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Effect of the use of dehydrated coffee husk and pulp as mulching in strawberry production on the incidence of pest mites and their natural enemies

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Abstract:

Mulching of soil beds of strawberry fields is usually done with polyethylene film in southern Minas Gerais state, Brazil. This material is relatively expensive and difficult to discard after use. In some countries, mulching is done with the use of organic material that could have an advantage over the use of plastic for its easier degradation after use, and for favoring edaphic beneficial organisms. Predatory mites (especially Gamasina, Mesostigmata) may be abundant in the soil and could conceivably move to the soil surface and onto the short-growing strawberry plants at night, helping in the control or pest arthropods. The two-spotted spider mite, Tetranychus urticae Koch, is considered an important strawberry pest in that region, where the fungus Neozygites floridana (Weiser & Muma) has found to infect it. Different mulching types could affect the incidence of this pathogen. Dehydrated coffee husk and pulp (DCHP) is a byproduct readily available in southern Minas Gerais, where could be used as organic mulching in strawberry beds. The temporary contact of that material with the soil of a patch of natural vegetation could allow its colonization by edaphic predatory mites helpful in the control of strawberry pests. The objective of this work was to compare the effect of mulching type on the population level of the two-spotted spider mite, associate mites and N. floridana, in studies conducted in a greenhouse and in the field. The use of DCHP increased the number of edaphic Gamasina on strawberry plants. The

	edaphic Gamasina Proctolaelaps pygmaeus (Müller) (Melicharidae) and Blattisocius dentriticus (Berlese) (Blattisociidae) were observed on strawberry leaflets, mainly in nocturnal samplings, indicating its possible daily migration from soil to plants. Lower levels of two-spotted spider mite occurred on plants from pots or soil beds mulched with DCHP instead of polyethylene film, apparently because coinciding with slightly higher levels of mites of the family Phytoseiidae and infection by N. floridana. Exposing DCHP onto the floor of natural vegetation did not result in higher diversity or levels of gamasine mites on DCHP. Complementary studies should be conducted to find ways to increase diversity and density of those organisms in strawberry beds, in an attempt to improve biological control of strawberry pests. Decision to use DCHP for mulching should also take into account other factors such as strawberry yield, costs and efficiency of weed management, to be evaluated in subsequent studies.

Introduction

Mulching is widely used in strawberry cultivation mostly to reduce weed development and incidence of plant pathogens, especially for reducing contact of fruits with the soil (Cook et al. 2006). The use of polyethylene film for mulching is very common in strawberry production in Brazil and elsewhere (Costa et al. 2014; Morra et al. 2016). However, the use of this material is prone to result in environmental disorder, especially for the difficulty in discarding it at the end of the growing cycle, due to its high persistence in the environment. In addition, the use of the film under tunnels can lead to reduced levels of relative humidity (Hanks et al. 1961; Forge et al. 2003; Castilho et al. 2015), with potential negative impact on some beneficial organisms, such as predatory mites and entomopathogenic fungi. These are considered important biological control agents of the two-spotted spider mite, *Tetranychus urticae* Koch (Croft et al. 1993; Klingen et al. 2008), one of the main strawberry pests.

Polyethylene film could potentially be replaced by organic material, as commonly used in countries such as Norway (Castilho et al. 2015) and China (Wang and Sun 1986; Li 2000). Organic mulching can bring recognized benefits such as increased temperature stability (Kęsik and Maskalaniec 2005), conservation of soil microorganisms (Brust 1994; Mathews et al. 2002), increased relative humidity (Erenstein 2003; Resende et al. 2005), enhanced soil conditioning and provision of nutrients to the crop over time.

Some authors have reported that the use of organic mulching can increase the abundance and diversity of soil Gamasina, a cohort of the order Mesostigmata (Lindquist et al. 2009) that includes many predatory mites. Using organic mulching (woody plant material and compost) in citrus orchards, Jamieson and Stevens (2006) reported increased Gamasina diversity and a parallel decrease in thrips (Insecta) abundance. About 50% reduction in the number of thrips pupae (usually found in the soil) was observed in avocado and onion cultivations with the use of organic cover (Hoddle et al. 2002; Jensen et al. 2003), leading the authors to suspect it to be a consequence of the observed increase in the incidence of predatory mites. Sánchez-Moreno et al. (2009) reported an increase in the population of nematode-associated predatory mites in tomato and corn cultivation with the use organic mulching.

Except for Astigmatina, the main pest mites (Eriophyidae, Tarsonemidae, Tenuipalpidae and Tetranychidae) are rarely found in the soil when conditions for their development are adequate. By far, the Gamasina most commonly found on plants belong to the family Phytoseiidae (McMurtry et al. 2015), but other gamasine families, including those considered typically soil inhabitants can also be found on plants in areas with high air humidity (Moraes et al. 2015). With few exceptions, predatory mites of the families Laelapidae, Macrochelidae, Ologamasidae, Parasitidae and Rhodacaridae are considered as almost exclusively edaphic (Krantz and Walter 2009; Carrillo et al. 2015). In other predatory mite families, some species are edaphic, while others can be found on plants. It is usually assumed that a particular species will not be at the same time edaphic and plant inhabiting, under adequate environmental conditions. However, these assumptions are based on observations conducted at daytime; the possible daily migration of mites from soil to plants and vice-versa has not received much attention.

The state of Minas Gerais is the main strawberry and coffee producer in Brazil. In the processing of coffee beans, dehydrated coffee husks and pulp (DCHP) are generated byproducts sold at relatively low prices, mostly for use as chicken bedding. Efforts have been dedicated to identify other uses for DCHP, one being for mulching of different crops (Braham and Bressani 1979; Oliveira and Franca 2015). Generated DCHP are not expected to contain appreciable number of Gamasida mites, given that the material is kept under dry conditions between its production and use. However, these could conceivably appear in the material during storage, after colonization by organisms such as fungi, nematodes, insects etc., on which they can feed (Carrillo et al. 2015). It may be expected that this process can be expedited by storing DCHP in environments where these mites are common, as on the floor of areas of natural vegetation. Similar procedure has been used in organic agriculture, for the production of "bokashi", a product obtained by placing an organic substrate onto the soil of areas of natural vegetation for colonization by naturally occurring microorganisms. That substrate is then fermented and later applied to agricultural soil for increasing biodiversity and re-establishing biological balance (Siqueira and Siqueira 2013).

In addition to increasing biodiversity, the expected increase in air humidity at the level of the plants in the tunnels may also enhance the development of pathogenic fungi. Species of the fungus *Neozygites* (Entomophthorales: Neozygitaceae) are considered important mortality factors of two-spotted spider mite when microclimatic conditions are appropriate (Dick and Buschman 1995). *Neozygites floridana* (Weiser & Muma) is a potentially important pathogen of the two-spotted spider mite in strawberry fields in Minas Gerais and elsewhere (Castilho et al. 2015).

