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Alain Pasquet, Nora Tupinier, Christophe Mazzia, Yvan Capowiez. Exposure to spinosad affects orb-web spider (*Agalenatea redii*) survival, web construction and prey capture under laboratory conditions. *Journal of Pest Science*, 2016, 89 (2), pp.507–515. 10.1007/s10340-015-0691-x . hal-01444631v2

HAL Id: hal-01444631

<https://hal.science/hal-01444631v2>

Submitted on 6 Apr 2023

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# Exposure to spinosad affects orb-web spider (*Agalenatea redii*) survival, web construction and prey capture under laboratory conditions

Alain Pasquet <sup>a</sup>, Nora Tupinier <sup>a</sup>, Christophe Mazzia <sup>b</sup>, Yvan Capowiez <sup>c</sup>.

**a** : CNRS, INRA, UR AFPA, Faculty of Sciences and Technologies, University of Lorraine, 54506 Vandoeuvre Les Nancy, France

**b** : UMR 7263 IMBE, UAPV - CNRS - IRD - AMU, Université d'Avignon, 301 rue Baruch de Spinoza, BP 21239, 84916 Avignon Cedex 09, France

**c** : INRA, UR 1115 "Plantes et Systèmes de Culture Horticoles", Site Agroparc, 84914 Avignon Cedex 09, France

Published in *Journal of Pest Science*. Available online: <https://doi.org/10.1007/s10340-015-0691-x>

## Keywords

Araneidae, insecticide, non-target effects, trap-building spiders.

## Abstract

Spinosad is a neurotoxic pesticide, which is currently used in IPM and organic agriculture. It can affect the survival and ecological function of spiders, which are natural enemies of important agricultural pests. In the laboratory, we carried out tests to determine lethal and sublethal effects of spinosad on mortality, web building, and web characteristics of *Agalenatea redii*. Spinosad has a lethal effect at the normal application rate (NAR, i.e.,

96 g ha<sup>-1</sup>) causing 35 % mortality (vs 0 % for control) after 4 days and 62 % mortality (vs 14 % for control) after 30 days. For the sublethal effects, web building was affected and fewer spiders built webs when exposed to spinosad (10/37 at NAR and 28/39 at half the NAR vs 35/37 for control group). No delay in web building was observed following exposure. Spider webs showed irregularities in the spiral-thread spacing (parallelism) when exposed to higher doses of spinosad (NAR and half of the NAR). Spinosad also affected prey capture: spiders exposed to spinosad (NAR) showed decreased prey capture efficiency (32 vs 73 % for control). These results showed that spinosad affects the spider predatory behavior (agriculture auxiliaries), which could modify their role in pest biocontrol.

## 1. Introduction

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Over the past 40 years, the use of chemical products to control pests has been increasing (Oerk and Dehne 2004). Pests cause major yield loss in crops and so are of great economic importance (Oerke 2006). Nevertheless, the use of pesticides is now under question and numerous cases of pest resistance are regularly reported (IRAC 2014). Broad-spectrum pesticides lacking selectivity are often still used and may also kill the beneficial species that ensure ecosystem services such as pollination or biocontrol (Power 2010). Several countries have introduced measures in their biocontrol programs (i.e., ban of the most toxic pesticides) to protect these beneficial species, particularly generalist predators (Desneux et al. 2007; Symondson et al. 2002).

Spiders are an important group among the polyphagous predators, with more than 44,000 species, which are widely

distributed in all biotopes, and can be highly abundant (more than 100 individuals per square meter in meadows, (Nyffeler and Benz 1987)). Some build and use traps (webs) to catch their prey and are recognized as important biocontrol agents in agriculture (Maloney and Drummond 2003; Nyffeler and Sunderland 2003). However spiders are generally sensitive to pesticides (Pekar 2012), and to preserve them, it is necessary to determine the selectivity of widely used pesticides. In this context, pesticides authorized in organic production, derived from mineral substances, or produced by microbes, animals or plants, are considered as an alternative to synthetic pesticides because they are thought to be more efficient and less harmful to the environment (Gupta and Dikshit 2010). These natural compounds are now widely used especially but not exclusively in organic agriculture. However, like their synthesized counterparts, natural pesticides may not only have an impact on the survival of natural enemies, but also on their predatory

behavior, limiting their influence on pest populations (Pekar 2012).

Among the different biological compounds, the spino-syne family, and spinosad in particular, is one of the most well known bio-pesticides. It is produced by fermentation of an actinomycete (*Saccharopolyspora spinosa*), and has strong neurotoxic effects and kills insects in a few days (Williams et al. 2003). It is rapidly degraded in the environment with a half-life of about 1 day (Zhao et al. 2007). Spinosad is now widely used throughout the world in both conventional and organic agriculture (Kirst 2010). Several studies have shown that spinosad can have lethal effects on spiders (for review see Pekar 2012), but only a few studies focused on sublethal effects (Benamu et al. 2007, 2013). These sublethal effects have been observed in different invertebrate species and especially in spiders (i.e., *Philo-dromus cespitum* (Rezác et al. 2010), *Araneus pratensis* (Benamú et al. 2007), or *Alpaida veniliae* (Benamú et al. 2013)). Spinosad may affect different aspects of spider physiology and ecology such as prey capture, reproduction, and generally all functions that are under neuronal control. These interactions could affect the spider's ability to build their trap and then to catch prey. Indeed, it has been shown that the central nervous system influences web construction (Samu and Vollrath 1992; Samu et al. 1992; Hesselberg and Vollrath 2004; Anotaux et al. 2012). In this context, the spider's orb web is a good model for studying the

