STUDIES ON THE SPIDERS AS POTENTIAL BIOLOGICAL CONTROL AGENTS IN AGROECOSYSTEMS

露地野菜栽培における生物的防除素材としての真正クモ類の有効性

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ENGLISH ABSTRACT

ABSTRACT

Firstly, the attractiveness of potentially beneficial flowering plants to spiders and other insect natural enemies was investigated in an agricultural field cultivated organically at Kiire, Kagoshima City, Japan, in 2013 and 2014. In 2013, five plant species, *Salvia farinacea* Benth., *Mentha spicata* L., *Foeniculum vulgare* Mill., *Fagopyrum esculentum* Moench and *Anethum graveolens* L. were compared for their attractiveness to spiders using a direct count method. *S. farinacea* attracted significant numbers of Thomisidae and *Me. spicata* attracted Theridiidae, while the other flowering plants attracted fewer numbers of spiders. In 2014, another set of five flowering plant species, *S. farinacea* Benth., *Matricaria recutita* L., *Achillea millefolium* L., *Petunia atkinsiana* D. Don ex Loudon, and *Alyssum maritimum* (L.) Desv. were compared for their attractiveness to natural enemies; Thomisidae, Ichneumonoidea, Chalcidoidea, Anthocoridae, and Syrphidae. Samples were collected weekly from each plant species using a sweeping net. Crab spiders and predatory bugs clearly preferred *S. farinacea* compared to other plants in this study, whereas chalcidoid wasps preferred *S. farinacea* and *Ac. millefolium*. This study may be the first to investigate the attractiveness of *S. farinacea* and *Me. spicata* to thomisid and theridiid spiders in an agricultural field.

Secondly, this study was aimed to identify the treatment that increases the populations of spiders. In 2013 and 2014, two different treatments, organic fertilizer and chemical fertilizer treatments were applied to the

experimental eggplant field and in 2014, I surrounded organic fertilizer plots with the flowering plants *Salvia farinacea* Benth., *Mentha spicata* L., and *Ocimum basilicum* L. Repeated measures ANOVA revealed significant influences of fertilizer type on the numbers of linyphiid spiders and Collembola in 2013. In 2014, the numbers of Collembola, thrips, and lycosid and linyphiid spiders were higher in organic fertilizer with flowering plants treatment comparing with the chemical fertilizer treatment. Moreover, the numbers of *Henosepilachna vigintioctopunctata* (F.) were significantly lower in the organic fertilizer with flowering plants treatment than in chemical fertilizers treatment. I expect that Thysanoptera and Collembola were important alternative prey for linyphiid and lycosid spiders and the use of organic fertilizer and flowering plants enhanced the density of these spiders, and may increase their effectiveness in suppressing the populations of *H. vigintioctopunctata* (F.).

Finally, analysis the gut content of *Pardosa* spiders by polymerase chain reaction (PCR), to detect the DNA of cotton aphid (*Aphis gossypii* Glover), played as an essential tool to check the probability of predation in the open field. Thirteen of total eighty individuals of these field samples of *Pardosa* spider were positive for DNA of cotton aphid. These results confirmed that *Pardosa* spiders are a very important predator and deserve more attention in biological control of cotton aphid.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Spiders are considered as an effective biological control agent in agricultural ecosystems because they are obligate predators, and they can feed on many insect pests. Many scientists reported that spiders can kill a greater number of prey than they can consume (they can kill more they can feed, more than 50 times) (Riechert and Lockley, 1984). Sheet-web spiders feed on many insects including Diptera, Hemiptera (particularly, aphids and leafhoppers), and beetles. Orb-web spiders prey on Hemiptera (leafhoppers), Diptera, and Orthoptera (grasshoppers). While, many of Orthoptera, Coleoptera, and Lepidoptera were consumed by Funnel-web spiders (Riechert and Bishop, 1990). Hunting spiders catch and feed on many insect pests, Orthoptera, Diptera, Thysanoptera, Hemiptera, Lepidoptera, and Coleoptera.

The management of fields to produce suitable habitats can improve the biological control in agricultural fields by enhancing the natural enemy populations (Alomar et al., 2006; Bianchi et al., 2006). Also, Riechert and Lockley (1984) and Rypstra et al. (1999) indicated that raising various habitats may provide the agroecosystem with different microclimates, microhabitats, alternative food resources, and web attachment locations, thus they may increase the spider numbers. Adding suitable refuges can increase the spider numbers (Sunderland and Samu, 2000). Jmhasly and Nentwig (1995) showed that the spider numbers increased while the numbers of aphid decreased when the strips of flowering plants were cultivated in the main field of wheat. The strips of flowering plants are essential in biocontrol to increase the numbers of predators and their alternative prey (Frank, 2003). Harwood et al. (2003) showed that many individuals of Thysanoptera were caught in the Linyphiidae web, and they expected that it was a good prey for linyphiid spider. Hatley and Macmahon (1980), Landis et al. (2000) and Jonsson et al. (2008) confirmed that increasing the various habitats can add different food resources, so they can enhance the density natural enemies. Additionally, Marc et al. (1999) demonstrated that it is very important to take care of the environment (i.e., habitat quality) to increase spiders. Provencher and Vickery (1988) and Rypstra et al. (1999) demonstrated that the value of intercropping to increase populations of spiders by enhancing spatial complexity and increasing more good habitats. Peterson et al. (2010) and Carrel et al. (2000) showed that Linyphiidae spiders feed on pollen. Pollen is an effective food for spiderlings, especially if prey populations are not enough. Also, many previous studies have confirmed the effectiveness of pollen for many natural enemies as an alternative food resource (Peterson et al., 2010; Messelink et al., 2014). In addition, for orb-weaving spiders (Araneidae), pollen is an essential component of the spiders' diet (Eggs and Sanders, 2013). Moreover, another study showed that some juveniles of orb-web spiders feed on pollen, especially when they recycle their webs (Smith and Mommsen, 1984).

On the other hand, the structure of soil and microclimate were changed to be more suitable for saprophagous insects by applying organic fertilizers, because organic fertilizers can enhance the population of saprophagous insects (Alderweireldt, 1994; Chen and Wise, 1999; Axelsen and Kristensen, 2000; Pfiffner and Luka, 2003). Moreover, Agustí et al. (2003) found that the number of spiders enhanced by *Isotoma anglicana* (Collembola), and this species could increase the number of spiders and hence is useful to control some insect pests such as aphids. Also, the richness of weeds and some invertebrates were higher in the organic fertilizer treatment than in the chemical fertilizer treatment (Dicks et al., 2013).

Polymerase chain reaction(PCR) technique has become an important tool for identifying prey species in the gut contents of predators to understand the trophic interactions under the natural conditions (Sunderland et al., 2005; King et al., 2008; Pompanon et al., 2012; Traugott et al., 2013; Gariépy et al., 2007). In the agroecosystems, Lycosidae spiders (*Pardosa* sp.) are regularly found in high numbers (Schmidt et al., 2005). Many studies have used some of the guts investigation for different predators to evaluate aphid predation (Harwood and Obrycki, 2005). In this study, the PCR was used to detect the DNA of *Aphis gossypii* from spider guts to evaluate the ground dwelling spider as an effective predator of cotton aphid under open field conditions.

The objectives of the present study were as follows.

1- To investigate the attractiveness of spiders and insect predators and parasitoids to some flowering plants to determine the suitable plants for natural enemies.

2- To determine the influence of a combination of organic fertilizers and flowering plants on the numbers of spiders and its importance for pest control.

3- To evaluate the spider efficiency as a predator under field conditions by molecular analysis (polymerase chain reaction).