The objective of this work was to compare the effect of mulches on the population level of the two-spotted spider mite and associate mites as well as on the incidence of *N. floridana*.

Material and methods

The study was conducted under greenhouse and field conditions.

Conditioning of coffee husk and pulp

Thirty-six jute bags (about 20 L each; mesh opening sufficient to allow flux of mites and other small organisms) of DCHP were acquired from a private company at Ouro Fino, Minas Gerais. Half of the bags were stacked and covered with a black polyethylene film to protect them from the rain; this material is subsequently referred to as non-pre-exposed mulching (treatment 1). The remaining jute bags were placed side by side on the floor of a disturbed forest fragment (about 500 m^2); this material is subsequently referred to as pre-exposed mulching (treatment 2). Rainfall in the area between the placement of the bags and the beginning of the experiment (about a month) was about 60 mm (Inmet 2016).

Greenhouse experiment

To evaluate the possible migration of Gamasina from the plant growing substrate and mulching onto strawberry plants at different times, forty-five 2.0-liter pots were filled to about 80% of their capacity with a growing substrate (Basaplant®, mixture of pine bark, peat, coal and vermiculite) and then one strawberry seedling of the 'Albion' cultivar was transplanted to each pot. Fifty days later, when plants were about 10 cm high and with about 15 leaflets, each plant was subjected to one of three treatments (15 plants per treatment), which corresponded to the coverage of the exposed surface of the growing substrate of each pot with: treatment 1, a layer about 4 cm thick of non-pre-exposed mulching; treatment 2, a similar layer of pre-exposed mulching; and treatment 3, a piece of black polyethylene film. The plants were maintained in a greenhouse at 22–38 ºC, 55–75% RH and about 13 h of daily photoperiod.

To evaluate the mites present in each type of organic mulching (treatments 1 and 2) and in the growing substrate (treatments 1–3), eight samples were taken from each type of mulching and from the substrate on the day the treatments were assigned. Each sample consisted of a volume of approximately 400 cm^3 (cylinder 5 cm high and 10 cm in diameter). Mites were extracted from each sample over a period of seven days into a container with 70% ethanol, using a set of modified Berlese funnels (Oliveira et al. 2001). Extracted mites were mounted in Hoyer's medium and identified to family by using taxonomic keys provided by Krantz and Walter (2009), to genera by using unpublished keys provided by the Ohio Summer Program, Agricultural Acarology, Columbus, Ohio, USA, and to species by using published descriptions and redescriptions of the species of each family.

After 1, 5, 10, 15, 20, 25 and 30 days of the experimental setup, mites on strawberry leaflets were evaluated at 7 and 11 PM, and 3 and 7 AM, by examining one leaflet of the median section of each plant with a hand lens. Mites found were collected with a fine brush in 70% ethanol and later mounted in Hoyer´s medium for identification. Soon after the last evaluation (August 10, 2015), a sample (about 400 cm^3) of mulching (treatments 1 and 2) or growth substrate (treatment 3) was collected from the top surface of each of eight pots of each treatment to determine the mites present, using the previously described procedure.

Field experiment

Setup

This experiment was conducted in a 0.2 ha grower´s strawberry field of the 'Albion' cultivar. The plants were cultivated in beds, each about 40 m long and containing three plant rows, with plants spaced at 35 cm between and within rows. The beds were covered with a black polyethylene film, except for the experimental areas, covered as subsequently described.

The experiment was initiated on July 10, 2015, three months after transplanting. Treatments were similar to those described for the greenhouse experiment (except that plants were grown in the soil, instead of in the growing substrate), using a randomized block design, with eight replicates. Each experimental plot corresponded to a 3.0 m long bed section (24 plants), maintaining a buffer area of 1.0 m between experimental plots in each bed, covered with black polyethylene film. The DCHP mulch layer was about 5 cm thick, as used by Filgueira (2000).

In the first three months after transplanting (period between transplanting and beginning of the evaluations), the fungicides fluazinam (Frowncide® 500 SC) and azoxystrobin (Amistar®) were applied bi-weekly and alternately for the control of the fungus diseases mycospherella and dendrophoma. Later, control of the diseases was only done by the removal of diseased leaves after each evaluation. Two-spotted spider mite was never controlled. Weed growing in plots of treatments 1 and 2 was periodically removed by hand. Fertilization was done, alternately once every twenty days with Visa Fertil® 14-5-8 (50 kg/ 10,000 plants at transplanting, by fertirrigation) and with Adubos Real® 12-6-12 (50 kg/ 10,000 plants, granulated). Plants were sprinkler irrigated in the first 15 days after transplanting and drip irrigated afterwards (30 min a day at a rate of 2 L/ plant/ h).

During the experiment (ended in December 16, 2015), average temperature was about 17.5 ºC (10.6–18.0), relative humidity was 88 % RH (76–94) and photoperiod ranged between 11–12 h (Inmet 2016).

Mites in the mulching/ soil

Three samplings were conducted to determine the mite species: immediately before, two and six months after the beginning of the experiment. At each date, a sample of 400 cm^3 of the DCHP mulch (treatments 1 and 2) or of soil under the polyethylene film (treatment 3) was collected from the surface of each experimental plot. This was done with the aid of the same metal cylinder used in the greenhouse experiment, pressed down near the central row of each bed, so that the top edge of the cylinder was in level with the surface of the coffee husk and pulp mulch or of the soil, according to the treatment. Each sample was placed in a polyethylene bag and stored in a cool box for transport to the laboratory. Mites were extracted from the samples as previously described.

Mites on plants

An extended evaluation of mites on strawberry plants (six monthly samplings, starting three months after transplanting) was carried out. Each sample consisted of five fully developed leaflets, taken at random from strawberry plants of the central line of each plot between 10:00 and 12:00 AM. The leaflets of each sample were stored in a container with 70% ethanol.

In a single date (October 15, 2015, three months after the beginning of the experiment, when each plant had 40-45 leaflets), two plants were randomly taken from the central row of each experimental plot at about 10 AM and other two plants were similarly taken at about 8 PM. Each plant was placed in a container with 70% ethanol.

Samples were taken to the laboratory to count the mites under a stereomicroscope and mount them in Hoyer's medium for identification. Infection of two-spotted spider mites by *N. floridana* was determined by the presence of fungus infective spores or hyphal bodies associated with each mite (Van der Geest et al. 2000).

Analyses

Statistical analyses of soil mites took into account only the Gamasina. The predominant species were calculated as proposed by Pinzón and Spence (2010). In both field and greenhouse experiments, mite densities on plants and soil were compared with Tukey's (parametric data), Kruskal-Wallis' (non-parametric data) tests in the R Development Core Team (2013) statistical program.