influence of drugs (Witt et al. 1968), medical products (Hesselberg and Vollrath 2004), and also pesticides (Pekar 2012) on behavior. All these products have an effect on the spider brain (Eberhard 2011). As orb-web building is the

consequence of stereotyped and successive behaviors (Eberhard and Hesselberg 2012), and is under brain control, the chemical or natural products that affect brain function might also influence web construction behavior and/or web design.

Spinosad is applied in a majority of organic apple orchards (Marliac et al. 2015) and even some 1PM orchards. When applied in orchards, this pesticide may affect the survival or behavior of the orb-web spiders inhabiting these orchards such as *Nuctenea umbratica*, *Araneus diadematus* and less frequently *Agalenatea redii* (Capowiez, personal observation). These orb-web spiders are assumed to prey on and thus decrease the abundance of the apple pest *Dysaphis plantaginea* in the fall (Wyss et al. 1995). *A. redii* is a smaller spider, which spins tiny webs and is found in high numbers in fallow lands. Thus we used it to investigate the lethal and sublethal effects of spinosad on mortality, web building, and web characteristics under laboratory conditions, where the direct effects of the pesticide could be explored. We hypothesized that spinosad would affect survival, web building, and orb-web characteristics.

## 2. Material and Methods

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### 2.1. Spider rearing

*Agalenatea redii* (Scopoli 1973) (Araneae, Araneidae) is a widely distributed orb-web spider in northern Europe (Roberts 1995). It builds a vertical orb web from March to July in the Palearctic zone. We collected spiders in a fallow near Nancy (East of France) in March and April 2014. Only adult or sub-adult females were collected. They were observed under the binocular microscope to confirm their sex and their stage of development. We only sampled females with eight legs, as the loss of legs may influence web construction and characteristics. Spiders were reared in small individual boxes (10 x 7 x 2.5 cm), where they could not build webs, under standard conditions (humidity 50 ± 5 %, temperature 19 ± 2 °C and light 12/12). These rearing conditions do not affect future web construction or web characteristics (Pasquet et al. 2014). They were fed once a week with two flies (*Lucilia caesar*) (Linnaeus 1758) (Diptera, Calliphoridae). Sub-adult individuals were observed daily until their last molt.

### 2.2. Spider exposure

Experiments were carried out on adult females (regardless of whether they molted in the laboratory or if they were captured as adults). They were all in the same nutritional state, having received and eaten two prey during the week before the exposure. All spiders were weighed (balance Sartorius BASIC BA 1 10S, precision 0.1 mg) before their exposure to spinosad to distribute them according to their mass into replicates with the same mass mean for each replicate, since it is known that mass influences web construction and characteristics (Venner et al. 2003).

Spiders were exposed to a solution of Success 4 (DowAgroSciences 2001) diluted in distilled water. Each spider was individually sprayed (with a commercial sprayer) twice for a few seconds at a distance of 15 cm and received an average of 270 (±20) µL of insecticide solution. The different concentrations of active ingredient tested were 192, 96, 48, and 24 mg a.i. L<sup>-1</sup> for doses of 2 x, 1x, 0.5 x, and 0.25 x, respectively, with 1 x corresponding to the normal application rate (NAR) in French apple orchards (96 g ha<sup>-1</sup>). Controls were treated with distilled water. At the 2 x and 1 x doses, 60 and 40 % of the spiders, respectively, died after 24 h; for the other doses (0.25 x and 0.5x) and the control group no mortality was observed. Thus, to test the sublethal effects of spinosad, the control group and the 0.25 x, 0.5 x, and 1 x groups were retained to assess the effects on web construction.

### 2.3. Web construction

Following exposure, all spiders were transferred to the experimental room. Four replicates were conducted during four successive weeks. For each replicate, the same number of spiders was exposed to each pesticide dose. A total of 37 to 39 spiders were used for the different groups (control n = 37, 0.25 x n = 37, 0.5 x n = 39 and 1 x n = 37); overall 150 spiders were included in the experiment.

For each replicate, we placed the spiders in a wooden frame (50 x 50 x 10 cm) enclosed by two windows. The frames were placed in a room with controlled temperature (19.± 2 °C) and artificial light (12/12). In each frame, we put a small dish of water to ensure a higher degree of humidity (between 55 and 60 %) than in the room. Just after the spider was introduced, a

prey (*Lucilia caesar*) was also placed in the frame to stimulate web building. Spiders were left for 7 days in the frames. During the first day, web building was surveyed every 4 h. The following days, the spiders were monitored in their frames twice a day (in the morning and evening). At each survey, the presence of a web and the status of the prey (alive, captured or eaten) were noted. Dead prey were collected and examined under a binocular microscope. A captured and eaten prey had silk around it and holes especially on the head or the thorax were clearly visible. A dead prey without capture was intact (no silk and no hole). When present, each web was photographed (Nikon D200- lens MicroNikkor 105). Webs that had been partially destroyed or were in a bad position (perpendicular to the windows) in the frame were not taken into account. At the end of the experiment, all surviving spiders were put back in the rearing room with the previous conditions. They were then surveyed every day for a further 3 weeks, in order to estimate possible long-term lethal effects of the treatment. Mortality was estimated after 72 h when the spiders were in the frames (short-term mortality) or after 4 weeks in their rearing boxes (long-term mortality).

