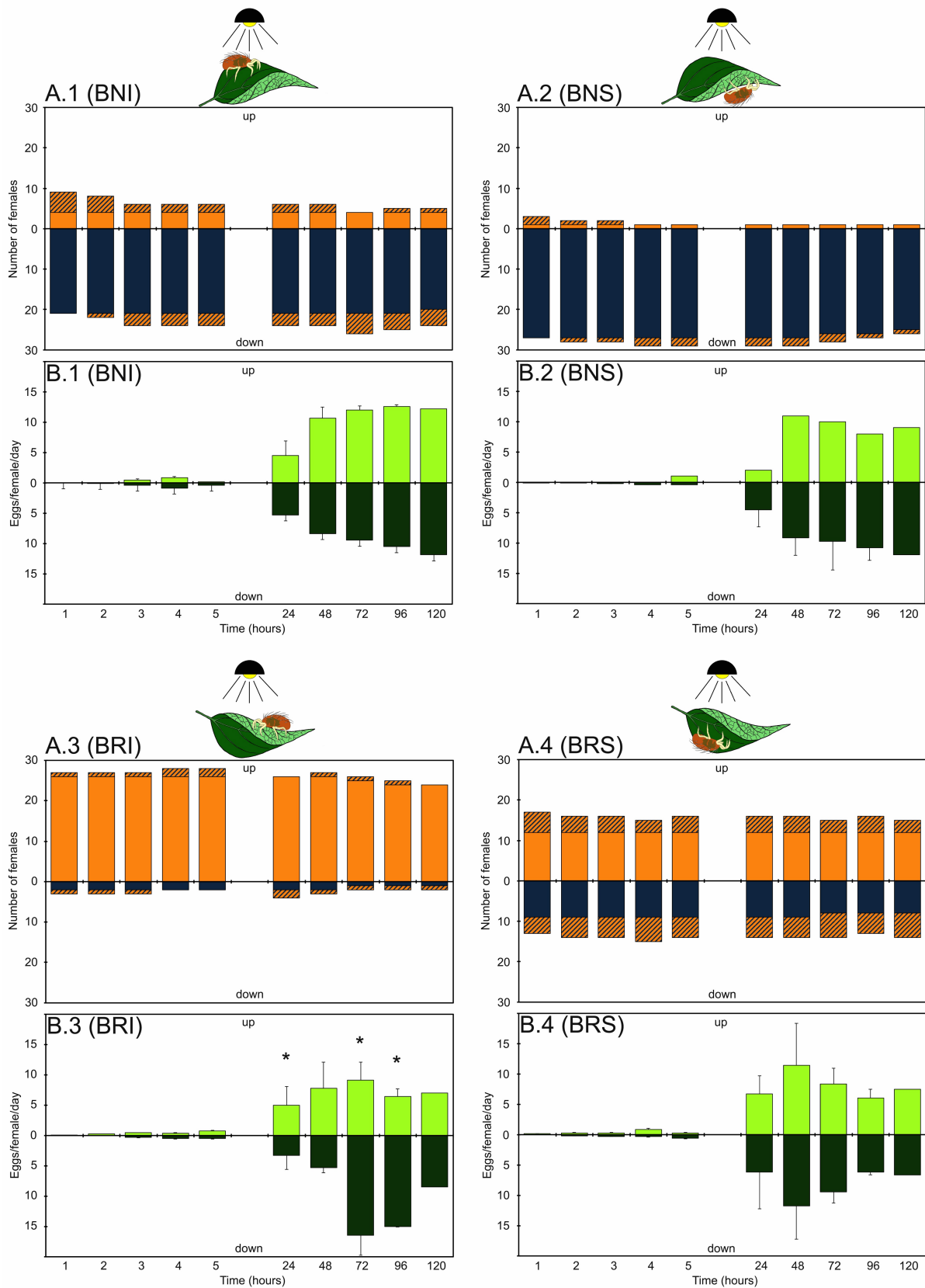


Figure 3 Distribution of *Tetranychus urticae* females (A) and daily oviposition rate (B) on bean leaves: A1, B1 (BNI); A2, B2 (BNS); A3, B3 (BRI); A4, B4 (BRS) (see also Table 1). ‘Up’ indicates the illuminated surface of the leaf and ‘Down’ the shaded one. The drawings indicate the light source, the orientation of the leaves and the initial position of mites. Uniform coloured histograms in A1–4 indicate the number of females that did not change their position after that occurred during the first hour. Striped histograms indicate the number of females that changed their position at least once during the test period. Bars in B1–4 histograms represent S.E. of means; asterisks indicate significant differences between the two leaf surfaces.