CHAPTER 1:

Attractiveness of spiders and insect predators and parasitoids to flowering plants

CHAPTER 1: Attractiveness of spiders and insect predators and parasitoids to flowering plants

1.1- INTRODUCTION

Biological control plays an essential role in an integrated pest management program to protect economically valuable plant crops from various pests. Currently, to enhance the numbers of natural enemies, it has beensocommon to use insectary plants to provide these insects with alternative food resources (e.g., pollen and nectar), a suitable microenvironment, and appropriate prey (Wolcott, 1942; Hocking, 1966; Sotherton, 1984; White et al., 1995; Jervis et al., 1992, 1996; Al-Doghairi and Cranshaw, 1999; Jackson et al., 2001; Heimpel and Jervis, 2005; Ambrosino et al., 2006; Wanner et al., 2006; Zurbrügg and Frank, 2006; Fiedler and Landis, 2007). The honey dew of aphids and the nectar and pollen of flowering plants have been proposed as such alternative diet resources, and their effects on the fecundity and the longevity of parasitoid wasps have been well studied (Leius, 1960; Powell, 1986; Jervis et al., 1992, 1996; Irvin et al., 1999;Johanowicz and Mitchell, 2000; Heimpel and Jervis, 2005; Winkler et al., 2005; Wäckers et al., 2008; Winkler et al., 2009). Landis et al. (2000) and Ambrosino et al. (2006) showed that many insect predators and parasitoid species feed on pollen and nectar. Because the nectar contains sugars (hexose, sucrose and glucose) and lipids, it can provide an effective auxiliary food resource (Bernardello et al., 1999;

Jackson et al., 2001). Numerous studies have examined the relationship between spiders and nectar, demonstrating nectivory by some spiders, e.g., crab spiders and jumping spiders, Thomisidae and Salticidae (Beck and Connor, 1992; Pollard et al., 1995; Taylor and Foster, 1996; Jackson et al., 2001; Taylor, 2004; Taylor and Pfannenstiel, 2008; Chen et al., 2010; Nelson and Jackson, 2013). Additionally, the high value of pollen as a food resource for natural enemies has been suggested by (Fiedler and Landis, 2007; Landis et al., 2000; Lundgren, 2009; Peterson et al., 2010;Messelink et al., 2014). Pollen is a highly effective resource for juvenile arthropods, e.g., predatory bugs and spiders, especially when prey numbers are extremely low. For example, it has been shown that when spiderlings were able to feed on pollen, they displayed considerable longevity compared with when they did not feed on pollen (Smith and Mommsen, 1984; Schmidt et al., 2013). Hence, it is beneficial to spiders and predatory bugs to adapt to different food resources under food scarcity (Smith and Mommsen, 1984; Cocuzza et al., 1997).

Therefore, the cultivation of plants rich in these substances can enhance the numbers of natural enemies in agroecosystems. However, it has been suggested that the attractiveness of flowering plants depends on many factors, including the number of suitable flowers, flower morphology, color, odor, timing of nectar production, whether the nectaries are exposed, and the extent of chemical stimulation (Tietjen and Rovner, 1982; Barth, 1993; Patt et al., 1997; Pare and Tumlinson, 1999; Heimpel and Jervis, 2005; Lavandero et al., 2005). Rocha-Filho and Rinaldi (2011) observed that the importance of inflorescences and its role to attract the crab spiders. Winkler (2005) recommended flowering plant species possessing numerous benefits for natural enemies and few benefits for pests as suitable insectary plants to enhance communities of natural enemies in open fields. Thus, studies of the relationships between insects and flowering plants are crucial to determine which plant species are effective for biological control.

The aim of the present study was to evaluate the attractiveness of several species of flowering plants for the spiders and insect natural enemies Syrphidae, Chalcidoidea, Ichneumonoidea, and Anthocoridae in a summer vegetable field in Japan. I chose nine candidates of flowering plants, yarrow, chamomile, petunia, sweet alyssum, mealy cup sage, spearmint, fennel, buckwheat, and dill, which are commonly planted as “companion plants” between vegetable crops. Previous studies indicated the importance of insectary plant species to attract the family Syrphidae and its role in the suppression of aphid populations (e.g., Kloen and Altieri, 1990; Colley and Luna, 2000; Martínez-Uña et al., 2013). Some studies discovered the attractiveness of some of these plants for predatory bugs (Cocuzza et al., 1997; Rizk et al., 2012; Kawamura et al., 2014). The attractiveness of such flowering plants to spiders in agricultural fields, however, is rarely reported.

1.2- MATERIALS AND METHODS

The 2013 study:

The study site was an organic vegetable field located in Kiire, Kagoshima City, Japan. In 2013, five flowering plant species, *Salvia farinacea* (mealy cup sage), *Mentha spicata* (spearmint), *Foeniculum vulgare* (fennel), *Fagopyrum esculentum* (buckwheat), and *Anethum graveolens* (dill), were compared for their attractiveness to spiders. The dimensions of the experimental field were 22 × 8 m. On 18 April, the field was divided into three rows, and plastic mulches were applied. The width of each row was 1 m, and the distance between rows was also 1 m. Each row contained five plots. On 16 May, the seedlings of each plant species were planted in the field. Each plant species was replicated twice in a completely randomized design, and each plot was 1 m^2 and contained three individuals of the same plant species.

Spiders were collected by the direct count from three individual plants of *S. farinacea*, *Fo. vulgare*, *Fa. esculentum,* and *An. graveolens* species. Spiders from *Me. spicata*, were collected from a 30×30-cm area, and the collection was repeated three times, because *Me. spicata* was widely spread and it was difficult to discriminate plant individuals. The time of collecting samples was 10 minutes per replicate.

The 2014 study:

Study site and design

A similar field trial was conducted in the same field in Kiire, from 23 June to 8 September 2014. On 11 May, the farm was divided into five rows, and subsequently plastic mulches were applied. The width of each row was 1 m, and the distance between rows was 0.5 m. Each row was divided into five plots 4 × 1 m in size. On 24 May, the seedlings of *S. farinacea*, *Matricaria recutita* (chamomile), *Achillea millefolium* (yarrow), *Petunia atkinsiana* (petunia), and *Alyssum maritimum* (sweet alyssum) were planted in the field, with each distributed five times using a completely randomized design. Each replicate covered an area of 1 m^2 (inside each plot); a distance of 3 m was maintained between replicates in the same row, and the distance between plots in different rows was 2 m. Nine plants were cultivated in each replicate, for a total of 45 plants in five replicates for each flowering plant species. The field was managed without chemical inputs and was hand weeded twice every month during the experiment.

Sampling

A sweeping net 36 cm in diameter with a 120-cm-long handle was used for collecting insects and spiders from the plant surfaces. The insects were collected weekly from each flowering plant species (on 23 and 30 June; 7, 14, 21, and 28 July; 4, 11, 18, and 25 August; and 1 and 8 September). Samples were collected using 15 double strokes (three double strokes from each replicate). After collection, the catch was emptied into glass jars and transferred directly to the laboratory for identification.

Statistical analysis

All data analysis was done with SPSS 15.0 for windows evaluation version. The data transformation converts to follow the normal distribution by using SPSS. Then, repeated measures analysis of variance (ANOVA), using the post hoc Tukey-Kramer test, was applied to compare the attractiveness among different flowering plant species.

1.3- RESULTS

Attractiveness to spiders (2013 study)

As shown in Figure 1, *S. farinacea*, *Me. spicata*, *Fo. vulgare*, *Fa. esculentum*, and *An. graveolens* attracted different spider families, *S. farinacea* and *Me. spicata* were the most attractive flowering plants for overall spiders, while *Fo. vulgare* and *Fa. esculentum* displayed intermediate attractiveness and *An. graveolens* was low in attractiveness.

Particularly, *S. farinacea* attracted significant numbers of crab spiders (Thomisidae), whereas the other flowering plants attracted small numbers of crab spiders. In contrast, *Me. spicata* attracted the highest numbers of Theridiidae, while other plants displayed low attractiveness to this spider family.

Attractiveness to spiders (2014 study)

The attractiveness of *S. farinacea*, *Ma. recutita*, *Ac. millefolium*, *P. atkinsiana*, and *Al. maritimum* to different families of spiders in 2014was shown in Figure 2. *S. farinacea* attracted large numbers of spiders, especially from the family Thomisidae, while the other plants attracted small numbers of individuals in this spider family. The numbers of spiders belonging to other families were low, and there were no significant differences among these plants in their attractiveness to the spiders. *S. farinacea*, *Ma. recutita*, *Ac. millefolium*, *P. atkinsiana*, and *Al. maritimum* attracted 11, 8, 10, 5, and 9 spider families, respectively; hence, *S. farinacea* attracted the highest diversity of spiders at the family level.