## 2.4. Web parameters

Photos of the webs were analyzed with ImageJ software (Schneider et al. 2012). Only 84 webs were analyzed; the other webs were destroyed when we opened the frames. Some parameters were assessed directly on the web; for that, we defined four sectors (Fig. 1) in four directions (high, low, right and left). In each sector, we manually counted the number of spiral turns, and measured the length of the internal and external radii that are the threads joining the hub to supports (Fig. 1). Web structure was then characterized according to investment in silk and its general shape by computing several parameters.

The length of the capture thread (CTL) measured the silk investment (Venner et al. 2001),

$$CTL = \pi/8(N_h + N_l + N_{le} + N_r)[(re_h - ri_h) + (re_l - ri_l) + (re_{le} - ri_{le}) + (re_r - ri_r)] \quad (1)$$

where  $N_h$ ,  $N_r$  are the number of spiral turns in the four sectors (h = high, l = low, le = left, r = right) (Fig. 1),  $re_h$ ,  $re_l$ ,  $re_{le}$ ,  $re_r$  are the length of the external and  $ri_h$ ,  $ri_l$ ,  $ri_{le}$ ,  $ri_r$ , the length of the internal radii, respectively, in these sectors.

The capture area (WA) is the surface of the web,

$$WA = \pi Ro^2 \quad (2)$$

where  $Ro = [(re_h - ri_h) + (re_l - ri_l) + (re_{le} - ri_{le}) + (re_r - ri_r)]/4$ .

The number of radii,

$$Nr \quad (3)$$

The symmetry of the web,

$$S = re_l/re_h \quad (4)$$

One parameter was added to characterize the lower section of the web below the center, which is the most important area for foraging activity (Nakata and Zschokke 2010). This parameter was the mean distance between two spiral turns calculated by dividing the height of the lower section of the web by the number of turns in this area minus one.

$$Mh = (re_l - ri_l)/(N_l - 1). \quad (5)$$

The deviance from parallelism between two consecutive spiral turns reflects the spider's motor coordination during web construction (Anotaux et al. 2012; Pasquet et al. 2013). For four web sectors without irregularities such as holes, missing spiral turns, units of thread stuck together between two radii (Fig. 1), all pairs of distances (right and left) between two consecutive spiral turns were computed along the web radii. To avoid successive deviations compensating for each other,  $DP_i$ , the current deviation from parallelism was computed using the following formula:

$$DP_i = \max(D_r, D_{le})/\min(D_r, D_{le}) \quad (6)$$

where for each pair of distance measurements, we divided the larger distance by the smaller distance, yielding a value  $>1$ , and average all values for the four sectors of the web. Higher values indicated higher deviance from parallelism and more net irregularity in spiral-thread spacing.

## 2.5. Data analysis

Data were first tested for homoscedasticity and normality using the Shapiro—Wilk method and Levene test. Only the web symmetry data failed to meet assumptions of normality even after log-transformation, and in this case, we used a non-parametric Kruskal—Wallis test. All the other parameters fitted the parametric conditions and we used an ANOVA. As weight is known to influence web characteristics (Venner et al. 2003), we used a one-way ANOVA to test for differences in spider mean weight between groups (doses) for each replicate (week) separately. Mortality, defined as the number of dead spiders, was analyzed at two different time scales: short-term (after 72 h) and long-term (after 4 weeks). Mortality was compared between the four groups with a  $\chi^2$  test. The same statistical method was used for the number of webs built (the first built by each spider) and the number of prey captured. The  $\chi^2$  test was replaced by a Fisher test when the conditions of the  $\chi^2$  test were not verified. For the analysis of the web parameters, we removed the webs where at least two sectors were destroyed (by prey capture). All web parameters were analyzed using a one-way ANOVA with group (dose) as a factor (PLSD Fisher tests between groups were applied when the results of the ANOVA were significant). We tested the effect of prey capture before web construction by using a two-way ANOVA, introducing "data on prey capture before web construction during the experiment" as a second factor. All statistical analyses were carried out using R software (version 3.0.1), and level of significance was set to 0.05.

### 3. Results

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#### 3.1. Spider mass and mortality

There was no difference in mean spider biomass between the four groups for each replicate (ANOVA; P-values ranged from 0.82 to 0.98) so in the analysis we combined all the data. Short-term mortality was significantly different between the four groups ( $x_2 = 28$ ;  $df = 3$ ;  $P < 0.001$ ) and significantly higher for the 1x group (32 %) than the others (Fig. 2). There was no significant difference between the other groups. The long-term mortality, assessed after 4 weeks, was different between the four groups ( $x_2 = 29$ ;  $df = 3$ ;  $P < 0.001$ ) and was higher for the 1 x group (62 vs 14 % for the control, 8 % for the 0.25x and 24 % for the 0.5x) (Fig. 2). There was no significant difference between the other groups.