Results

Greenhouse experiment

Mites in the mulching or growing substrate

The total numbers of mites collected from samples of treatments 1, 2 and 3 were respectively 1467, 1263 and 189. The Gamasina comprised respectively about 73, 64 and 65% of all mites collected in both samplings (Table 1). The following species were classified as predominant in both samplings: treatment 1 – *Blattisocius dentriticus* (Berlese), *Macrocheles* sp., *Proctolaelaps pygmaeus* (Müller) and *Parasitus* sp.; 2 –*Macrocheles* sp. and *P. pygmaeus*; and 3 – *Digamasellus* sp. and *Gaeolaelaps* sp..

Although major differences were observed for the number of some particular species between sampling dates, some declining and others increasing in numbers from the first to the second sampling (Table 1), no significant effect of sampling dates on numbers of Gamasina as a whole was observed (Table 2). Combining the gamasine mites of the two sampling dates, the highest mean number was found in treatment 1 followed by treatment 2, the number being much lower in treatment 3**.**

Mites on plants

The phytoseiids were the predominant gamasine group on strawberry plants, but the average numbers were not significantly different between treatments (Kruskal-Wallis test, $p > 0.05$). Among the groups considered edaphic Gamasina (Blattisociidae, Macrochelidae, Melicharidae, Ologamasidae and Parasitidae), more mites were collected from strawberry plants of treatment 1 (0.7 ± 0.1 mites/ leaflet) along the experiment (Kruskal-Wallis test, $p < 0.05$), with very few or no mites found on plants of treatments 2 and 3.

About 93% of all Gamasina collected in treatment 1 were identified as *P. pygmaeus* (288 specimens); other species collected were *Macrocheles* sp., *Gamasiphis* sp., *Parasitus* sp. and *B. dentriticus*. The only 12 specimens found in treatment 2 were identified as *P. pygmaeus*, *Parasitus* sp. and *B. dentriticus* In both treatments, the Gamasina collected on plants were also the most numerous in the substrate (except *Gamasiphis* sp.). Taking into account only the plants of treatment 1, the peak number of *P. pygmaeus* on strawberry plants was observed at 11:00 PM (Figure 1). The pattern of variation indicated a tendency for reduction in the number of that species on plants in daytime.

Field experiment

Mites in the mulching or soil

The total numbers of mites collected from beds of treatments 1, 2 and 3 were respectively 4579, 2290 and 653, of which 50, 54 and 51% were Gamasina, but none was phytoseiid (Table 3). The following Gamasina were classified as predominant (in at least one sampling date): treatment 1 – *B. dentriticus*, *Lasioseius* sp. 2., *Macrocheles* sp., *P. pygmaeus* and *Parasitus* sp.; 2 – *Lasioseius* sp., *Macrocheles* sp. and *P. pygmaeus*; and 3 – *B. dentriticus*, *P. pygmaeus*, *Parasitus* sp. and *Pergamasus* sp. In treatments 1 and 2, the acarid *Tyrophagus neiswanderi* (Johnston & Bruce) was the most numerous mites in the second and third evaluations, while in treatment 3 this was also the most numerous in the second evaluation. The lower proportions of Gamasina in the field than in the greenhouse experiment seem to be due to the high numbers of *T. neiswanderi*.

In the three treatments, the highest number of Gamasina was determined on the second sampling date (September 8), although the difference between numbers of the first and second samplings was not significant in treatment 2 (Table 4). Combining the gamasine mites of the three sampling dates, the highest mean number was determined in treatment 1, followed by treatment 2 (Table 4).

Population dynamics of the two-spotted spider mite and associated organisms

The population level of the two-spotted spider mite was low throughout the experimental period, but it was significantly lower in treatment 1 (0.1 ± 0.1 mite/ leaflet), followed treatments 2 (0.6 ± 0.3) and 3 (1.7 ± 0.7). Two-spotted spider mite population increased in treatments 2 and 3 from the first to the second month (Figure 2), followed by a reduction in the following month, which coincided with a sharp increase in rainfall and a discreet increase in *N. floridana* infection and phytoseiid density. A new upsurge in treatment 3 occurred in the fourth month, but it was contained in the last two months, with a new parallel increase in rainfall and *N. floridana* infection; phytoseiid population also increased in the last two month, but only in treatment 3 probably because of prey scarcity in other treatments. The dynamics of the non-phytoseiid Gamasina seemed to be related to the dynamics of *T. neiswanderi*, and both increased after the heavy rainfall in September.

Representatives of nine Gamasina families were also collected, of which Phytoseiidae was by far the most diverse (10 species), representing respectively 17, 13 and 15% of the mites in treatments 1–3. Predominant Gamasina species were the phytoseiids *Neoseiulus californicus* (McGregor) in all treatments and *Phytoseiulus macropilis* (Banks) in treatment 3, and the blattisociid *B. dentriticus* in treatment 1. Other non-phytoseiid Gamasina (*Macrocheles* sp., *P. pygmaeus* and *Parasitus* sp.) were rarely collected.

Mites on strawberry plants in diurnal and nocturnal evaluations

Combining the data of the two sampling times within each treatment, the highest number of uninfected two-spotted spider mite was found in treatment 3, and the lowest, in treatment 1 (Table 6), as also observed in the previous subsection (mites in diurnal evaluations only). The number of phytoseiids was also highest in treatment 3, while difference between treatments 1 and 2 was not significant. Other Gamasina were significantly more numerous in treatment 1 and less numerous in treatment 2. *Tyrophagus neiswanderi* was also more numerous in treatment 1, while the difference between treatments 2 and 3 was not significant. The numbers of infected two-spotted spider mites was low, so that statistical comparison was considered meaningless.

Comparing the numbers of mites between sampling times, no significant differences were observed for uninfected two-spotted spider mites within any of the three treatments (Table 6). For the number of phytoseiids, significant difference was only observed in treatment 3, in which the number in the diurnal evaluation was higher. For other Gamasina, significant differences were observed for all treatments, the numbers at nocturnal evaluations being always higher. For *T. neiswanderi*, the nocturnal evaluation was higher in treatment 1; statistical comparisons were not conducted for other species, because the number of mites was low, regardless of the evaluation period.

Pooling data of both sampling times, totals of 1907, 1136 and 2400 mites were collected from plants of treatments 1, 2 and 3 respectively (Table 7). Of these, the total numbers of Gamasina were respectively 349, 61 and 280 in diurnal samplings and 467, 147 and 242 in nocturnal samplings.

Predominant Gamasina were: treatment 1 - *B. dentriticus*, *P. pygmaeus*, *N. californicus* and *Neoseiulus anonymus* (Chant & Baker) and *Phytoseiulus macropilis* (Banks) (both samplings); treatment 2 - *B. dentriticus* and *P. pygmaeus* (nocturnal), and *P. macropilis* (both samplings); and treatment 3 - *Neoseiulus* sp. (diurnal), and *B. dentriticus* and *P. macropilis* (both samplings).