Attractiveness to insect natural enemies

The five flowering plant species, *S. farinacea*, *Ma. recutita*, *Ac. millefolium*, *P. atkinsiana*, and *Al. maritimum*, were compared for their attractiveness to the insect natural enemies ichneumon wasps, chalcid wasps, predatory bugs, and hoverflies.

S. farinacea attracted a large number of predatory bugs of the family Anthocoridae, whereas the other flowering plants were less attractive to these insects. *S. farinacea* and *Ac. millefolium* were the most attractive flowering plants for chalcid wasps. There were no significant differences in the abundances of Syrphidae among the plants (Fig. 3).

Relationship between seasonal abundance of arthropod natural enemies and flowering period

The seasonal abundance of the crab spider and other insect natural enemies on the flowering plants was shown in Table 1. The flowers of *Ma. recutita* and *P. atkinsiana* bloomed two weeks earlier than those of the other plants, and *Ma. recutita* finished blooming in mid-August; the end of its flowering stage was approximately one month earlier than that of the other plants in this study. *Al. maritimum* had the latest and shortest flowering period among the flowers studied in 2014. The crab spider and anthocorid predatory bugs increased markedly on *S. farinacea* as soon as its flowers bloomed. Other insect natural enemies on the other flowering plants increased in the middle of the flowering period.

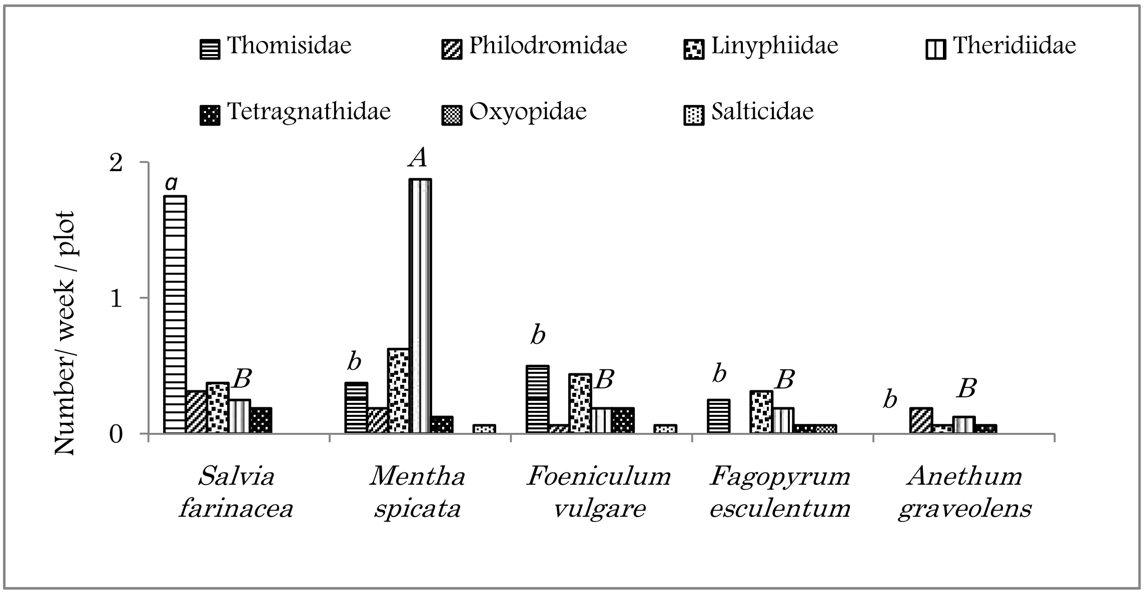

Fig. 1: Attractiveness of flowering plants to spider families in 2013. Different italic letters above the bars indicate significant differences between flowering plants according to Tukey-Kramer's multiple comparisons ($p < 0.05$).

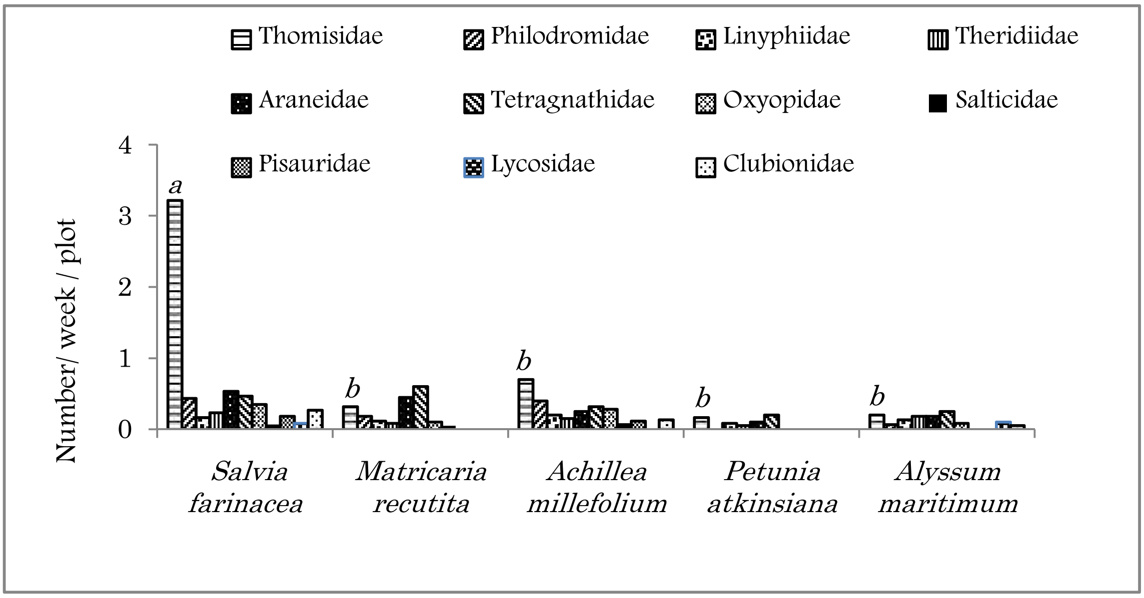

Fig. 2: Attractiveness of flowering plants to spider families in 2014. Different italic letters above the bars indicate significantly different density of spider collected between flowering plants according to Tukey-Kramer's multiple comparisons ($p < 0.05$).

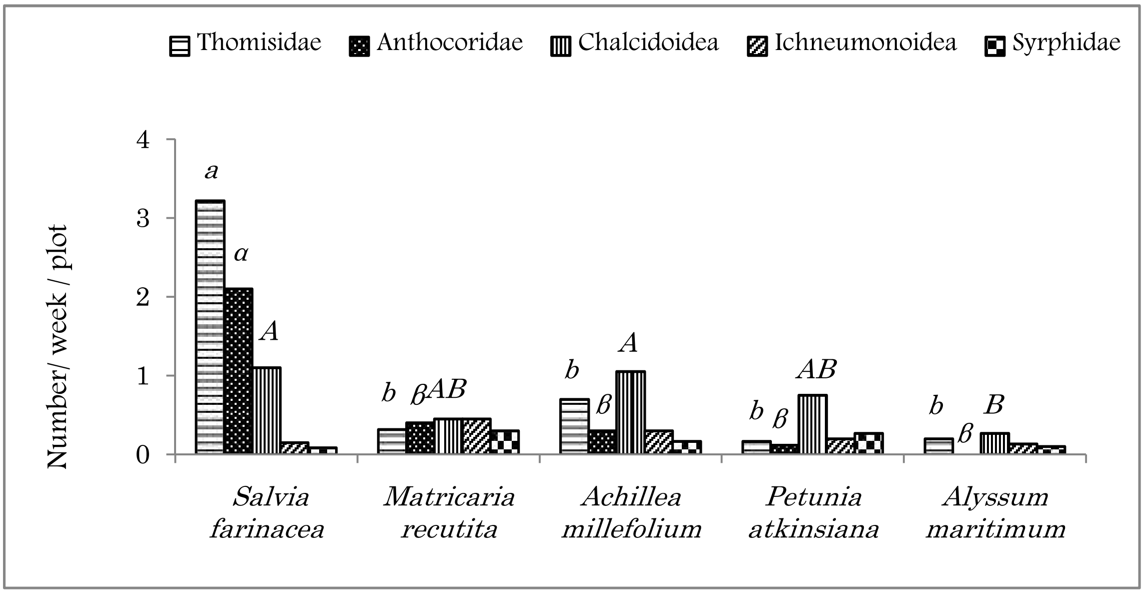

Fig. 3: Evaluation of attractiveness of flowering plants to different natural enemies in 2014. Different italic letters above bars indicate significantly different density of each spider family collected between flowering plants according to Tukey-Kramer's multiple comparisons (p<0.05).