#### 3.2. Web construction

Among the 150 spiders tested, 107 spun at least one web, and the number of constructions was significantly different between the four groups ( $x_2 = 46$ ;  $df = 3$ ;  $P < 0.001$ ); the groups exposed to 0.5 x and 1 x made the lowest number of webs (30 % for 1 x and 73 % for 0.50 x vs 95 % for the control and 87 % for the 0.25x) (Fig. 3). There was no significant difference between the groups in the time it took building their first web ( $x_2 = 3.81$ ;  $df = 3$ ;  $P = 0.28$ ).

#### 3.3. Web parameters

Not all the webs made by the spiders could be used to analyze parameters: only 29, 30, 22 and three webs for the control,

0.25x, 0.5 x, and the 1x groups, respectively, were taken into account. Spinosad or the capture of a prey before web construction did not have an effect on most of the measured or calculated parameters (Table 1); this was the case for the length of the spiral thread (CTL), the surface of capture, and the number of radii. There was also no interaction between groups and prey capture before web construction for these three parameters (CTL,  $F_{3, 53} = 0.22$ ;  $P = 0.87$ ; surface of capture,  $F_{3, 53} = 0.42$ ;  $P = 0.74$  and number of radii,  $F_{3, 53} = 1.47$ ;  $P = 0.23$ ) (Table 1). The prey capture before web construction did affect the symmetry ( $F_{3, 53} = 4.07$ ;  $P = 0.05$ ) and also the mean distance between two turns of the capture spiral in the lower section of the web ( $F_{3, 53} = 5.20$ ;  $P = 0.02$ ). For symmetry, groups and prey capture before web construction had an interacting effect ( $F_{3, 53} = 2.76$ ;  $P = 0.05$ ) but not for the mean distance ( $F_{3, 53} = 1.53$ ;  $P = 0.22$ ). Deviation from parallelism was the only characteristic affected by spinosad ( $F_{330} = 4.63$ ;  $P < 0.01$ ) and this parameter was significantly higher for both the 0.5 x and 1x doses compared to the control (Table 1).

#### 3.4. Prey capture

The number of spiders that captured and ate at least one prey during the time (1 week) of each experiment in the wooden frames, with or without a web, was significantly lower for the spiders in the group exposed to spinosad at NAR ( $x_2 = 15$ ,  $df = 3$ ,  $P < 0.01$ ) than for the other groups (Fig. 4). There was no significant difference between the other groups.

### 4. Discussion

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Our study examined both the lethal and sublethal effects of spinosad, which is widely used in organic and TM systems, on web-building spiders. The effects of this insecticide on the survival, web building, and prey capture behavior of an orb-weaving spider (*A. reidii*) common in fallows in France and sometimes observed in apple orchards (Capowiez personal observations) was investigated. Spinosad had a short-term effect (after 72 h) but also a long-term effect (until 4 weeks) on spider survival.

Exposure to spinosad also affected the web-building rate, prey capture, and consumption. Some of these results are consistent with previous laboratory or field studies, but there are also some differences with other studies. Our results show that spinosad modifies spider behavior, which could have significant implications on their important role as biocontrol agents in most crops.

#### 4.1. Effect on spider survival

Spinosad's lethal effects on insect predators (Filgus et al. 1999; Cisneros et al. 2002; Galvan et al. 2005; Mandian et al. 2007; Rimoldi et al. 2008; Dağh and Bahsi 2009; Jalali et al. 2009) and parasitoids (Haseeb et al. 2004; Ahmed and Maqsood 2006; Desneux et al. 2004; Agrawal and Brar 2006) have been reported previously in many studies. Similarly our

present findings suggest that this insecticide has a strong effect on the populations of pest enemies even at its normal application rate in field applications. Spinosad's effect depends on the predator species, and it appears to be strong for spiders (Benamú et al. 2013) compared to that found for other groups especially parasitoids (Williams et al. 2003; Schneider et al. 2004, 2008; Benamti et al. 2010). We also investigated its long-term impact on spiders for the first time and found that under favorable food supply conditions (laboratory) a significant number of the spiders exposed to the normal application rate still died up to 4 weeks later (62 % for the NAR concentration compared to 14 % for the control). This suggests that under field conditions, repeated applications of this pesticide might have dramatic effects on orb-web spider species.