Discussion

Mites in the growing substrate

The similar abundances of Gamasina in the pots at the beginning and at the end of the greenhouse experiment suggested the stability of the experimental setup within its 1-month duration. The observed higher abundance of Gamasina in pots of treatments 1 and 2 (both with DCHP) than in pots of treatment 3 (surface covered with polyethylene film) was expected, given that the predominant Gamasina in this study were mainly fungivorous and/ or predaceous (Carrillo et al. 2015) and that the presence of organic mulching would favor the maintenance of fungi and small invertebrates (including mites) that could serve as their prey.

Conversely, the small difference between treatments 1 and 2 in terms of composition and number of the edaphic mites suggested that the maintenance of the organic mulch in the patch of natural vegetation did not result in enrichment of the substrate. Enrichment would probably occur if maintenance were longer, which however would not be desirable in this case, as it could accelerate decomposition of the material and consequently shorten its effect as a protective covering of the soil. An alternative could be the addition of a supplementary food source to attract and/or arrest edaphic Gamasina, as for example free-living nematodes, onto which many Gamasida are known to feed (Carrillo et al. 2015).

As the organic mulching material was the same in the greenhouse and field experiments, it is not surprising that the predominant Gamasina in treatments 1 and 2 in the greenhouse were about the same as in the field experiment. Obviously, the predominant species in treatment 3 in the greenhouse were different from those in the field, because of the difference in the substrate, commercial planting substrate in the greenhouse and soil in the field. In the field experiment, the uniform pattern of variation among treatments in the number of Gamasina along the sampling dates (higher number in the second sampling, September 8) suggested that treatments had nothing to do with it. Rather, the variation could be related to climatic factors. As reported in the study of the population dynamics, rainfall in this month was intermediate between a lower level in July (55 mm; middle of the dry season in the region) and a higher level in December (366 mm; middle of the rainy season).

Mites on plants

The low incidence of the two-spotted spider mite contrasted with the high population levels regularly seen in growers’ fields in southern Minas Gerais. Low incidence was also reported by Castilho et al. (2015), in a study conducted in the same region, and could be due to the nonuse of pesticides in the experimental field for arthropod control, unusual in grower´s fields. The higher abundance of this mite in treatment 3 suggested the microenvironment in beds of this treatment to be more favorable to it, in part because of the lower levels of air relative humidity, known to favor the two-spotted spider mite (Duso et al. 2004; Castilho et al. 2015). Lower levels of relative humidity conceivably occur in areas mulched with polyethylene (Cadavid et al. 1998; Costa et al. 2007). Kivijärvi et al. (2002) also reported higher levels of two-spotted spider mite in beds mulched with polyethylene film than with dry grass, barley or wheat straw, and pine bark.

Higher levels of relative humidity with the use of DCHP would in parallel favor *N. floridana*. Thus, it was expected that mulching with DCHP would increase infection of two-spotted spider mites by that fungus. The low incidence of the fungus during the experimental period could be related to the low levels of two-spotted spider mites and/ or to the application of fungicides to control plant diseases in three months preceding the first evaluation. The decrease of the infection levels in treatments 1 and 2 from November to December seems related to markedly low levels of two-spotted spider mite in these treatments, which could have resulted from the more significant increase of the infection level in the same treatments the month before (October to November).

The positive effect of the organic over plastic mulching on predatory mites and *N. floridana* cannot be determined by comparing their level of occurrence in different treatments, given the non-linear relation along time between predator/ pathogen and prey/ host. Another difficulty in evaluating that effect refers to the unequal variation of other factors (other natural enemies, different prevailing abiotic conditions etc) among treatments. In any case, the markedly higher phytoseiid population levels in treatment 3 in November and December seems related to the tendency of the two-spotted spider mite to increase on plants of this treatment after September. The increase in the population of the two-spotted spider mite between September and October might have allowed the increase in predator population and the subsequent reduction in prey population. This interpretation is supported by the observed higher numbers of two-spotted spider mites and of phytoseiids in treatment 3 in the experiment to determine the effect of sampling time. The predominant phytoseiids in this study, *N. californicus* and *P. macropilis*, are known as efficient control agents of the two-spotted spider mite (McMurtry et al. 2013). These are used by some growers of southern Minas Gerais to control this pest.

In the greenhouse experiment, the much higher number of edaphic Gamasina on leaflets of treatment 1 was a function of the much higher abundance of *P. pygmaeus* in that treatment. That was one of the few non-phytoseiid Gamasina found on leaflets. Although most numerous at night, they were also found on leaflets at daytime, which is not unusual for melicharids (Moraes et al. 2015). *Blattisocius dentriticus* was found in much lower number than *P. pygmaeus* in the substrate in the greenhouse experiment, which explains its rare occurrence on strawberry leaflets.

A relevant question at this stage refers to the reason for finding *P. pygmaeus* and *B. dentriticus* on strawberry leaflets, especially of treatment 1. In the greenhouse experiment, large numbers of Gamasina (probably *P. pygmaeus*) were seen roaming on the surface of the substrate in the pots at night, but not at daytime. The possible role of *P. pygmaeus* as a biological control agent on strawberry (or other crops) remains to be determined. A revision of the feeding habits of *Proctolaelaps* and other melicharid species was presented by Moraes et al. (2015). As they summarized, mites of this genus have been repeatedly reported in association with fungi and nematodes. *Proctolaelaps pygmaeus* has also been reported to develop and oviposit on the acarid *Rhizoglyphus robini* Claparède (Metwalli et al. 1991), suggesting that in the present work it could be feeding on *T. neiswanderi*, likewise an Acaridae, in the substrate and on strawberry leaflets.

Proctolaelaps pygmaeus has been reported to feed on the two-spotted spider mite (Mathys and Tencalla 1959), but it was not considered by the authors a good predator of that pest. In laboratory studies on two other *Proctolaelaps* species, *P. bickleyi* Bram and *P. bulbosus* Reis, Moraes & Gondim Jr. (Lawson-Balagbo et al. 2008; Galvão et al. 2011) a diet of the two-spotted spider mite was found unsuitable for develop to adulthood and oviposition. Further investigation should be conducted to confirm the level of importance of *P. pygmaeus* as a predator of the two-spotted spider mite on strawberry plants. Yet, this predator was suspected to cause depletion of a laboratory colony of *Drosophila*, by feeding on its eggs as summarized by Moraes et al. (2015). This is important in the context of the region where this study was conducted, given the recent first report of *Drosophila suzukii* (Matsumura, 1931) in southern Minas Gerais (Andreazza et al, 2016). This is a serious strawberry pest in Europe (Kinjo et al. 2014). In Brazil, it was first found in 2013 (Deprá et al. 2014) in Rio Grande do Sul state, where it has already been reported to cause economic losses (Santos 2014). Evaluation of the effect of *P. pygmaeus* on *D. suzukii* in southern Minas Gerais is warranted.