Table 1: Spiders and insect natural enemies occurrence and flowering period in 2014.

Plant	Natural enemies	Jun. 23	30	Jul. 7	14	21	28	Aug. 4	11	18	25	Sept. 1	8
Salvia farinacea	Thomisidae	2		10	13	9	16	28	16	24	31	18	26
	Ichneumonoidea						1		2	2	1	3	
	Chalcidoidea		2		3	5	7	6	9	8	12	8	6
	Syrphidae					3						2	
	Anthocoridae		5	9	19	15	22	14	10	8	6	11	7
Main flowering period				♣	♣	♣	♣	♣	♣	♣	♣	♣	♣
Matricaria recutita	Thomisidae		1	3	3	5		3	4				
	Ichneumonoidea	2	6	4	3		5	4	2	1			
	Chalcidoidea	3	5	5	2	3		3	2	4			
	Syrphidae	1	4	2	1	3	4	2	1				
	Anthocoridae	1	4	3	2	4	7	2		1			
Main flowering period		♣	♣	♣	♣	♣	♣	♣	♣				
Achillea millefolium	Thomisidae			2	1	5		4	6	5	8	6	5
	Ichneumonoidea				1		3	4	1	3	5	3	
	Chalcidoidea	1	3		3	5	6	9	4	7	12	7	6
	Syrphidae				3	1	4	1				1	
	Anthocoridae					2	3	2	5	2	3	1	
Main flowering period				♣	♣	♣	♣	♣	♣	♣	♣	♣	
Petunia atkinsiana	Thomisidae				3	1`				2		4	
	Ichneumonoidea				2			1	3		2	4	
	Chalcidoidea			5	3	6	3	8	9	5	3	2	
	Syrphidae	3	2		4	2	1	3				1	
	Anthocoridae					1		4	2	1			
Main flowering period		♣	♣	♣	♣	♣	♣	♣	♣	♣	♣	♣	
Alyssum maritimum	Thomisidae					1		1		3	1	4	2
	Ichneumonoidea							1	3		2	2	
	Chalcidoidea					1	1		3	5		2	4
	Syrphidae					1			1	3		1	
	Anthocoridae												
Main flowering period							♣	♣	♣	♣	♣	♣	♣

1.4- DISCUSSION

In this study, specific flowering plants were identified as serving as food resources for the natural enemies of Thomisidae, Anthocoridae, Chalcidoidea, Syrphidae, and Ichneumonoidea.

Attractiveness to spiders

S. farinacea attracted a large number of thomisid spiders, which was an unexpected result, whereas the other flowering plants attracted fewer of these spiders. Several studies have indicated nectivory in various spider families, including species of crab spider (Beck and Connor, 1992; Taylor, 2004; Chen et al., 2010). Some previous studies suggested that the enhancement of crab spider numbers was affected by the inflorescence of the plants and that high inflorescence was the main factor in attracting crab spiders (Nentwig, 1993; Rocha-Filho and Rinaldi, 2011). Also, the exposed nectaries of *S. farinacea* probably supplied the natural enemies with more nectar than did the hidden nectaries. Additionally, during the first season, spearmint attracted large numbers of individuals of the Theridiidae family, which is consistent with observations by Rizk et al. (2012), who collected 98, 71, 73, and 73 individuals of spiders in spearmint, castor bean, roselle, and red pepper, respectively.

Me. spicata attracted more theridiid spiders than *S. farinacea* and the other flowering plants did in 2013. This attractiveness of *S. farinacea* to theridiid spiders was similar to that of *S. farinacea* to thomisid spiders. The attractiveness of *Me. spicata* to parasitic wasps and other predators has been reported in previous studies (Maingay et al., 1991; Jiménez et al., 1997; Al-Doghairi and Cranshaw, 1999). This study is the first to demonstrate the attraction of theridiid spiders to *Me. spicata*. Thus, *Me. spicata* is also a potential candidate as an insectary plant; however, *S. farinacea* is considered to be superior to *Me. spicata* as an insectary plant because the height of *S. farinacea* is approximately five times longer than *Me. spicata* so spiders can move easily from *S. farinacea* to the main crop, and the flowering period of *Me. spicata* is approximately one month shorter than that of *S. farinacea* (unpublished data). Also, the role of inflorescence in *S. farinacea* is considerably greater than that in the other plants in this study, because the composition of its flowers appears to be highly suitable for several natural enemies.

Attractiveness experiment for insect natural enemies

Highly significant numbers of chalcid wasps and anthocorid bugs were attracted by *S. farinacea* and *Ac. millefolium* during only the flowering period. With respect to chalcid wasps, one possible explanation has been proposed, namely that flowering plants may have independently evolved spectral signals that maximize color discrimination by Hymenoptera (Dyer et al., 2012). The longevity and fecundity of many parasitoids were affected by feeding on pollen (Powell, 1986; Johanowicz and Mitchell, 2000). Also, among flowering plants, it was very clear that the period of flowering was associated with an abundance of natural enemies (Table 1).

The predatory bugs of family Anthocoridae were attracted in significant numbers by *S. farinacea*, whereas the other flowering plants tested here demonstrated very low attractiveness to these bugs. The attractiveness of *S. farinacea* to anthocorid bugs corresponded well with the results of a previous study (Kawamura et al., 2014). The predatory bug *Orius* sp. was shown to depend on the distribution of its prey, including thrips (Elimem and Chermiti, 2013). One of the most common pests to *S. farinacea* is the family Thripidae (Pobożniak and Sobolewska, 2011). Moreover, in the experimental field, large numbers of non-pest thrips, *T. coloratus* Schmutz, were attracted by the flower of *S. farinacea* (unpublished data), and the fecundity of an anthocorid bug was enhanced when its diet included pollen (Cocuzza et al., 1997).

No differences were detected among the five flowering plants in their attractiveness to Syrphide. These results did not agree with findings reported by Sadeghi (2008), who reported that *Ma. recutita* and *P. atkinsiana* were the most attractive flowering plants to Syrphidae. The relative attractiveness of insectary plant species depends on numerous factors including the availability of suitable flowers, the distance to these resources, the type and intensity of chemical stimulation, flower colors, the availability of pollen and nectar, the timing of the nectar production, and the availability of refuges (Tietjen and Rovner, 1982; Barth, 1993; Patt et al., 1997; Pare and Tumlinson, 1999; Heimpel and Jervis, 2005; Lavandero et al., 2005). On the other hand, syrphid flies and chalcid wasps can clearly distinguish and easily choose their favorite flowers from various nectar resources (Leius, 1960; Macleod, 1992; Cowgill et al., 1993; Lovei et al., 1993; Lunau and Wacht, 1994; Colley and Luna, 2000; Martínez-Uña et al., 2013). Due to limitations in the data gathered, I could not determine why were unable to detect differences in the numbers of the syrphid hoverfly.