#### 4.2. Effects on web construction

Building a web is necessary for web spiders to capture prey but also has energetic costs. Thus spiders optimize their building by constructing webs when environmental factors are favorable to minimize the relative costs of a web, i.e., when potential prey are present in the environment (Pasquet et al. 1994). In some studies of spiders exposed to pesticides, sublethal effects were observed (Tietjen and Cady 2007) and in some cases spiders even stopped building webs (Benamä

et al. 2013). However, in these studies it was difficult to definitively conclude that the absence of web building was a direct effect of the pollutant on the spiders; it could also be an indirect effect due to the decrease in prey especially after insecticide application. In contrast, in our experimental design where fresh prey was provided, we could conclude that the absence of web construction was a direct effect of spinosad on spider behavior. This effect could result from damage to the central nervous system by spinosad, which is a neurotoxic product (Haynes 1988; Foelix 2011), resulting in motor coordination problems, but this is not the only possible hypothesis. Alternatively, the insecticide may affect silk production, and then subsequently have an impact on web building. Such an effect involving silk production was previously reported in arthropods (Schneider et al. 2004).

For example, neurotoxic insecticides such as spinosad were shown to have an action on cocoon building by the larval instars of the parasitoid. *Hyposoter didymator*.

### 4.3. Effects on web structure

Surprisingly, exposure to spinosad did not influence all web characteristics. Some effects were observed for the NAR (lower number of radii, lower CTL, and higher mean distance between two spiral turns), but because only a few webs were spun and many were not complete, these differences were not significant. Only the parameter 'parallelism' was significantly affected at the lower spinosad doses (0.5 x). Parallelism in orb-web spiders is the result of specialized behaviors during the construction of the sticky spiral (Foelix 2011). It is affected by behavioral errors (Toscani et al. 2012) and if spinosad affects neuronal structures, this parameter could be especially sensitive to the treatment. Indeed, substances that influence neural structure functioning can affect web-building behavior, as shown for drugs or neurotransmitters (Witt et al. 1968; Hesselberg and Vollrath 2004). Various chemical products had a strong effect on web building, and the webs spun were abnormal and sometimes did not even look like orb-webs. Similar results were found with neuronal insecticides on invertebrates (Benamú et al. 2013). In our study, some webs were not measured because they were too deformed and most of the parameters could not have been computed on these webs. This suggests that there were too many anomalies during web construction. A similar phenomenon was seen under different conditions i.e., with age (Anotaux et al. 2012), or due to environmental factors (presence of prey, type of prey, abiotic factors—temperature, wind, humidity—anchorage points of the web on the vegetation) (Heiling and Herberstein

2000). In the present case, the effects appear to be concentration dependent; above the 0.5 x concentration, there was some damage to the brain, which caused death, or the absence of construction. Below this concentration, the spiders could recover their ability to build a new web.

### 4.4. Effects on prey capture

Our results showed that following exposure to NAR, prey capture decreased significantly. Pesticides can affect prey capture in two ways. First the predator may reject or reduce consumption of contaminated prey as reported for *Coccinella* (Wiles and Jepson 1994) or in the parasitoid *Aphidius rhopalosiphi* (Longley and Jepson 1996). This effect is not known in spiders. Contaminated prey could also expose the predator eating them to the toxin and thus lead to changes in behavior but this type of scenario requires further investigation. Second, the lack of prey as a consequence of pesticide use may affect the web site choice and web construction. Despite a historical emphasis on web-based vibratory cues, spiders also use chemical cues when choosing a good foraging place. For instance, changes in chemical and seismic cues from prey may lead to shifts in spider microhabitat use and web architecture, which may alter their functional role in the ecology community (Johnson et al. 2011; Pruitt et al. 2011).

In conclusion, spinosad appears to have adverse effects on the function of a group of beneficial species useful for biocontrol. Although spinosad is approved for use in organic production, which may lead consumers to believe it is safe for non-target organisms, our study clearly demonstrates its lethal and sublethal effects on orb-web spiders. This insecticide not only has some effects on spider behavior, but also influences spider populations through affecting fecundity or adult longevity, as has been found with other insecticides (Saber and Abedi 2013). Here we demonstrated that the spinosad has a strong effect on spider survival and web building. This could reduce the influence of spiders as a biocontrol agent (Wyss et al. 1995). Spinosad may also reduce the population of adult females that build webs and the opportunity for survivors to catch prey through disrupting their abilities to build webs. In this context, it would be useful to limit the exposure of spiders to the spinosad especially during the adult life stage.

**Table 1. Mean (and SE) of the web parameters made by spiders exposed to control (water) and different concentrations of spinosad (0.25x, 0.5x et 1x)**

Groups	Control	0.25x	0.50x	1x
Number of radii	24 (1.0)	23 (0.6)	23 (1.2)	19 <sub>2</sub> (2.6)
CTL (cm)	841 (93)	854 (70)	922 (80)	485 (80)
Capture area (cm <sup>2</sup> )	138 (17)	161 (18)	172(30)	155 (62)
Symmetry	1.79 (0.09)	2.00 (0.10)	1.77 (0.15)	1,88 (0.31)
Mean distance (mm)	3.42 (0.22)	3.73 (0.19)	3.71 (0.28)	5.44 (0.88)
Deviance from parallelism	1.280 (0.013)a	1.344 (0.019)a	1.387 (0.033)b	1.525 (0.079)b

For the parameter 'deviance from parallelism', values bearing a same letter are not significantly different

