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The effects of cypermethrin on *Tenuiphantes tenuis* (BLACKWALL, 1852): development of a technique for assessing the impact of pesticides on web building in spiders (Araneae: Linyphiidae)

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Abstract: The construction and design of webs are fundamental units of behaviour in spiders and can be used as an indicator of the impact of environmental stressors (for example pesticides) on their health. Very few studies have quantified web building by spiders that produce three dimensional webs, with the majority of published works concentrating on web building in orb weavers. An arena was developed to allow the filming of Linyphiidae to take place during the construction of sheet webs. The methods described are considered sensitive enough to detect the effects of exposure to different levels of the pesticide cypermethrin on *Tenuiphantes tenuis*. Exposure to high levels of cypermethrin resulted in increased mortality and reduced levels of activity detected through filming.

Key words: spiders, *Tenuiphantes tenuis,* cypermethrin, locomotion, mortality

Introduction

Spiders can be divided into two main categories in terms of foraging techniques; active hunting spiders and those that capture prey by means of a web. Web building spiders comprise a large proportion of spider species producing webs of many varying sizes, shapes and architectures. The construction and design of a web is one of the fundamental units of behaviour in spiders (HERBESTEIN, TSO 2000) which can range from simplistic webs to some often highly complex structures. All web building spiders utilise their web in some way for prey capture and some spiders use it as a moulting or mating platform, as cocoon support, or as a sun shield (ZSCHOKKE 1994). Therefore, it is possible that exposure to environmental stressors, such as agrochemicals, may result in disturbances in the web building activity and web appearance. However, there is a current paucity of knowledge regarding the process of web building in many spider families which needs to be addressed before these more applied questions are examined.

Testing the effects of pesticides on web building behaviour is limited to a handful of works which mainly comprise of studies on two dimensional orb webs. JOHANSEN (1967) was the first to describe the significant disruptions to web building associated with exposure to pesticide. The changes in the geometric pattern of the web were evident, even when low doses were sprayed directly onto the web (JOHANSEN 1967) which has since been shown to be a particularly efficient collector of small droplets of pesticides (SAMU *et al.* 1992). Subsequently, the sublethal effects of several pesticides were tested on *Larinioides sclopetarius* (CLERCK, 1757) (LENGWILER, BENZ 1994). This was the first time that pesticides had been applied topically to web building spiders to allow an exact amount of pesticide to be applied directly to the spiders. The effects varied

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with each pesticide, ranging from no effects with pirimicarb applications to increased mortality, delays to web building and a reduction in web size being associated with exposure to diazipon (Lengwiler, Benz 1994). However in European crops, orb weavers are generally not abundant and Linyphiidae are the dominant web building family.

Testing pesticide effects of web building behaviour in Linyphiidae spiders has yet to be documented, in part due to the complex nature of the web. Indeed, linyphiid web building per se had previously not been described prior to Benjamin *et al.* (2002), and Benjamin, Zschokke (2004). Benjamin, Zschokke (2004) were able to film web building by limiting the size that the spider was able to build the web in and thus were not able to quantify changes in behaviour.

The aim of the current study was to develop a protocol for assessing the impacts of commonly applied pesticides (e.g. cypermethrin) on the web building behaviour of *Tenuiphantes tenuis* (Blackwall, 1852) which is a common agricultural spider throughout Europe. The design had to allow spiders to build webs of differing sizes and enable the assessment of the changes in the size of those webs. Although filming would initially be used, subsequent monitoring was designed to be able to be conducted by eye. Thus the removal of part of the arena was required to allow this, and for feeding to take place.

Methods

Experimental Development

Initially an attempt was made to directly observe spiders beginning to build a web. This was to determine when web building took place (to allow accurate filming of the process) and what the best method of capturing this behaviour was and to gauge whether it was possible to collect data on web building by merely observation alone. However, spiders never initiated web building whilst an observer was present and often began building only several hours after the departure of the observer (this was assessed by returning to the arena at regular intervals). Therefore, it was concluded that to efficiently capture the process, filming would be required. It was also noted that during early developmental stages *T. tenuis* did not build a complete web for several days once placed in an arena. This was related to individuals building webs in the acclimation chambers during an initial starvation period. Those individuals that built webs within the acclimation chambers did not then build a web within the experimental arena. Spiders were therefore sequestered in small plastic vials during the acclimation and starvation period in order to limit the amount of web produced prior to experimentation. This resulted in individuals building connecting threads during the first evening in the arena and producing the beginnings of the sheet portion of the web during the second night.