reached ($p=0.26$, $C.I.$ 0.11–0.53), but was very different to that observed in the same kind of test with bean leaves ($P<0.001$). As a matter of fact, only four females stayed on the illuminated abaxial surface and 11 moved towards the adaxial shaded surface, remaining in these positions until the end of the test or death. The other females changed their position multiple times (Figure 4A3). On the contrary, in LRS tests (Figure 4A4), about half of the females stayed in their initial position until the end of the tests and only one moved onto the illuminated surface staying there for the whole test period ($p=0.06$, $C.I.$ 0.009–0.34) (Table 2). Fifteen females changed their position multiple times. This behaviour differed significantly from that in the corresponding tests on bean leaves ($P<0.001$).

The behaviour of females in all the abovementioned tests on lemon leaves, did not change after 24 hours in comparison with that observed after 1 hour or after the last position of females observed at 5th hour. Similarly, female's behaviour was mainly conditioned by the position recorded in the previous time interval, for the entire test period.

The oviposition rate of *T. urticae* females was strongly influenced by the type of leaf (factor 1) ($F = 1174.02$; $df = 1, 966$; $P < 0.001$), but also by the orientation of the leaf surface (factor 2) ($F = 14.57$; $df = 1, 966$; $P = 0.000$), time (factor 3) ($F = 17.09$; $df = 4, 966$; $P < 0.001$) and the initial position of the female (factor 4) ($F = 8.27$; $df = 1, 966$; $P = 0.004$). It should be mentioned that interactions between factors 1-2-3 and factors 1-2-4 were also significant ($F = 7.92$; $df = 4, 966$; $P < 0.001$ and $F = 4.95$; $df = 1, 966$; $P = 0.026$ respectively); therefore, these factors were analysed conjointly. No differences were found for the oviposition rate between the two leaf surfaces during the first 5 hours in all tests. As regards bean leaves (Figure 3B1–4), the oviposition rate recorded on the first day was significantly lower than at subsequent times ($F=10.73$; $df = 4, 966$; $P < 0.001$), while no differences were noted among the latter ($F = 1.28$; $df = 1, 28$; $P = 0.267$) (Table 4). On the other hand, no differences were noted for the oviposition rate among the set of tests on lemon leaves (Figure 4B1–4). With regards leaf orientation, *T. urticae* females laid more eggs on the “naturally” oriented bean leaves (9.15 and 9.18 eggs/female/day for BNI and BNS tests respectively) than on “reversed” oriented leaves (7.01 and 7.87 eggs/female/day for BRI and BRS tests respectively), regardless of initial position of the mite ($F = 4.00$; $df = 1, 545$; $P = 0.046$), but no differences were found among tests on lemon leaves ($F = 2.25$; $df = 1, 400$; $P = 0.135$). On the other hand, no differences were found in the daily oviposition rate between the illuminated and the shaded surface of leaves in any test ($F = 0.53$; $df = 1, 545$; $P = 0.466$ and $F = 0.76$; $df = 1, 400$; $P = 0.552$ for bean and lemon leaves respectively) except BRI. In the latter test, oviposition was higher during the first day on the illuminated abaxial surface ($F = 9.6$; $df = 1, 28$; $P=0.004$), while it was lower during the 72 and 96h intervals ($F = 4.46$; $df = 1, 26$; $P = 0.044$ and $F = 8.23$; $df = 1, 25$; $P = 0.008$ respectively) (Figure 3B3 and Table 4).

Discussion

No difference in the oviposition rate of *T. urticae* females was observed between the adaxial and abaxial surface of bean leaves, indicating the same food quality of both leaf surfaces. Consequently, selection of the abaxial or adaxial surface does not seem to be related to the search for better food quality or for an easier way to obtain food. Similar results were reported by Sakai *et al.* (2012), although the latter authors reported greater fecundity on the adaxial bean leaf surface facing downward. Some authors have speculated on differences in the epidermal thickness or the adaxial-biased palisade mesophyll (Tateishi 1987; Vasquez *et al.* 2008). However, the epicuticular wax deposits on bean leaves are distributed as amorphous films (Jeffree 1986) and do not differ significantly between the two leaf surfaces: $0.9 \mu\text{g cm}^{-2}$ and $0.6 \mu\text{g cm}^{-2}$, for the adaxial and abaxial surface respectively (Baker & Hunt 1981), allowing mites to feed easily on both surfaces. This lack of difference is also supported by the survivorship of females, which was high and similar on both surfaces (Figure 2A). On the contrary, the higher mortality on lemon leaves, in particular that recorded on the adaxial surface, and the lower