Relationship between the seasonal abundance of arthropod natural enemies and flowering periods

The abundance of flowering plants, the competition between various insectary plants, and their distribution all played important roles in the attractiveness of pollinators (Thomson, 1981). Rocha-Filho and Rinaldi (2011**)** suggested that inflorescences affected the attraction of crab spiders. Additionally, the present study showed that *S. farinacea* attracted high numbers of different natural enemies, including crab spiders (Thomisidae) and predatory bugs (Anthocoridae) as predators and chalcid wasps as parasitoids, compared with the other flowering plants examined here. Especially, crab spiders and predatory bugs were attracted in highly significant numbers by *S. farinacea*. Thus, planting *S. farinacea* can be an effective tool for biological control in agricultural fields**.**

CHAPTER 2:

The effect of organic fertilizers and flowering plants on sheet-web and wolf spider populations (Araneae: Lycosidae and Linyphiidae) and its importance for pest control

CHAPTER 2: The effect of organic fertilizers and flowering plants on sheet-web and wolf spider populations (Araneae: Lycosidae and Linyphiidae) and its importance for pest control

2.1- INTRODUCTION

To produce organic vegetables and other crops, it is essential to utilize indigenous natural enemies of pests in combination with strategic cultivation techniques, such as planting flowering plants that attract beneficial insects and applying organic fertilizers. Spiders are effective natural predators in field crops, but their effects depend on their densities in agroecosystems (Riechert and Lawrence, 1997; Marc et al., 1999; Landis et al., 2000; Symondson et al., 2002; Schmidt et al., 2003). They kill and consume a large number of prey daily (Riechert and Lawrence, 1997; Riechert and Maupin 1998). Hunting spiders decreased numbers of herbivorous Coleoptera in an old field in Tennessee (Riechert and Lawrence, 1997).

The quality of organic materials and the plant structure are very important to increase the soil organism densities (Yeates et al., 1997). Besides, the diet for most Collembola species is soil fungi or decaying of plant materials (Verma and Paliwal, 2010). Also, organic fertilization by manure application improves soil quality and structure, and it enhanced the populations of saprophagous insects such as springtails (Collembola) and midges (Diptera)(Alderweireldt, 1994; Chen and Wise, 1999; Nyffeler, 1999; Axelsen and Kristensen, 2000; Pfiffner and Luka, 2003). These prey are very important for the survival of their predators (Alderweireldt, 1994; Chen and Wise, 1999; Nyffeler, 1999; Axelsen and Kristensen, 2000). Hendawy and Abul-Fadl (2004) have reported greater densities of lycosid and linyphiid spiders in organic fertilization fields than in chemical fertilization fields. Also, Birkhofer et al. (2008) indicated that organic fertilizer had a positive effect on the ground-dwelling spiders. Additionally, numbers of sheet-web weavers spiders, (Linyphidae) had a positive respond to Collembola (Birkhofer, 2007). The organic fertilizers treatment supported species richness of weeds, numbers of earthworm and density and diversity of some invertebrates higher than chemical fertilizers treatment (Dicks et al., 2013). Öberg (2007) reported that the densities of lycosid and linyphiid spiders increased in response to organic treatment. Lycosid and linyphiid (Araneae) spiders are commonly found in arable land in central and northwestern Europe (Toft, 1989; Feber et al., 1998; Samu and Szinetár, 2002; Pfiffner and Luka, 2003; Clough et al., 2005; Öberg and Ekbom, 2006), and play an essential role in suppressing aphid populations (Luczak, 1979; Nyffeler and Benz, 1987; Mansour and Heimbach, 1993; Lang, 2003; Öberg and Ekbom, 2006). On the other hand, Linyphiid spiders can be dispersed by the wind, whereas lycosid spiders walk (Luczak, 1979; Weyman et al., 2002). Linyphiid spiders occasionally caught Coleoptera as prey (Nentwig, 1983). Alderweireldt (1994) indicated that sheet-web weavers depend on web captured prey or from time to time by the direct hunt.

Proper habitat management can enhance the populations of natural enemies for biological control in agricultural ecosystems (Alomar et al., 2006; Bianchi et al., 2006). Providing good refuges can enhance the density of spiders (Sunderland and Samu, 2000). Some studies demonstrated that the spider populations increased and aphid populations decreased when the wheat field contained strips of flowering plants (Jmhasly and Nentwig, 1995). The strips of flowering plants play an essential role in biological control by enhancing the predators and the alternative prey densities (Frank, 2003). Furthermore, the Lycosid spiders can feed on thrips (Sahito et al., 2013). Likewise, some scientists indicated that high numbers of Thysanoptera were captured at the web of linyphiid spiders and they expected that it was a suitable prey for spiders (Harwood et al., 2003). Marc et al. (1999) indicated that it is necessary to manage the environment (i.e., habitat quality) to enhance the communities of spiders. Spider communities are very sensitive to sources of environmental changes, such as soil pollutants and chemical pesticides. Diverse habitats provide the abundance of various food resources and thus can increase the populations of natural enemies (Hatley and Macmahon, 1980; Landis et al., 2000; Jonsson et al., 2008).

Flowering plants such as mealy cup sage (*S. farinacea*) can play an essential role in enhancing the natural enemies of crab spiders, predatory bugs, and chalcidoid wasps (El-Nabawy et al., 2015). Peterson et al. (2010) and Carrel et al. (2000) reported that Linyphiidae also feeds on pollen. Many scientists have reported the value of pollen as a food resource for natural enemies (Landis et al., 2000; Jackson et al., 2001; Fiedler and Landis, 2007; Lundgren, 2009; Peterson et al., 2010; Messelink et al., 2014). Pollen is an excellent food resource also for spiders, particularly for spiderlings, when prey populations are insufficient. Pollen increases the longevity of spiderlings (Vogelei and Greissl, 1989).

Many insect pests attack the family Solanaceae, *Henosepilachnavigintioctomaculata* (the large 28-spotted ladybird beetle) (Coleoptera) is considered as a serious pest of eggplant in Japan (Nakamura, 1987). The size of *H. Vigintioctomaculata*, is greater than other ladybird beetle species and this species moves slowly, and when anything disturbing, it escapes to the ground surface (Kalaiyarasi and Ananthi, 2015). Then it can fly for just a short distance (Hao et al., 2006).

In this study, I evaluated the effects of field treatments on spider populations. I tested the effects of organic versus chemical fertilizers, and then the impact of growing flowering plants in an organic fertilizer plots to attract spiders.

2.2- MATERIALS AND METHODS

Study site

The experimental field was located in Kiire, Kagoshima prefecture, Japan, and experiments were carried out during the summers from 10 April till 19 September 2013 and from 18 April till 18 Sept 2014. The total area of the experimental farm was 800 m^2. The farm was divided into two treatment plots (treated with organic or chemical fertilizers) with two replicates. Each replicate was 150 m^2, consisting of five rows (10 m × 1 m) in the center of the field. The rows were covered with black plastic mulch. Each row was planted with ten plants of eggplant (*Solanum melongena* L.) seedlings, for a total of 50 plants in each replicate. Planting was carried out on 6 May 2013 and 25 May 2014.

Table 2: Chemical and organic fertilization in 2014.

Treatment	Fertilizers type	Weight (kg)	N%	P%	K%
Chemical fertilization per 3a	Chemical fertilizers	144	48	48	48
Organic fertilization per 5a	Oilcake	200	18	8	4
	Microbe fertilizer	200	8	16	8
	Cattle manure	1000	22	28	30
	Total	1400	48	52	42

In addition, each replicate was surrounded by sorghum (*Sorghum bicolor* [L.] Moench.) as a wind buffer. An area of 200 m^2in the middle of the field separated the replicates. The quantities and composition of organic and chemical fertilizers are shown in Table 2.

The experimental design was similar in 2013 and 2014, except that in 2014, some flowering plants were added to the organic plots. Three species, namely, mealy cup sage (*Salvia farinacea*), spearmint (*Mentha spicata*), and basil (*Ocimum basilicum*), were planted in the organic plot in three alternating rows. A fourth plant species (*Cosmos bipinnatus*) was intercropped between eggplants. Weeding was done manually twice a month, using a brush cutter. No chemical pesticides were applied throughout the cultivating seasons. For meteorological data, I used the local weather data published by Japan Meteorological Agency.