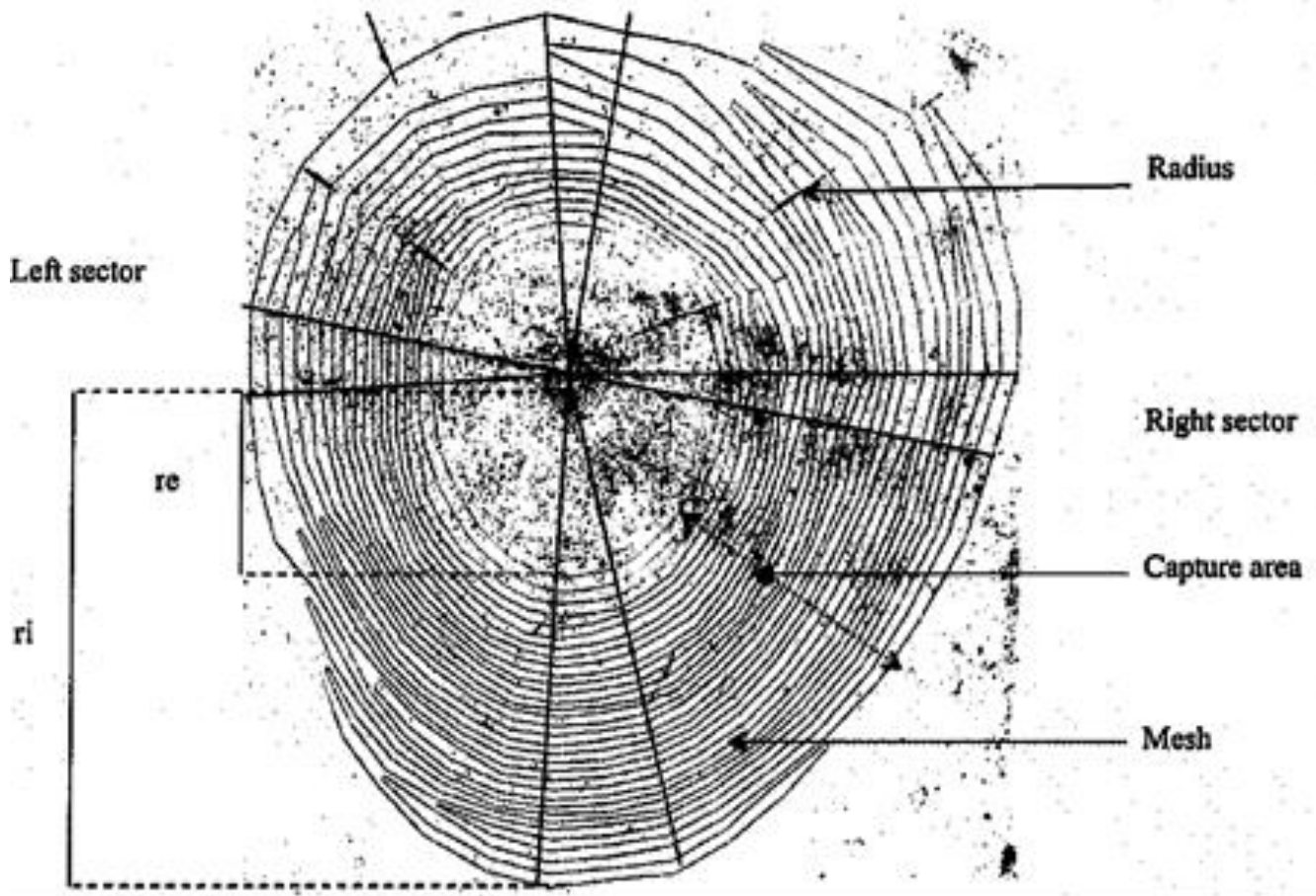


Figure 1. *Agalenatea redii* web showing the parameters measured and used to characterize construction.  $r_e$  external radius,  $r_i$  internal radius in the four sectors