As mentioned for *P. pygmaeus*, the possible role of *B. dentriticus* as a biocontrol agent on strawberry (or other crops) remains to be determined. As summarized by Moraes et al. (2015), *B. dentriticus* has been reported to develop and reproduce on *Tyrophagus putrescentiae* (Schrank) (Rivard 1960). This suggests that in this work it could be feeding on *T. neiswanderi*.

The much larger number of Gamasina in the beds with DCHP led to the conclusion that this type of mulch contributed to increasing the availability of those predatory mites in strawberry fields. In supporting that conclusion, the predominant Gamasina determined in the DCHP on strawberry beds two and six months after the beginning of the work were basically the same as determined from that substrate from samples taken from the bags immediately before starting the field experiment. This would be expected, given that edaphic mites are much more numerous in the litter than in the mineral fraction of the soil (Carrillo et al. 2015), associated with the fact that the use of polyethylene film in treatment 3 eliminated possible litter accumulation. It is worth pointing out that edaphic mites were found on the leaflets even in treatment 3, what could be related to their migration from plots of other treatments. The fact that this did not occur in the first sampling date corroborate that hypothesis.

By conducting monthly surveys of leaves of the tree *Genipa americana* L. (Rubiaceae) during one year, at day and nighttime, Parecis-Silva et al. (2016) observed that the phytoseiids *Euseius citrifolius* Denmark & Muma and *Euseius concordis* (Chant) foraged for prey on the leaf surface during the day, whereas *Agistemus floridanus* Gonzalez (Stigmaeidae) foraged especially at night. Studying the relationship between phytophagous and predatory mites on cassava plants (*Manihot esculenta* Crantz), Onzo et al. (2003) noted that at daytime the phytoseiid *Typhlodromalus aripo* De Leon remained protected between the developing leaflets in the plant growing tips, moving at night to neighboring young leaves in search of prey.

The results of the present work in a way resemble those of Onzo et al. (2003), cassava growing tips having an analogous function as DCHP mulching, harboring predators that at night visited the surrounding substrates, seemingly in search of prey. Despite reported differences in relation to groups foraging at different periods, faunistic compositions of arthropods on plants have been reported as rather similar in diurnal and nocturnal samplings (Novotny et al. 1999; Saigusa et al. 2000). However, those studies have dealt with mite fauna of relatively tall plants. For being low growing, strawberry plants could facilitate movement of mites from soil to plants and vice-versa.

The similar densities of infected or uninfected two-spotted spider mites in samples collected at day and at nighttime were expected, given that tetranychids are not expected to move from plants to soil and vice-versa on a daily basis. Conversely, the observed higher numbers of non-phytoseiid Gamasina on leaflets sampled at night in the three treatments are compatible with what was observed in the greenhouse experiment. The reason for this is not known, but could be related to the higher humidity, lower temperature and/ or absence of light at night.

Concluding remarks

The use of DCHP for mulching resulted in lower incidence of two-spotted spider mites compared to the use of plastic, and this could be paralleled by a discreet but higher rate of incidence of *Neozygites* fungus infection between October and November, a more effective impact of phytoseiids between July and October, but not to an increased number of edaphic Gamasina onto the strawberry plants at night.

While the results of this study were illustrative concerning the determination of the mite fauna in strawberry fields in southern Minas Gerais and the movement of mites between soil and plants, complementary studies should be conducted to confirm the results suggesting the lower two-spotted spider mite incidence on plants from beds with organic mulch. More conclusive results would be expected with higher incidence of two-spotted spider mites in the field.

Factors to be taken into account in complementary studies should include the determination of the: a) possible effect of the enrichment with free-living nematodes of the DCHP to be maintained on the soil of the patch of natural vegetation, to attract more predatory Gamasina; b) potential prey items of *B. dentriticus* and *P. pygmaeus* in strawberry plantations, including their possible role on the incidence of *D. suzukii*; c) impact of DCHP on other environmental factors (weed and plant pathogen incidence) and on strawberry yield; d) cost and environmental benefits of the use of DCHP.

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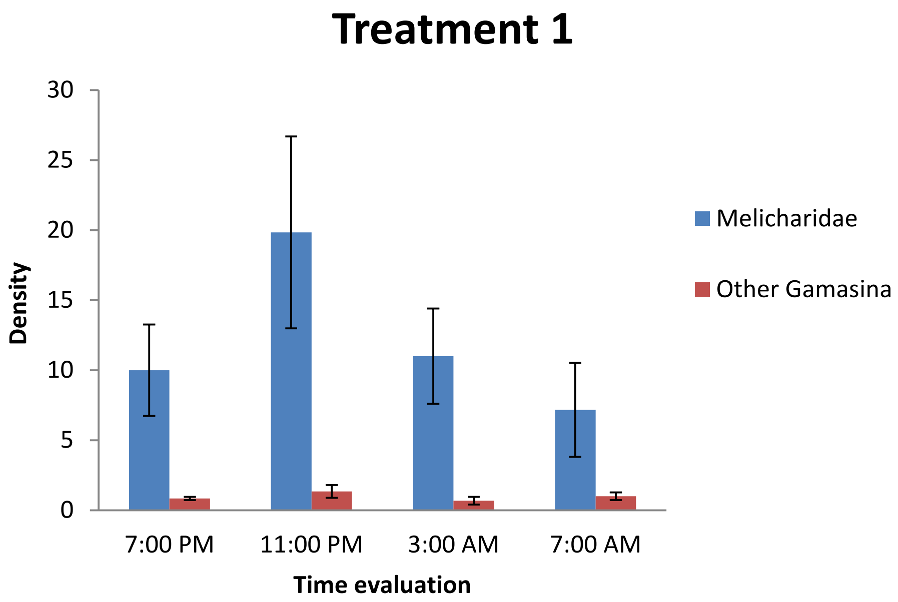

Figure 1. Mean number of Melicharidae (*Proctolaelaps pygmaeus*) and other Gamasina (Blattisociidae, Macrochelidae, Ologamasidae and Parasitidae) on 15 strawberry leaflets in Treatment 1: Coffee husk and pulp from jute bags stored in the experimental field protected from the rain (n = 7, each corresponding to an evaluation date 1, 5, 10, 15, 20, 25 and 30 days from beginning of the study).