Sampling

A) Pitfall traps

Pitfall traps were used to collect ground-dwelling spiders and Collembola. Each trap pot was 10 cm in diameter and 7.5 cm deep and was buried with its top just at the soil surface. Approximately 40 ml propylene glycol was added to trap pots to prevent dead spiders and insects from decaying. A plastic rain roof cover was placed over the trap, about 5 cm above the trap. Eight traps were used, with two in each replicate. The traps were used twice a month and the samples were emptied into plastic jars and transferred directly to the laboratory for analysis. The collected spiders and Collembola were counted and identified under a binocular microscope and preserved in 70% alcohol in glass vials. Spiders were identified using the appropriate keys developed by Kaston (1953) and Chikuni (1989).

B) Population of two insect pests, cotton aphid and 28-spotted ladybird beetle in 2014

Ten eggplant leaves were randomly collected weekly from each plot. They were preserved individually in plastic bags, and any aphids on the leaves were identified and counted. The aphid populations in this study consisted of *Myzus persicae* and *Aphis gossypii*.

This study confirmed the number of 28-spotted ladybird beetle, *Henosepilachna vigintioctomaculata,* by a direct count in the field. Each replicate was divided into two parts, the eastern and western halves. In total, 10 leaves were selected randomly in each half of the replicate, for a total of 20 leaves per replicate. The beetles were counted and the counts were recorded.

By ten double strokes of sweep net (36-cm in diameter and 90-cm long handle) from each plot, insect pests were collected to measure how treatment affected by habitat structure. After collections, each catch was kept in a glass jar until identified.

Data analysis

Repeated measures ANOVA was used to determine the effect of fertilizer type on the numbers of lycosid and linyphiid spiders (SPSS, 2006). In addition, the correlations between spider numbers and numbers of some alternative prey were analyzed by the Pearson correlation coefficient. The analysis used the four pitfall traps from each treatment and served as replicates and the different dates as repeated measures to improve the significance of experimental results.

2.3-RESULTS

Family composition of spiders collected by pitfall traps

During the 2013 and 2014 seasons, 961 spiders, representing 12 families, were captured with pitfall traps (Table 3). Lycosidae and Linyphiidae spiders represented 68.76–59.4 % and 23.94–28.85%, during the two seasons, respectively, of the total number of trapped spiders. These two spider families were the primary ground-dwelling predators in the eggplant field.

Fluctuations of populations of linyphiid and lycosid spiders, and Collembola

In 2013, the number of linyphiid spiders differed between organic and chemical fertilizer plots, particularly in mid-July, after that the population in each plot declined until the end of the season (Fig. 4a; $F_{1,6} = 6.92$, $p < 0.05$). During the seasonal transition of 2013, the differences in Collembola counts were identified between the two plot types ($F_{1,6} = 87.04$, $p < 0.01$). Counts were higher in plots treated with organic fertilizer, particularly early in the season, from mid-May until mid-July. The counts were very low from the end of July to the end of summer. The fluctuations of the populations of linyphiid spiders and Collembola were very similar after mid-June. But, there were not higher numbers of lycosid spiders in organic fertilizer plots than chemical fertilizer plots (Fig. 4b; $F_{1,6}$ =1.66, p=0. 22).

Table 3: Total number of spiders (and the families to which they belong) collected during 2013 and 2014 in different treatment plots.

Spider family	Total no. trapped in 2013			Total no. trapped in 2014		
	Organic	Chemical	%	Organic	Chemical	%
Lycosidae	178	161	68.76	165	113	59.4
Linyphiidae	71	47	23.94	83	52	28.85
Theridiidae	12	11	4.67	15	17	6.84
Gnaphosidae	1	2	0.61	3	1	0.86
Clubionidae	0	0	0	0	1	0.21
Ctenidae	3	1	0.81	0	0	0
Hahniidae	1	0	0.2	1	0	0.21
Pisauridae	0	0	0	4	1	1.07
Salticidae	1	0	0.2	2	1	0.64
Nesticidae	0	0	0	1	0	0.21
Oxyiopidae	1	0	0.2	2	0	0.43
Tetragnathidae	2	1	0.61	5	1	1.28
Total number	270	223	100	281	187	100

In 2014, there were large differences in the numbers of both spider families and Collembola between the two types of plot (Fig. 5; Linyphiidae, $F_{1,6}$ =31.68, $p < 0.01$; Lycosidae, $F_{1,6}$ =10.82, $p < 0.05$; Collembola, $F_{1,6}$ =41.36, $p < 0.01$). The number of linyphiid spiders was higher in organic plots than in chemical plots from mid-June to mid-July, and then the population in each plot declined until the end of the season. The counts of Collembola were similarly high in organic plots from early June to mid-July. Clear differences between organic and chemical treatments were observed during June, and from late July to late August. The seasonal fluctuations of lycosid spider counts differed from Collembola counts.

Fluctuations of the populations of cotton aphid, 28 spotted ladybird beetle, Collembola and *Thrips coloratus* Schmutz in 2014

An acute outbreak of aphids was observed during June in the plot treated with chemical fertilizers while, the differences between the two treatments were not significant (Fig. 6a, $F_{1,2}$ =1.66, p=0. 33). In addition, there were fewer 28-spotted ladybird beetle numbers in organic fertilizer with flowering plants treatment than in chemical treatment, especially in July and the early part of September (Fig. 6b, $F_{1,6}$ =8.45, $p < 0.05$). The population density of pests in the organic treatment remained low, compared to the chemical treatment, throughout the season. On the other hand, the numbers of non-pest *T. coloratus* Schmutz were significantly higher in the organic fertilizers with flowering plants plot than the chemical fertilizer plot especially during August (Fig. 6c, $F_{1,2}$ =169.92, $p < 0.01$).

Correlation between the numbers of lycosid and linyphiid spiders and insects

To identify different factors fluctuating spider population density, I studied the Pearson correlation coefficient between spiders and prey insects. In 2013, linyphiid spider counts were positively influenced by Collembola ($r = 0.48$, $p < 0.01$). Additionally, the correlation between lycosid spider and Collembola is significantly positive ($r = 0.26$, $p < 0.05$). Similarly, in 2014 Collembola affected the numbers of Lycosidae ($r = 0.34$, $p < 0.01$) and thrips positively affected numbers of Linyphiidae ($r = 0.35$, $p < 0.5$).