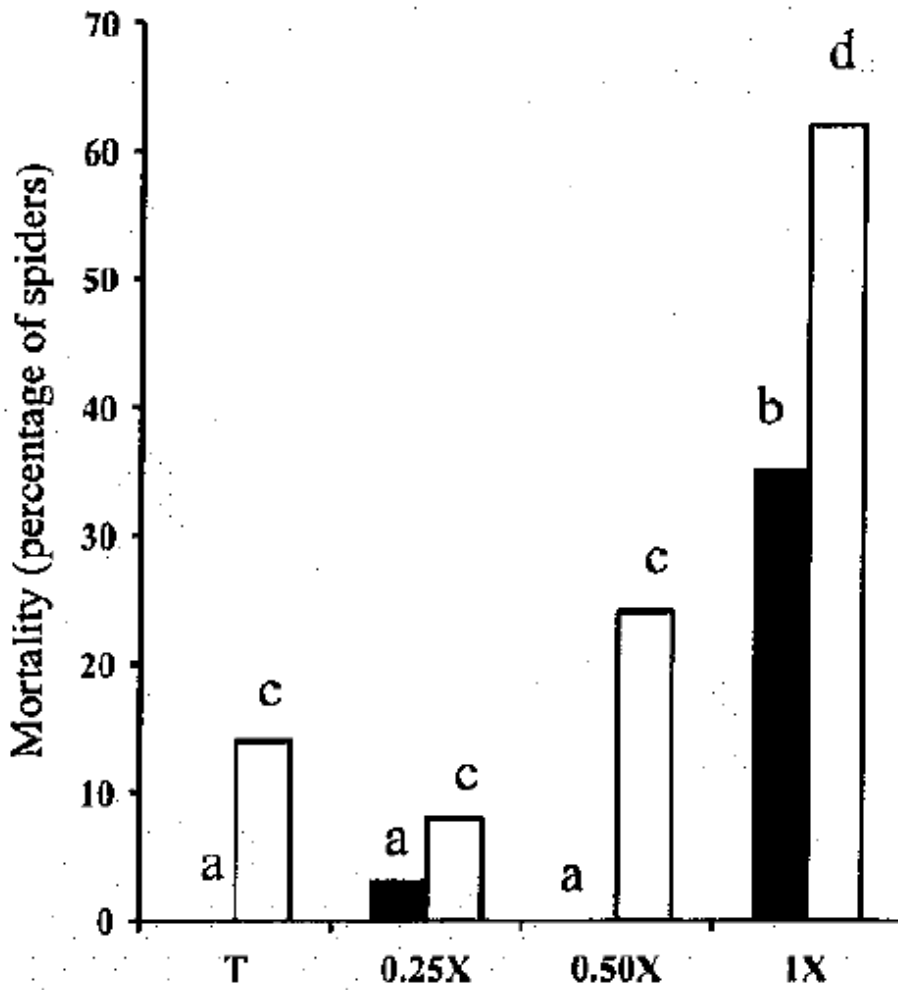


Figure 2. Spider mortality after short-term (72 h-shaded) and long-term (30 days non-shaded) exposure to water (control = T) or different spinosad concentrations (0.25x, 0.5 x, and 1x with 1x corresponding to NAR of 96 mg of active ingredient 1.-1); the same letters show non-significant differences ( $P < 0.001$  between the 1 x and the other doses for short-term (a, b) and long-term (c, d))