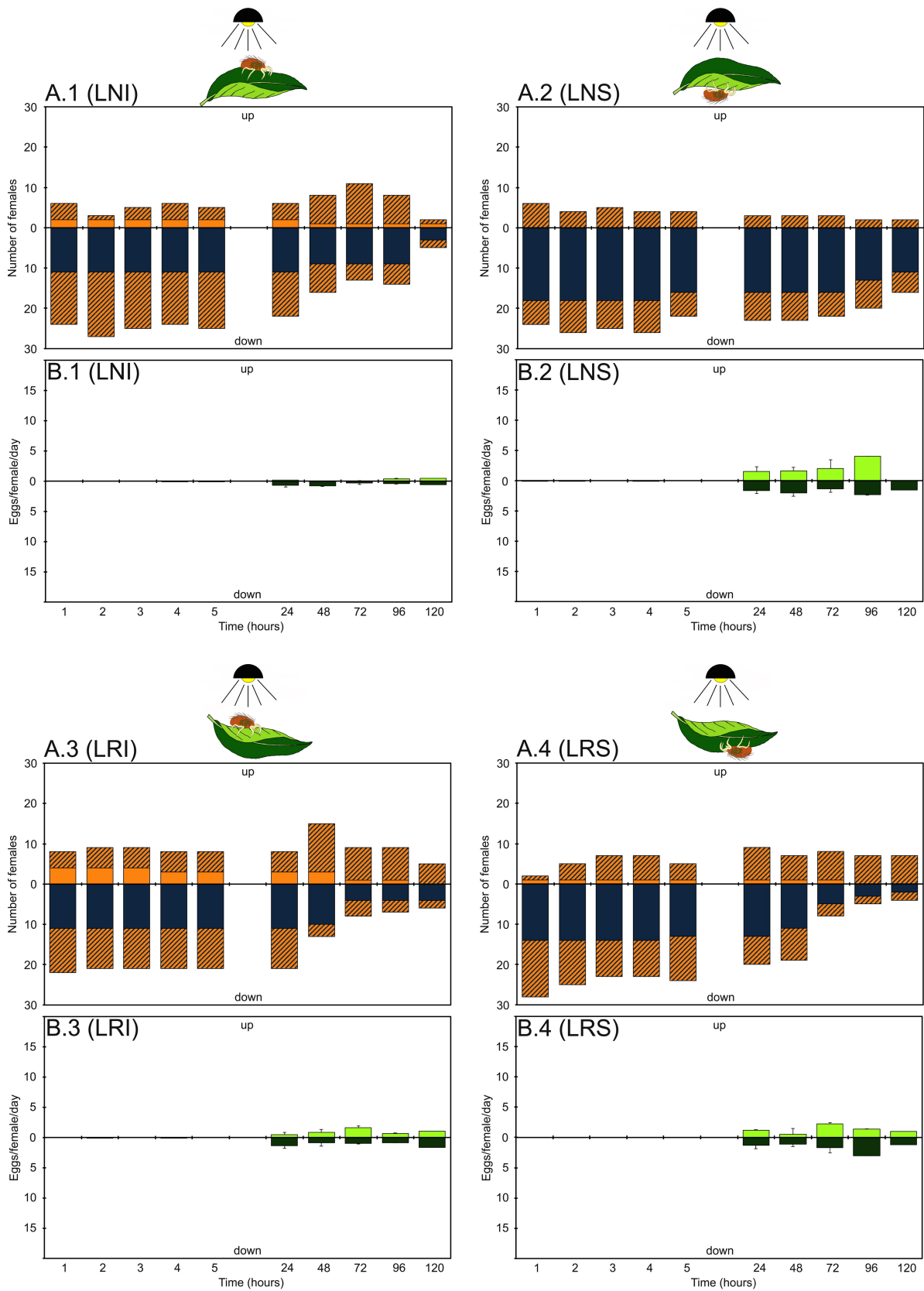


Figure 4 Distribution of *Tetranychus urticae* females (A) and daily oviposition rate (B) on lemon leaves: A1, B1 (LNI); A2, B2 (LNS); A3, B3 (LRI); A4, B4 (LRS) (see also Table 1). ‘Up’ indicates the illuminated surface of the leaf and ‘Down’ the shaded one. The drawings indicate the light source, the orientation of the leaves and the initial position of mites. Uniform coloured histograms in A.1–4 indicate the number of females that did not changed their position after that occurred during the first hour. Striped histograms indicate the number of females that changed their position at least once during the test period. Bars in B1–4 histograms represent S.E. of means.