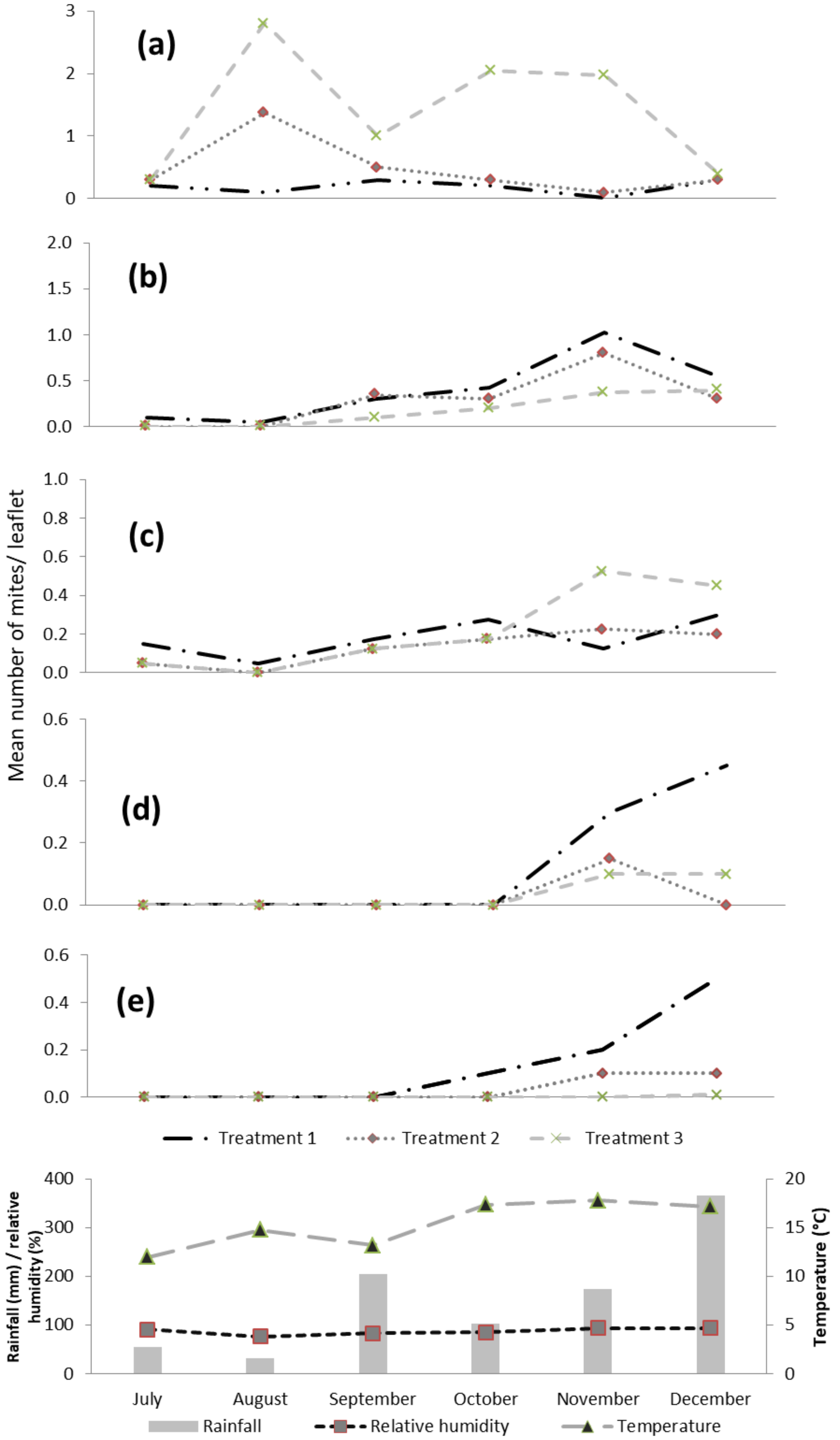

Figure 2. Average number of mites / leaflet, (a): *T. urticae*, (b): *N. floridana* infected *T.urticae*, (c): Phytoseiidae, (d): *Tyrophagus neiswanderi*, (e): Others Gamasina and (f): rainfall, humidity and temperature (Inmet, 2016) between July 10 and December.

Table 1. Mites extracted from 8 samples (400 cm^3) of the top layer of the substrate of pots in the greenhouse test. Treatments: 1, mulched with dehydrated coffee husk and pulp; 2, mulched with the same material, previously maintained on the floor of a fragment of natural vegetation; 3, mulched with black polyethylene film. Samples taken one day before beginning and at the end of the experiment.

	Treatment 1		**Treatment 2**		**Treatment 3**	
Taxa	**Jul 10**	**Aug 10**	**Jul 10**	**Aug 10**	**Jul 10**	**Aug 10**
Sarcoptiformes, Oribatida, Astigmatina						
Acaridae						
Tyrophagus neiswanderi	82	75	135	33	0	0
Sarcoptiformes, other Oribatida						
Galumnidae						
-	3	10	10	48	24	6
Suctobelbidae						
-	0	0	0	16	7	9
Parasitiformes, Mesostigmata, Gamasina						
Ascidae						
Cheiroseius ornatus	0	3	0	6	7	4
Protogamasellus sp.	3	1	39	0	0	0
Males	0	0	0	1	0	0
Blattisociidae						
Blattisocius dentriticus	44*	36*	8	24	0	0
Blattisocius everti	52	8	0	10	0	0
Lasioseius sp. 1	14	1	12	0	0	0
Lasioseius sp. 2	3	7	4	0	0	0
Immatures	23	0	3	0	0	0
Males	9	0	0	0	0	0
Digamasellidae						
Dendrolaelaps sp.	0	0	0	8	9	2
Digamasellus sp.	0	32	0	29	17*	21*
Multidendrolaelaps sp.	0	0	0	11	9	2
Laelapidae						
Gaeolaelaps sp.	0	17	0	0	21*	8*
Macrochelidae						
Glyptholaspis sp.	1	4	6	12	0	0
Holostaspella sp.	2	2	5	3	0	0
Macrocheles sp.	31*	58*	138*	41*	0	0
Melicharidae						
Proctolaelaps pygmaeus	325*	91*	59*	67*	0	0
Tropicoseius sp.	3	0	0	0	2	0
Immatures	25	48	31	51	0	0
Males	23	16	0	14	0	0
Ologamasidae						
Athiasella sp.	0	0	1	0	0	0
Gamasitus sp.	2	0	1	0	1	0
Neogamasellevans sp.	1	0	2	0	0	0
Immatures	1	0	0	0	0	0

Males	1	0	4	0	0	0
Pachylaelapidae						
Zygoseius sp.	16	0	9	9	7	4
Males	0	0	3	0	0	0
Parasitidae						
Parasitus sp. (deutonymph)	53*	51*	15	34	2	7
Pergamasus sp. (deutonymph)	5	29	2	26	0	0
Larva/protonymph	22	9	88	28	0	0
Parasitiformes, Mesostigmata, Uropodina						
Uropodidae						
-	5	11	3	15	5	2
Trombidiformes, Prostigmata						
Cheyletidae						
-	59	89	4	128	0	0
Cunaxidae						
-	2	27	17	35	2	7
Tydeidae						
-	10	22	7	8	3	1
Partial totals	820	647	606	657	116	73
Overall totals	1467		1263		189	

(-) Non identified to genus /species level. (*) Predominant species

Table 2. Mean number (± SE) of Gamasina mites per sample (393 cm^3) taken from pots in the greenhouse test. Treatments: 1, mulched with dehydrated coffee husk and pulp; 2, mulched with the same material, previously maintained on the floor of a fragment of natural vegetation; 3, mulched with black polyethylene film. Samples taken one day before the beginning and at the end of the experiment; n= 8 samples/ treatment.