On determining that filming would be required in order to compare behaviours successfully, a suitable arena in which spiders would build a web was required. A number of designs were attempted, many of which resulted in the spider not building a web or the spider building its' web on the roof of the arena or on the sides, but not in the middle of the arena where filming was possible. The type of arena used by Benjamin, Zshockke (2004) was deemed as unsuitable as it did not allow the spider to vary the size of the web that was built and would not allow the easy and continued monitoring of web size and development once filming had ceased.

Final Arena

Arenas (154 mm diameter) consisted of clear, plastic, circular containers with a transparent lid that allowed filming to take place (Fig. 1). A cardboard base, with a grid of 36 vertical, wooden uprights (60 mm high and set 20 mm apart), was inserted into the arena base. Approximately

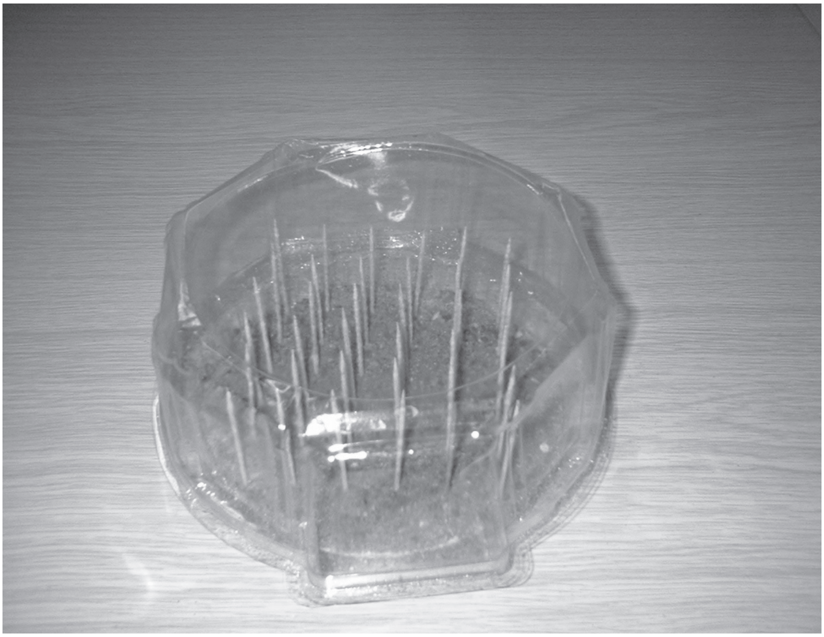

Fig. 1. Final design of web building arena used to examine the effects of a pesticide on *T. tenuis*.

20 mm depth of silica sand was added to the arena and sprayed with distilled water to solidify the sand and provide moisture. In order to prevent spiders from attaching the web to the arena lid petroleum jelly was applied to the sides of the removable lid, allowing individuals to walk on that area but not allowing web points to be attached (S. Zschokke pers. comm.).

Treatments

All individuals (*n*=20) were collected from an area of grassland (Chorlton, Manchester, UK) that has not been sprayed with insecticides for over 10 years. An acclimation and starvation period of ten days was imposed on all individuals during which time they were sequestered in small plastic vials.

Spiders were exposed to a topically applied droplet (0.05 µl) of either distilled water or technical grade cypermethrin. All individuals were anaesthetised, using CO_2, prior to droplet application in order to reduce movement, thus ensuring all droplets were applied successfully. Following recovery from the effects of the CO_2, spiders were placed in the centre of the arena which was placed directly underneath a video camera.