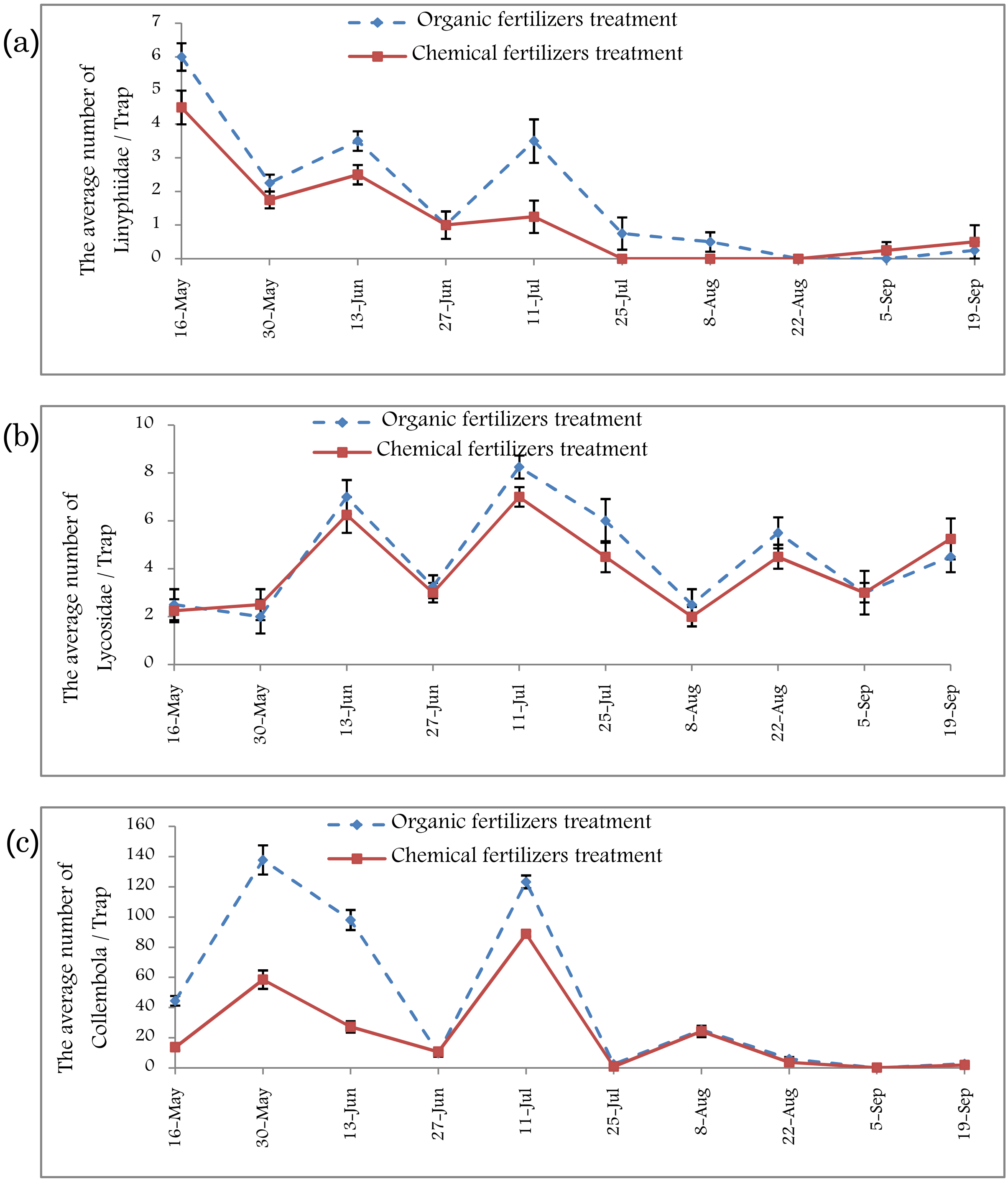

Fig. 4: Fluctuations of the populations of Linyphiidae (a) and Lycosidae spiders (b) and Collembola (c) captured by pitfall traps, according to plot type in 2013.

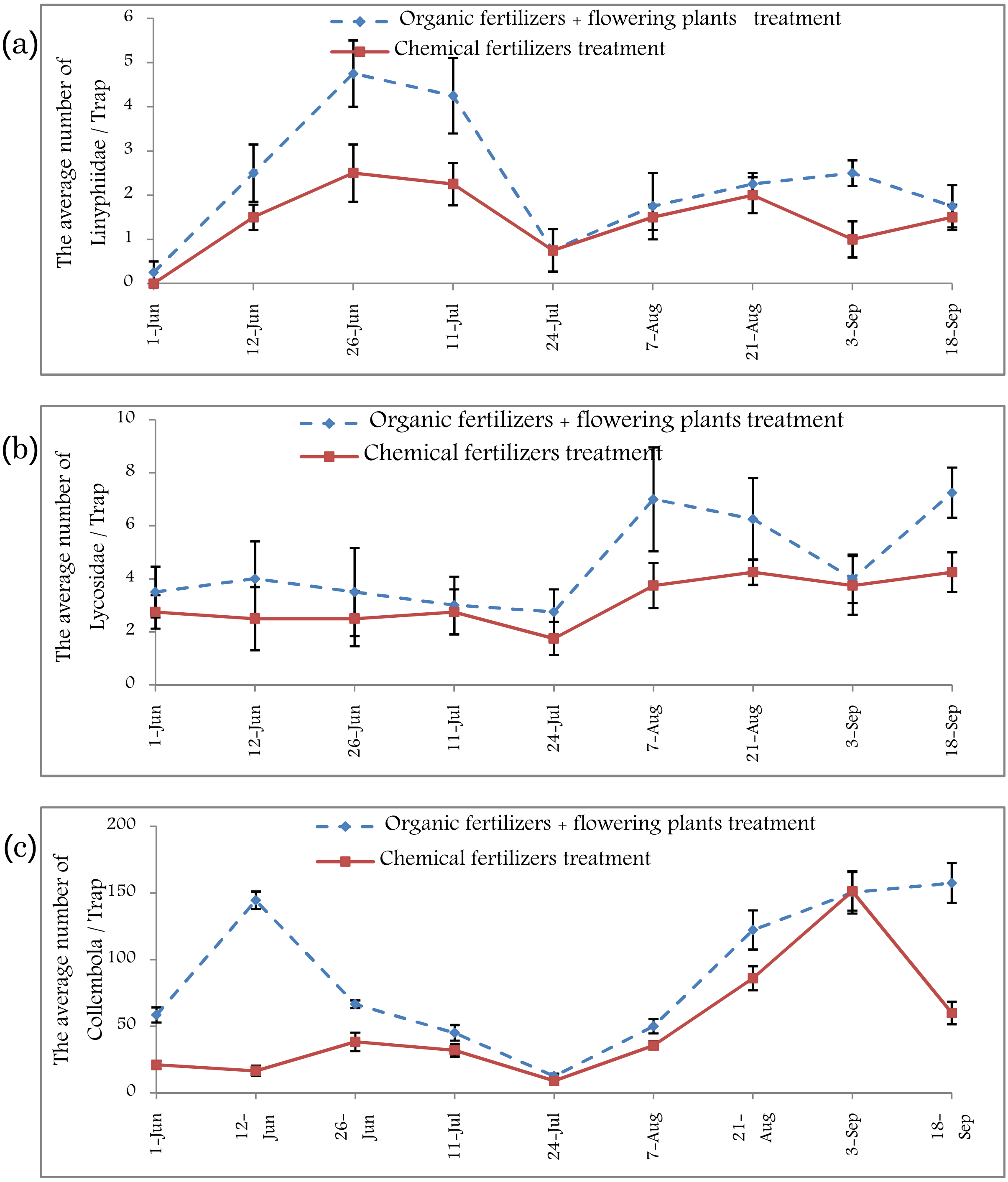

Fig. 5: Fluctuations of the populations of Linyphiidae (a) and Lycosidaespiders(b) and Collembola (c) captured by pitfall traps, according to plot type in 2014.