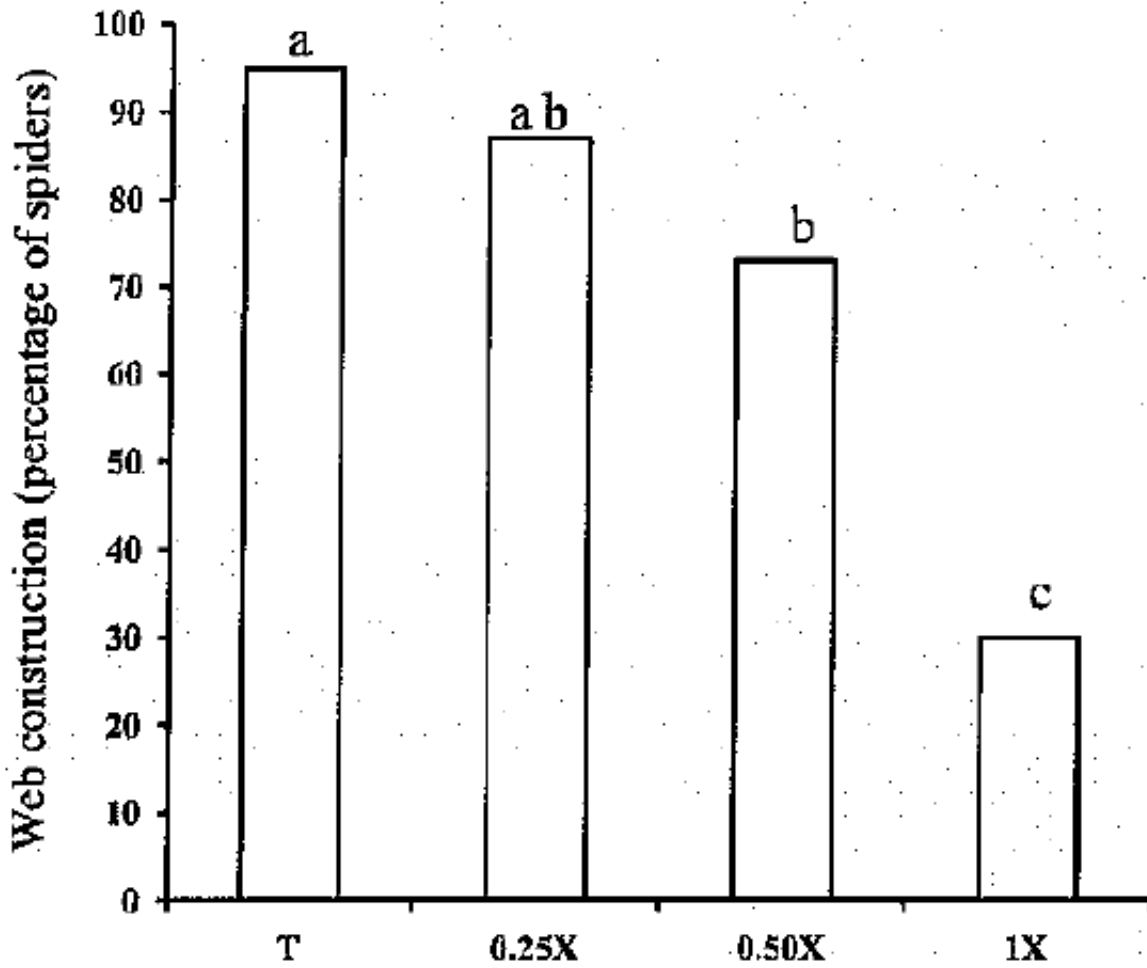


Figure 3. Percentage of spiders which build at least one web after exposure to water (control = T) or to different spinosad concentrations 0.25 x, 0.5 x, and 1x with 1x corresponding to the NAR of 96 mg of active ingredient L-5; the same letters show non-significant differences (P <0.05 between the 0.5 x and the control and P < 0.001 between the 1 x and the other doses)

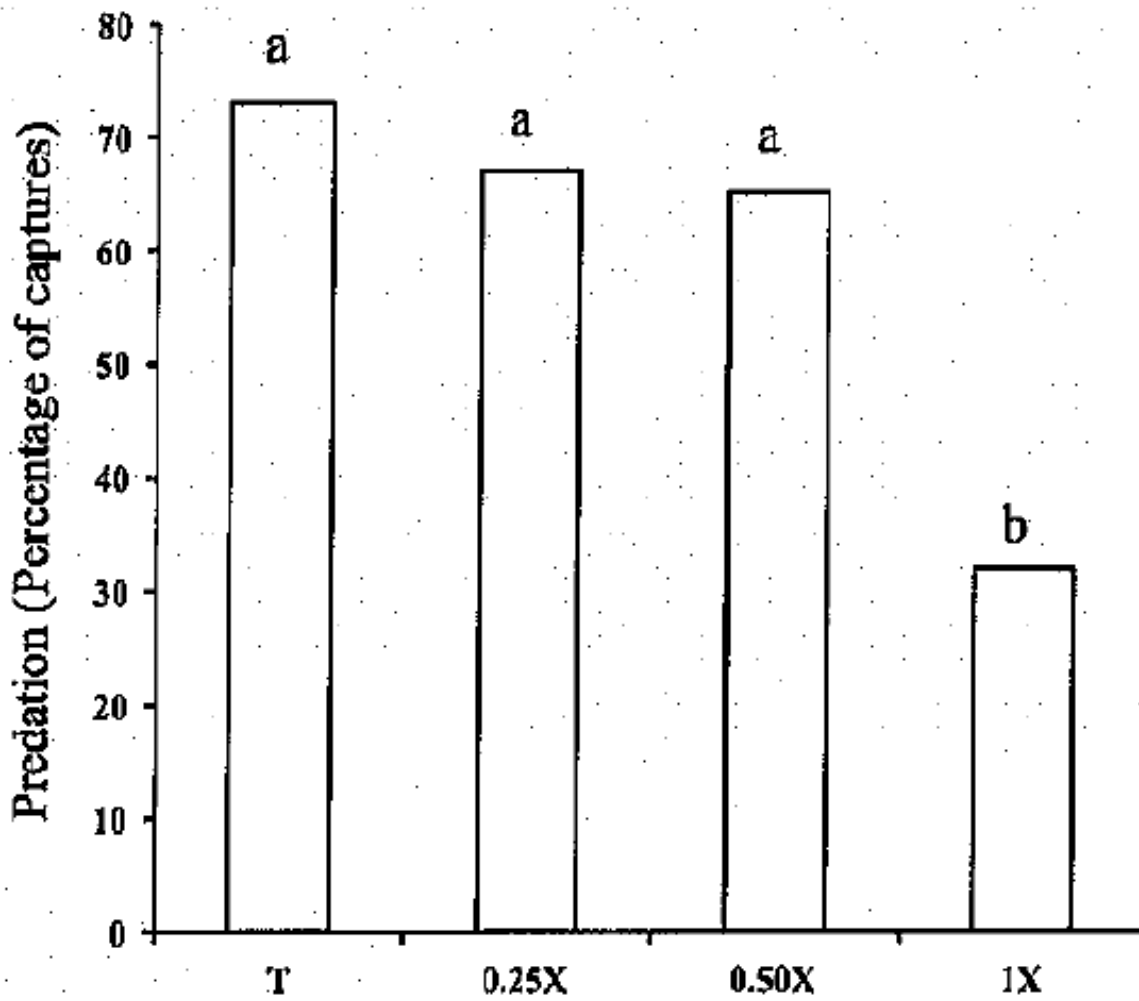


Figure 4. Prey capture rate (first prey caught with or without a web) by spiders exposed to water (control= T) or different spinosad concentrations 0.25x, 0,5x, and 1 x with 1 x corresponding to the NAR of 96 mg of active ingredient L-1); the same letters show non-significant differences ( $P < 0.001$  between the 1x and the other doses)

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## 6. Acknowledgements

The University of Lorraine (Unit of Research in Functions and Animal Product, URAFPA) supported this study. We thank Joel Couturier who helped us rear the spiders

in the laboratory and two anonymous reviewers for their helpful comments on an earlier draft of the paper.