Sampling date	**Treatments**		
	1	**2**	**3**
July 10	82.3 ± 5.5 a	53.7 ± 3.7 a	9.4 ± 1.7 a
August 10	51.6 ± 7.7 a	46.1 ± 4.8 a	6.0 ± 1.4 a
Mean total	67.0 ± 3.7 C	50.3 ± 3.1 B	7.7 ± 1.1 A

Within each treatment, no statistical differences were observed between sampling dates (Tukey for treatments 1 and 2, Kruskal-Wallis for treatment 3; $p > 0.05$). Mean totals were significantly different from each other (Kruskal-Wallis test, $p < 0.001$).

Table 3. Mites extracted from 8 samples (400 cm^3) of the top layer of the substrate (organic mulch/ soil) of a strawberry field at Bom Repouso, Minas Gerais. Treatments: 1, mulched with dehydrated coffee husk and pulp; 2, mulched with the same material, previously maintained on the floor of a fragment of natural vegetation; 3, mulched with black polyethylene film. Samples taken one day before beginning, 2 and 6 months after the beginning of the experiment.

	Treatment 1			**Treatment 2**			**Treatment 3**		
Taxa	**Jul 10**	**Sep 8**	**Dec 16**	**Jul 10**	**Sep 8**	**Dec16**	**Jul 10**	**Sep 8**	**Dec 16**
Sarcoptiformes, Oribatida, Astigmatina									
Acaridae									
Tyrophagus neiswanderi	82	1416	605	135	540	211	0	252	5
Sarcoptiformes, other Oribatida									
Galumnidae									
-	3	0	23	10	32	24	0	2	8
Parasitiformes, Mesostigmata, Gamasina									
Ameroseiidae									
Ameroseius sp.	0	8	4	0	1	0	0	0	0
Ascidae									
Asca sp.	0	0	2	0	0	0	0	0	0
Cheiroseius sp.	0	0	1	0	0	4	0	0	0
Protogamasellus sp.	3	0	0	39	1	3	0	2	0
Immatures	0	0	0	0	0	16	0	0	0
Blattisociidae									
Blattisocius dentriticus	44*	191*	32*	8	33	0	0	22*	0
Blattisocius everti	52	0	0	0	0	0	0	0	0
Lasioseius sp. 1	14	12	10	12	21	9	0	13	0
Lasioseius sp. 2	3	37*	19*	4	66*	5*	0	9	0
Immatures	23	0	1	3	1	1	0	2	0
Males	9	0	1	0	0	2	0	14	0
Laelapidae									
Gaeolaelaps sp.	0	5	14	0	7	9	0	4	12
Immatures	0	9	0	0	0	8	0	0	5
Males	0	2	0	0	1	1	0	2	7
Macrochelidae									
Glyptholaspis sp.	1	2	5	6	0	1	0	2	0
Holostaspella sp.	2	0	4	5	0	3	0	0	0
Macrocheles sp.	31*	11*	18*	138*	8*	9*	3	1	0
Immatures	0	0	2	0	0	4	0	0	0
Melicharidae									
Proctolaelaps pygmaeus	325*	727*	294*	59*	335*	132*	0	101*	13*
Tropicoseius sp.	3	0	0	0	0	0	0	0	0
Immatures	25	33	0	31	5	11	0	33	0
Males	23	40	0	0	0	5	0	2	0
Ologamasidae									
Athiasella sp.	0	0	0	1	0	0	0	0	0
Gamasitus sp.	2	0	0	1	0	0	2	0	0
Neogamasellevans sp.	1	0	2	2	0	0	0	0	0
New genre	0	5	0	0	1	2	0	1	2

Immatures	1	0	0	0	0	0	0	0	0
Males	1	5	1	4	0	0	0	1	0
Pachylaelapidae									
Zygoseius sp.	16	0	0	9	0	0	0	0	0
Males	0	0	0	3	0	0	0	0	0
Parasitidae									
Parasitus sp. (deutonymph)	53*	41*	3*	15	20	7	22*	13*	8*
Pergamasus sp. (deutonymph)	5	36	7	2	31	8	5*	5*	8*
Immatures (larva, protonymph)	22	39	12	88	28	6	3	9	1
Males	0	2	2	0	0	9	0	4	0
Podocinidae									
Podocinum sp.	0	0	0	0	0	0	0	1	0
Parasitiformes, Mesostigmata, Uropodina									
Uropodidae									
-	5	0	0	3	0	0	0	0	0
Trombidiformes, Prostigmata									
Cheyletidae									
-	59	51	0	4	25	12	7	43	0
Cunaxidae									
-	2	4	21	17	0	3	0	0	0
Tydeidae									
-	10	0	0	7	23	0	4	0	0
Partial totals	820	2676	1083	606	1179	505	46	538	69
Overall totals		4579			2290			653	

(-) Not identified at the genus /species level. (*) Predominant species

Table 4. Mean number (± SE) of Gamasina mites per sample (393 cm^3) taken from the top layer of the substrate (organic mulch/ soil) of a strawberry field at Bom Repouso, Minas Gerais. Treatments: 1, mulched with dehydrated coffee husk and pulp; 2, mulched with the same material, previously maintained on the floor of a fragment of natural vegetation; 3, mulched with black polyethylene film. Samples taken one day before the beginning of the experiment and one month later; n= 8 samples/ treatment.

Sampling	**Treatments**		
	1 (1)	**2 (2)**	**3 (2)**
July 10	82.6 ± 3.0 b	53.7 ± 3.7 a	4.4 ± 0.7 b
September 8	150.6 ± 8.0 a	69.9 ± 6.9 a	30.1 ± 3.7 a
December 16	54.2 ± 6.8 b	31.8 ± 2.0 b	7.0 ± 1.2 b
Mean total	95.8 ± 7.7 A	51.8 ± 3.5 B	13.8 ± 2.2 C

In each row, different low case letters indicate statistical differences between sampling dates; (1) Kruskal-Wallis and (2) Tukey tests, both for $p < 0.05$. Different capital letters indicate statistical differences between treatments.

Table 5. Mites collected from 240 strawberry leaflets sampled monthly at daytime from July 10 to December 16, 2015. Treatments: 1, mulched with dehydrated coffee husk and pulp; 2, mulched with the same material, previously maintained on the floor of a fragment of natural vegetation; 3, mulched with black polyethylene film. Bom Repouso, Minas Gerais.