oviposition rate on both surfaces, indicates a) the worse food quality of lemon leaves than of bean leaves, or at least shows a clear preference of mites for bean constituents and b) greater difficulty feeding on the adaxial side of lemon leaves. Besides the presence of secondary toxic metabolites on *Citrus* leaves (Hasegawa & Miyake 1996), there is also a greater amount of granular waxes ($31 \mu\text{g cm}^{-2}$) (Baker *et al.* 1975), with a thicker layer on the adaxial surface (Jeffree 1986), making piercing of tissues by *T. urticae* females more difficult.

Both survival and oviposition rates recorded in our experiments were significantly higher on bean leaves, and similar to those obtained by other authors on the same host plant (Modarres Najafabadi *et al.* 2014). On the contrary, both the survivorship and oviposition rate were significantly lower on lemon leaves. This could seem paradoxical, because *T. urticae* is considered a dangerous pest on various *Citrus* species (McMurtry 1985; Bruessow *et al.* 2010; Jacas & Urbaneja 2010). Moreover, various authors have shown rapid adaptation of the species to a new host (Fry 1990; Agrawal 2000). However, some authors (Gotoh *et al.* 1993; Fytrou & Tsangarakou 2014) showed the presence of distinct host races with a preference for the plant of origin and Aguilar-Fenollosa *et al.* (2012) reported the weak performance of *T. urticae* populations originating from *Festuca arundinacea* Schreber (Fam. Poaceae) and reared on *Citrus clementina* Tanaka. It is possible that the one-month period on lemon fruits, adopted in our experiments, was not enough for complete adaptation of our populations to the new host.

The results of the choice tests confirm the above discussion and provide new data on the influence of light in choosing a leaf surface for residence and feeding. In particular, *T. urticae* females chose the shaded surface of leaves in naturally oriented leaves, regardless of their initial position, and they made this choice within the first hour of tests. On the other hand, also Li and Margolies (1991) found no influence of the initial placement of mites on their final choice in similar tests on corn leaves using *Oligonychus pratensis* (Banks). According to Barcelo (1981), *T. urticae* females did not avoid visible UV-A wavelengths (the same wavelengths used in our experiments), while being able to detect visible light but also the more dangerous UV-C radiation (McEnroe & Dronka 1969; Ohtsuka & Osakabe 2009). Naegele *et al.* (1966), using the visible spectrum on *T. urticae* females, showed phototaxis at 375 nm and photokinesis at 525 nm, but it is believed that this phototaxis is also influenced by environmental factors and the level of leaf damage (Suski & Naegele 1963). Therefore, the selection of the abaxial

Table 4 Statistics for the daily oviposition rate between the adaxial and abaxial surface of leaves in the two-choice tests during the test period. Asterisks indicate significant differences between the two leaf surfaces ($P < 0.05$). (1) Type of leaf; (2) Orientation of leaf; (3) Surface of initial position of female