Each spider was filmed, at an ambient temperature of 20°C (±1), over an 18 hour period in both light and dark (simulated by red light) conditions (3L/12D/3L). A time lapse video recorder (Panasonic Model AG-6010) was used to continuously record activity resulting in 24 hours film for every 1 hour of video tape. Filming took place in two dimensions only by placing the camera directly over the top of the arena (Fig. 2). Previous work has filmed spider movement from above and the side to allow a three dimensional view of the web building behaviour (Benjamin, Zschokke 2004). However, this was not possible in this experiment as it was necessary to be able to remove the arena lid to allow for accurate web size analyses throughout. Since this required petroleum jelly to be added to the arena walls, filming through the arena sides was not possible. As only one individual was filmed per night, the collection of spiders and the initiation of the starvation period were carried out in stages in order to ensure individuals were starved for similar lengths of time.

Fig. 2. Arena in position for filming showing the camera, red lights and time lapse recorder.

Each morning, once filming had ceased, the arena lid was removed and the amount of silk deposited, the position and the state of the spider were recorded and if a sheet portion of web had been produced, one prey item (*Sinella curriseta*) was introduced. Each individual was then monitored over a 30 day period assessing the changes in web size and spider condition, as well as recording the production of egg sacs and the number of hatchlings from those egg sacs.

Statistical analyses

Data were heavily skewed and transformation did not result in data meeting the assumptions of normality. Therefore, Mann Whitney *U* tests (StatView 5, SAS) were used to compare longevity after behavioural observations, the time spent moving, the distance travelled and the number of wooden uprights used in web building between treated and untreated individuals.

Results

Spiders exposed to cypermethrin died significantly earlier than individuals treated with distilled water (U=13, df=18, P=0.0049) with individuals in the treated cohort dying on average ten days after treatment and those in the control treatment surviving for the duration of the monitoring period (30 days; Fig. 3a). Exposure to cypermethrin also resulted in significantly reduced levels of movement during filming (U=16.5, df=18, P=0.0199); significantly shorter distances travelled (U=13, df=18, P=0.0090); and significantly lower numbers of uprights used in web building (U=13, df=18, P=0.0053). Treated individuals moved on average a distance of 1.14 m (±0.75) compared to control treated individuals that travelled a mean distance of over 3.25 m (±0.70) (Fig. 3b). This took place during a mean total movement time of 17.5 min (±12.4) for treated individuals and 32 min (±7.3) for control treated individuals (Fig. 3c). The size of web produced by the end of the trial, assessed by the number of uprights used in web attachment, was reduced to an average of ten uprights in the treated cohort whereas individuals in the control cohort used an average of 34 uprights (Fig. 3d).

Discussion

Exposure of *T. tenuis* to cypermethrin resulted in reductions in locomotor and web building behaviour and, as previously shown in *Pardosa amentata* (SHAW *et al.* 2004), culminated in high levels of mortality. The techniques used to assess these changes in behaviour were suitable for the current study but modifications are advised for future works in this area. The arenas allowed spiders to build webs of varying sizes, dependant on the exposure to pesticides. The results pro-