	Treatments		
Taxa	**1**	**2**	**3**
Sarcoptiformes, Oribatida, Astigmatina			
Acaridae			
Tyrophagus sp.	37	8	16
Sarcoptiformes, other Oribatida			
Oribatida			
-	14	9	4
Parasitiformes, Mesostigmata, Gamasina			
Blattisociidae			
Blattisocius dentriticus	31*	1	4
Macrochelidae			
Macrocheles sp.	1	0	0
Melicharidae			
Proctolaelaps pygmaeus	4	3	5
Parasitidae			
Parasitus sp.	2	0	0
Phytoseiidae			
Amblydromalus limonicus	0	1	7
Amblyseius chiapensis	2	0	3
Arrenoseius urquharti	5	1	1
Galendromus annectens	0	2	0
Neoseiulus anonymus	1	0	2
Neoseiulus californicus	17*	8*	12*
Phytoseiulus macropilis	7	7	17*
Proprioseiopsis cannaensis	0	1	2
Thyphodromips mangleae	4	1	0
Typhlodromus (Anthoseius) transvaalensis	9	0	0
Immatures	10	14	29
Males	3	5	21
Trombidiformes, Prostigmata			
Cheyletidae			
-	4	0	0
Cunaxidae			
-	1	1	0
Tetranychidae			
Tetranychus urticae	32	145	421
Neozygites floridana infected *T. urticae*	116	78	52
Tydeidae			
-	39	14	13
Total	339	299	609

(-) Not identified at the genus /species level. (*) Predominant species

Table 6. Mean number (± SE) of mites per strawberry leaflet sampled at 10:00 AM and 8:00 PM on October 15, 2015. Treatments: 1, mulched with dehydrated coffee husk and pulp; 2, mulched with the same material, previously maintained on the floor of a fragment of natural vegetation; 3, mulched with black polyethylene film. Bom Repouso, Minas Gerais (n= 16 plants/ sampling, each plant with 40-45 leaflets).

Mite group	**Treatment 1**			**Treatment 2**			**Treatment 3**		
	10 AM	**8 PM**	**Total**	**10 AM**	**8 PM**	**Total**	**10 AM**	**8 PM**	**Total**
T. urticae	1.5 ± 0.2 a	1.5 ± 0.3 a	1.5 ± 0.2B	1.9 ± 0.2 a	2.6 ± 1.4 a	2.3 ± 0.2AB	5.0 ± 0.9 a	5.4 ± 0.8 a	5.2 ± 0.6 A
Phytoseiidae	0.5 ± 0.0 a	0.4 ± 0.0 a	0.4 ± 0.0B	0.3 ± 0.0 a	0.4 ± 0.0 a	0.3 ± 0.0 B	1.4 ± 0.0 a	0.9 ± 0.0 b	1.2 ± 0.1 A
Other Gamasina	1.7 ± 0.3 a	2.7 ± 0.3 b	2.1 ± 0.2A	0.1 ± 0.0 a	0.6 ± 0.1 b	0.3 ± 0.2 C	0.3 ± 0.1 a	0.6 ± 0.1 b	0.5 ± 0.1 B
T. neiswanderi[1]	0.3± 0.1 a	1.9± 0.3 b	1.1 ± 0.1 A	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 B	0.2 ± 0.0 a	0.4 ± 0.1 a	0.2 ± 0.0 B
N. floridana infected *T. urticae*[2]	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0

Numbers in the same row followed by the same low case letter within a treatment or by the same capital letter across treatments are not significant different by Kruskal-Wallis test (p> 0.05); [1]Statistical comparison considered meaningless for treatments 2 and 3; [2]Statistical comparison considered meaningless for all treatments.

Table 7. Mites extracted from 160 strawberry leaflets sampled at 10:00 AM or 8:00 PM on October 15, 2015. Treatments: 1, mulched with dehydrated coffee husk and pulp; 2, mulched with the same material, previously maintained on the floor of a fragment of natural vegetation; 3, mulched with black polyethylene film. Bom Repouso, Minas Gerais (n= 2 plants/ sampling, each plants had 40-45 leaflets).

	Treatment 1		**Treatment 2**		**Treatment 3**	
Taxa	**10 AM**	**8 PM**	**10 AM**	**8 PM**	**10 AM**	**8 PM**
Sarcoptiformes, Oribatida, Astigmatina						
Acaridae						
Tyrophagus neiswanderi	40	306	14	24	33	67
Sarcoptiformes, other Oribatida						
Suctobeldidae						
-	2	2	4	0	0	3
Parasitiformes, Mesostigmata, Gamasina						
Ameroseiidae						
Ameroseius sp.	0	1	0	0	0	0
Ascidae						
Gamasellodes sp.	0	2	0	1	0	0
Blattisociidae						
Blattisocius dentriticus	235*	333*	5	55*	23*	36*
Blattisocius everti	1	2	0	1	4	2
Lasioseius sp. 1	8	3	0	1	0	0
Lasioseius sp. 2	0	3	0	0	0	2
Males	3	2	0	0	0	5
Laelapidae						
Pseudoparasitus sp.	1	0	0	0	0	0
Macrochelidae						
Glyptholaspis sp.	0	1	0	0	0	0
Holostaspella sp.	0	0	0	0	0	1
Melicharidae						
Proctolaelaps pygmaeus	12*	48*	8	25*	21	44
Immatures	3	9	2	2	3	5
Males	0	3	0	3	3	3
Ologamasidae						
Gamasiphis sp.	1	1	0	0	0	0
Parasitidae						
Parasitus sp. (deutonymph)	7	2	0	1	1	0
Phytoseiidae						
Amblyseius sp.	5	3	1	0	1	3
Arrenoseius sp.	2	3	2	0	3	6
Euseius sp.	0	0	4	0	4	0
Iphiseioides sp.	0	1	0	0	0	0
Neoseiulus anonymus	1	3*	2	4	7*	2*
Neoseiulus californicus	14*	4*	3	6	25*	3*
Phytoseiulus macropilis	37*	31*	27*	24*	101*	88*
Typhlodromalus marmoreus	3	0	0	0	0	0

Typhlodromus (Anthoseius) ornatos	0	1	0	0	0	0
Immatures	9	5	6	24	47	22
Males	7	6	1	0	37	20
Trombidiformes, Prostigmata						
Cheyletidae						
-	2	2	1	0	0	0
Tetranychidae						
Tetranychus urticae	239	233	302	418	801	859
Neozygites floridana infected *Tetranychus urticae*	73	45	84	64	45	39
Tydeidae						
-	40	107	11	6	5	26
Partial totals	745	1162	522	614	1164	1236
Overall totals	1907		1136		2400	

(-) Not identified at the genus /species level. (*) Predominant species