| (1) | Tests | | | 24h | 48h | 72h | 96h | 120h |
|-------|----------|-------------|---------------------------|-----------------------|-----------------------|------------------------|------------------------|-------------------------|
| | (2) | (3) | | | | | | |
| Bean | Natural | Illuminated | <i>F (df)</i> <i>P</i> | 0.64 (1, 28) 0.432 | 0.66 (1, 28) 0.424 | 1.84 (1, 28) 0.185 | 0.47 (1, 28) 0.497 | 0.06 (1, 27) 0.812 |
| Bean | Natural | Shaded | <i>F (df)</i> <i>P</i> | 1.28 (1, 28) 0.267 | 0.21 (1, 28) 0.650 | 0.00 (1,27) 0.951 | 0.29 (1, 26) 0.597 | 0.72 (1, 25) 0.404 |
| Bean | Reversed | Illuminated | <i>F (df)</i> <i>P</i> | 9.60 (1,28) 0.004* | 2.65 (1, 28) 0.114 | 4.46 (1, 26) 0.044* | 8.23 (1, 25) 0.008* | 0.57 (1, 24) 0.458 |
| Bean | Reversed | Shaded | <i>F (df)</i> <i>P</i> | 0.15 (1,28) 0.698 | 0.00 (1, 28) 0.983 | 0.19 (1, 27) 0.667 | 0.01 (1, 27) 0.910 | 1.48 (1, 27) 0.235 |
| Lemon | Natural | Illuminated | <i>F (df)</i> <i>P</i> | 3.96 (1, 26) 0.057 | 3.79 (1, 22) 0.064 | 1.83 (1, 22) 0.189 | 0.19 (1, 20) 0.667 | 0.04 (1, 5) 0.846 |
| Lemon | Natural | Shaded | <i>F (df)</i> <i>P</i> | 0.88 (1, 24) 0.356 | 0.19 (1, 24) 0.665 | 1.60 (1, 23) 0.219 | 1.80 (1,20) 0.195 | 17.64 (1, 16) 0.001* |
| Lemon | Reversed | Illuminated | <i>F (df)</i> <i>P</i> | 4.16 (1, 27) 0.051 | 0.00 (1, 26) 0.972 | 0.10 (1, 15) 0.757 | 0.12 (1, 14) 0.736 | 3.27 (1, 9) 0.104 |
| Lemon | Reversed | Shaded | <i>F (df)</i> <i>P</i> | 0.00 (1, 27) 0.948 | 1.56 (1, 24) 0.224 | 0.00 (1, 14) 1.00 | 3.49 (1, 10) 0.091 | 1.91 (1, 9) 0.200 |

shaded bean leaf surface by the mites should be ascribed to factors other than light. Sakai *et al.* (2012) attributed this choice to gravity, but in our experiments *T. urticae* females remained on the illuminated abaxial surface of bean leaves when this was their initial position (BRI tests). This behaviour indicates that a) the mite can recognize differences between the two leaf surfaces and b) the leaf architecture of the abaxial bean leaf surface seems to be a priority factor. The arrangement of raised veins (in relief) on the abaxial bean leaf surface offers the mite the possibility of creating the complicated web needed for protection and egg laying more rapidly and more easily, and this seems to be a priority in comparison to the influence of visible light or gravity. Other mite species also showed the ability to differentiate the adaxial and abaxial sides of the leaf, i.e., *O. pratensis* can clearly distinguish the adaxial and abaxial corn leaf surface (Li & Margolies 1991), whereas two phytoseiid mites, *Kampimodromus aberrans* (Oudemans) and *Typhlodromus pyri* Scheuten, clearly prefer the tomentous abaxial grape leaves (Duso 1992; Duso & Pasqualetto 1993).

On the other hand, mites initially put on the adaxial shaded bean leaf surface seemed to be confused. About the half of the females moved towards the illuminated abaxial surface, preferring this to the shade. It is noteworthy that about a third of females changed their position multiple times during the test period, indicating the difficulty of choosing between the shade and the leaf surface most appropriate to their needs. This behaviour was not recorded on reversed lemon leaves, where *T. urticae* females clearly preferred the shaded leaf surface, regardless of their initial position, even though about half of the females changed their position multiple times during the test period. This restlessness indicates the reluctance of females to stay and feed on the smooth adaxial lemon leaf, confirming observations reported for the no-choice tests, but also the negative influence of visible light when associated with an inadequate leaf structure and bad food conditions, as also argued by Suski and Naegele (1963).

It is well known that foliar surfaces influence both microclimate and leaf chemistry (Juniper & Southwood 1986), affecting the behaviours of microarthropods (Ferro & Southwick 1984; Wilmer 1986). Various authors have also shown the negative effect of UV-B radiation on *T. urticae*, describing the abaxial-biased distribution, as a consequence of avoiding deleterious wavelengths (Ohtsuka & Osakabe 2009). It could be inferred, that in greenhouses covered with UV-blocking films, *T. urticae* could equally infest the adaxial or abaxial leaf surfaces. However, according to our results, *T. urticae* choice depends more on the leaf surface morphology, than on the various light wavelengths. The two spotted spider mite is one of the most polyphagous known species, being able to feed on more than 1,100 plant species (Migeon & Dorkeld 2021), which is why it is feared by growers. However, the species have geographic and plant-adapted demes (Aguilar-Fenollosa *et al.* 2012; Fytrou & Tsangarakou 2014) that should involve different control approaches.

Further genetic and behavioural investigations of various *T. urticae* demes, inhabiting different plant species in natural and agricultural ecosystems, could provide new data for the rational and ecofriendly management of this interesting as well as damaging species.

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