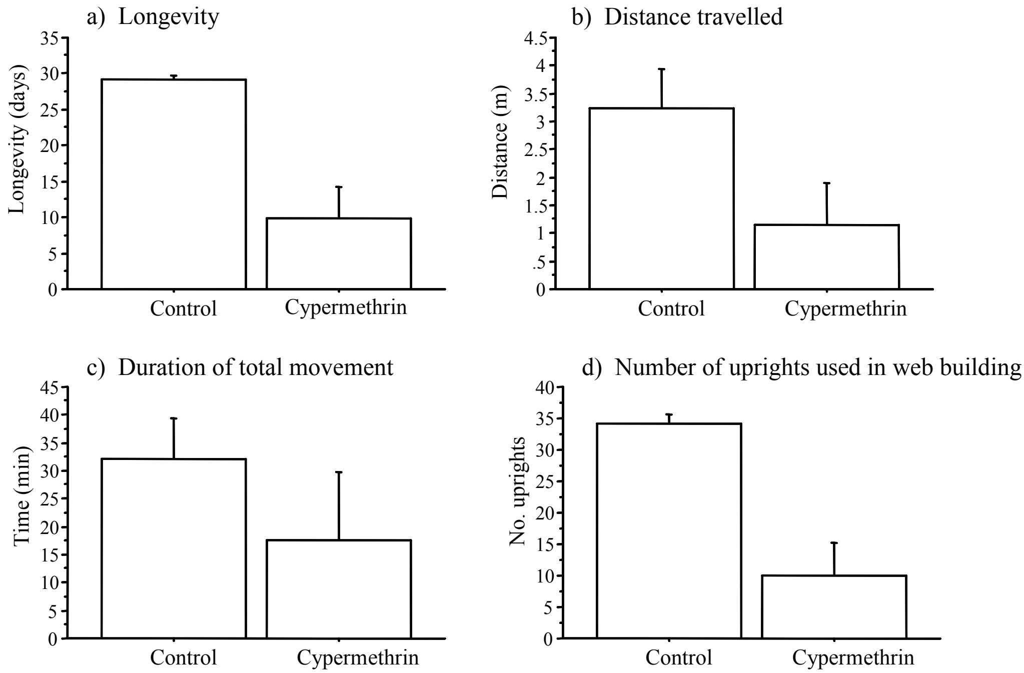

Fig. 3. Mean (± S.E.) responses of *Tenuiphantes tenuis* to droplets of cypermethrin or distilled water (Control).

duced detectable effects that were relatively easy to identify from the films produced. In future experiments it would be advised to increase the arena size in order to provide an area larger than the average size of the web of the particular spider species being studied. This may be a difficult assessment to make as the web building behaviour of many Linyphiidae is still unknown. However, the arena size that is currently being used may be sufficient for an initial assessment of web size for individual species prior to testing the effects of external stressors.

Filming of spiders gives an accurate assessment of the immediate impact of exposure to a pesticide and helps to give an insight into the sublethal changes in behaviour and, potentially the time scales over which these changes take place. The filming achieved only low levels of contrast between the spider and the background, mainly as a result of the small size of the spider. However, if a better contrast between the spider, the background and the wooden structures was achieved then a system such as Videomex-V (Columbus Instruments, Columbus, Ohio, USA) or Ethovision (Noldus Information Technology™) could be used in combination with the current arena. These are automated video based digital-data collection systems that allow the collection of data regarding activity patterns whilst filming is taking place. This system has been previously used to quantify the level of movement within a number of organisms including wolf spiders (Walker *et al.* 1999), mice (Murphy *et al.* 2001) and fish (Qian *et al.* 2001). The use of such a system would dramatically reduce the amount of time required for data extraction post filming. This could also facilitate the testing of exposure to pesticides via residual contact by treating some parts of an arena with pesticide and the remaining parts with distilled water. Videomex or Ethovision can then assess the levels of activity in each area of the arena to determine whether avoidance of treated areas occurs, as in true of some mites (Holland, Chapman 1994) and ladybirds (Singh *et al*. 2001) or how the level of effects are related to the time spent in treated areas.

Currently the impact of cypermethrin on the behaviour of *T. tenuis* is concurrent with effects observed in *P. amentata* (Baatrup, Bayley 1993, Shaw *et al.* 2004, 2006). This demonstrates that despite pyrethroid pesticides being of a lower potency to non-target organisms than many other pesticides (e.g. organophosphates) there are still a high level of sublethal impacts of cypermethrin. This insecticide can potentially impact upon the feeding efficiency of individuals due to delays in web building (SHAW, unpublished data) and dispersal as a result of the paralysis of hind legs in *P. amentata* (BAATRUP, BAYLEY 1993, SHAW *et al.* 2004, 2006).

The current work has highlighted a need to investigate the impact of repeated exposure to this, and other pesticides, and the effect of different modes of exposure (topical, residual and ingestion) in order to gain a more realistic view of what may occur in a treated crop. Furthermore, field based assessments are required to provide the most reliable results to be extrapolated into real environmental situations.

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References

BAATRUP E., M. BAYLEY 1993. Effects of the pyrethroid insecticide cypermethrin on the locomotor-activity of the wolf spider *Pardosa amentata* – quantitative-analysis employing computer-automated video tracking. - *Ecotoxicology and Environmental Safety*, **26** (2): 138-152.

BENJAMIN S. P., M. DÜGGELIN, S. ZSCHOKKE 2002. Fine structure of sheet-webs of *Linyphia triangularis* (Clerck) and *Microlinyphia pusilla* (Sundevall), with remarks on the presence of viscid silk. - *Acta Zoologica*, **83**: 49-59.

BENJAMIN S. P., S. ZSCHOKKE 2004. Homology, behaviour and spider webs: web construction behaviour of *Linyhpia hortensis* and *L. triangularis* (Araneae: Linyphiidae). - *Journal of Evolutionary Biology*, **17**: 120-130.

HERBESTEIN M. E., I.-M. TSO 2000. Evaluation of formulae to estimate the capture area and mesh height of orb webs (Araeoidae, Araneae). - *Journal of Arachnology*, **28**: 180-184.

HOLLAND J. M., R. B. CHAPMAN 1994. A comparison of the toxic and sublethal effects of fluvalinate and esfenvalerate on the two-spotted spider mite (Acari, Tetranychidae). - *Experimental and Applied Acarology*, **18** (1): 3-22.

JOHANSON R. R. 1967. The effect of DDT on the webs of *Araneus diademata*. - *Memoranda Societatis pro Fauna et Flora Fennica*, **43**: 100-104.

LENGWILER U., G. BENZ 1994. Effects of selected pesticides on web building behaviour of *Laranoides sclopetarius* (Clerck) (Araneae, Araneidae). - *Journal of Applied Entomology*, **117**: 99-108.

MURPHY QIAN X., Y. CUI, B. XIONG, S. XIE, X. ZHU, Y. YANG 2001. Spontaneous activity was unaffected by ration size in Nile tilapia and gibel carp. - *Journal of Fish Biology*, **58** (2): 594-598.

SAMU F., G. A. MATTHEW, D. LAKE, F. VOLLRATH 1992. Spider webs are efficient collectors of agronomical spray. - *Pesticide Science*, **36**: 47-51.

SHAW E. M., M. WADDICOR, A. M. LANGAN 2006. Impact of cypermethrin on feeding behaviour and mortality of the spider *Pardosa amentata* in arenas with artificial 'vegetation'. - *Pest Management Science*, **62**: 64-68

SHAW E. M., C. P. WHEATER, A. M. LANGAN 2004. Do pesticide applications influence feeding and locomotory behaviour of *Pardosa amentata* (Clerck) (Araneae, Lycosidae)? - *Arthropoda Selecta*, *Special issue*, **1**: 297-305.

SINGH S. R., K. F. WALTERS, G. R. PORT 2001. Behaviour of the adult seven spot ladybird, *Coccinella septempunctata* (Coleoptera: Coccinellidae), in response to dimethoate residue on bean plants in the laboratory. - *Bulletin of Entomological Research*, **91**: 221-226.

Walker S. E., S. D. Marshall, A. L. Rypstra, D. H. Taylor 1999. The effects of hunger on locomotory behaviour in two species of wolf spider (Araneae, Lycosidae). - *Animal Behaviour*, **58**: 515-520.

Zschokke B. S. 1994. Web construction of the orb weaving spider *Araneus diadematus* Cl. Unpublished PhD Thesis, Basel, Switzerland, 177 p.

Ефектът на циперметрина върху *Tenuiphantes tenuis* (Blackwall, 1852): разработване на техника за оценка на влиянието на пестициди върху изграждането на мрежи при паяците (Araneae: Linyphiidae)

Е. Шоу, Ф. Уетър, М. Ланган

(Резюме)

Начинът на изграждане на мрежата и нейната структура са основни поведенчески характеристики на паяците и могат да бъдат използвани като индикатори за определяне влиянието на различни вредни вещества (например пестициди) върху тях. В настоящата статия е представен експеримент, при който в лабораторни условия паякът *Tenuiphantes tenuis* е третиран с разтвор с различна концентрация на пестицида циперметрин. Наблюдавана е по-висока смъртност и по-ниска двигателна активност сред тези екземпляри, които са изложени на действието на циперметрин с по-висока концентрация. Представена е методика за заснемане на линифиидни паяци по време на строежа на техните мрежи. За целта в лабораторни условия е построена специална арена, над която е монтирана камера.