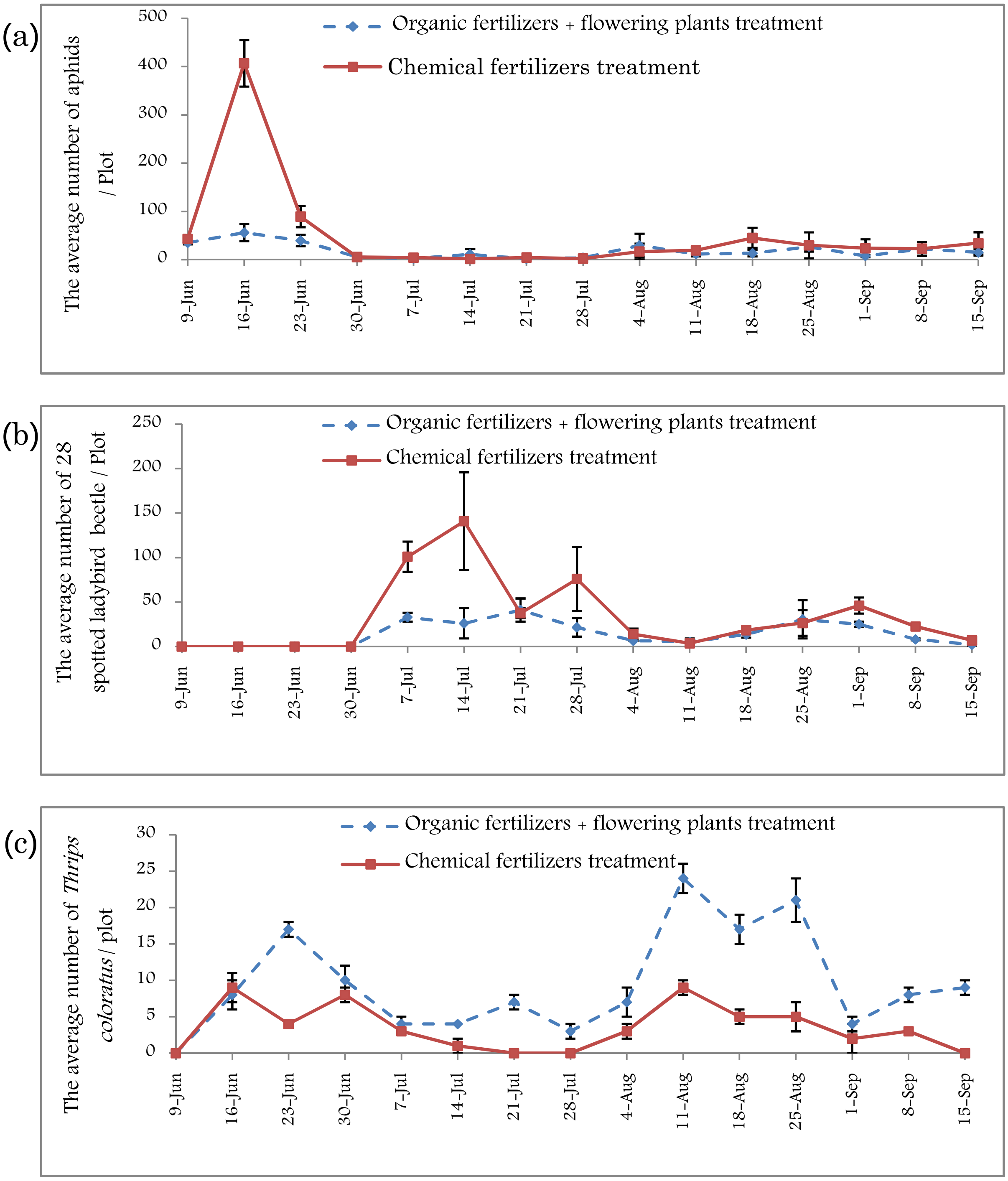

Fig. 6: Fluctuations of the populations of aphids (a) (counted by microscope), 24 spotted ladybird beetles (b) (counted directly) and *T. coloratus* Schmutz. (c) (collected by sweeping net) between the organic and chemical treatments in 2014.

2.4- DISCUSSION

Factors contributing to increase the linyphiid and lycosid spiders densities

Various factors can contribute to increases in spider population density (Pfiffner and Luka, 2003). This study compared spider and some insect populations between organic and chemically treated field plots in 2013 and 2014 separately. The chemical treatments were the same in both years, whereas the organic treatments differed. In 2013, the organic plot was treated solely with organic fertilizer, whereas in 2014 it was treated with the organic fertilizer and also flowering plants were added to the plot.

In 2013, organic fertilizer treatment enhanced the density of Linyphiidae spiders and Collembola, and there was a significant positive correlation between linyphiid spiders and Collembola counts. Birkhofer (2007) showed that sheet-web weavers spiders, Linyphiidae, had a positive relationship with alternative or non-pest prey (Collembola). Pfiffner and Luka (2003) found the numbers of saprophagous insects such as the Collembola increased by using organic fertilizer. Moreover, Collembola species feed on soil fungus or some of the plant decaying material (Verma and Paliwal, 2010) and the organic fertilizers are very essential to rise the soil organism densities (Yeates et al., 1997).

In 2014, differences in lycosid and linyphiid spider counts for the two treatments were evident, there are many reasons that may be responsible for boosted spider density in the organic fertilizers and flowering plants treatment: (1) Organic fertilizers can have indirect effects to enhance the spider population by increasing the alternative prey density. Moreover, organic fertilization can improve the soil structure and microclimate, which is very important for saprophagous insects such as Collembola (Alderweireldt, 1994; Chen and Wise, 1999; Nyffeler, 1999; Axelsen and Kristensen, 2000; Pfiffner and Luka, 2003). The present results are consistent with these previous studies because a positive relationship was found between the numbers of Collembola and Lycosidae. Also, a positive relationship was found between the numbers of Collembola and linyphiid spider in 2013 and between Collembola and lycosid spiders in both years. Birkhofer (2007) showed the importance of Collembola to linyphiid spider as alternative prey. In addition, Dicks et al. (2013) indicated that weeds richness and density and diversity of some invertebrates increased in the organic fertilizer treatment higher than the chemical fertilizer treatment. (2) The flowering plants may provide suitable refuges for the lycosid and linyphiid spiders. Sunderland and Samu (2000) found the density of spiders can enhance by increasing suitable refuges. Malumbres-Olarte et al. (2012) reported a positive relationship between the number of lycosid spiders and the diversity of plant species. (3) Flowering plants change the habitat composition or may enhance the density of alternative prey and this point was confirmed by Frank (2003) he found that the strips of flowering plants are very important to enhance the numbers of alternative prey and also, Landis et al. (2000) indicated that change the habitat composition can enhance the alternative prey of spiders. This study showed that during

August high numbers of non-pest *T. coloratus* Schmutz were attracted in the organic fertilizers and flowering plants treatment and maybe they played an important role to increase the numbers of spiders. Furthermore, high numbers of non-pest thrips (*T. coloratus* Schmutz) were attracted to *Salvia farinacea* Bench (El-Nabawy, unpublished data). Sahito et al. (2013) found that family Lycosidae, *Hippasa agelenoides* feed on thrips. Also, Harwood et al. (2003) reported that many individuals of Thysanoptera, were found at web-sites of the linyphiid spider, and the authors expected that Thysanoptera was an efficient prey for linyphiid spider. (4) Flowering plants also can provide spiders with alternative food resources (pollen) and some previous studies have indicated the value of pollen in the good ecosystem as an alternative food resource for linyphiid spider and its role to raise the fecundity (Peterson et al., 2010). Also, Vogelei and Greissl (1989) reported that pollen can enhance the spiderlings longevity.

Populations of insects in organic and chemical plots in 2014

In June 2014, the number of Linyphiidae spiders in the organic fertilizer plots was about twice as high as that in the chemical fertilizer plots which led to the aphid counts in the chemical treatment plots were temporarily higher than those in the organic plots. While, from the end of June until the end of August the number of Collembola and linyphiid spiders had the same trend. Thus, the higher density of linyphiid spiders might be maintained by partially changing the main prey from aphids to Collembola and *T. coloratus* Schmutz. Interestingly, the numbers of aphids (Fig. 6a) in the organic fertilizers with flowering plants treatment were clearly higher than the chemical fertilizers treatment especially in the first of the season during June while by using statistical analysis no significant differences were detected between the organic fertilizers with flowering plants treatment and the chemical fertilizers treatment. These results are not consistent with those reported by Luczak (1979), Nyffeler and Benz (1987), Mansour and Heimbach (1993), and Lang (2003), all of whom reported that lycosid and linyphiid spiders can affect aphid populations.

The numbers of 28-spotted ladybird beetle were lower in organic plots with flowering plants treatment comparing with chemical fertilizers treatment and I think the first reason is that increases in lycosid spiders may suppress populations of the beetles. Generally, most lycosid spiders dwell on the soil surface, and the 28-spotted ladybird beetle inhabits leaf surfaces. Many of the 28 spotted ladybird beetles were collected in my pitfall traps (unpublished data), and especially this species is bigger than other ladybird beetles and moves slowly, and if any disturbance happens, it falls to the ground (Kalaiyarasi and Ananthi, 2015) and I believe that the main disturbing things were rain and wind because Japan is also the country exposed to a tropical storm (typhoon) moreover, Japan considers generally a rainy country and the rainy days numbers during this study were 10, 6, 12 and 8 days during June, July, August and September, respectively. So I think that lycosid spiders could easily feed on this beetle. Some previous studies have also indicated that lycosid spiders can feed on coleopteran insect pests. Uetz et al. (1992) reported that *Lycosa* spiders prey on

Dermestes beetle, and Maloney (2002) found that lycosid spiders consume blueberry flea beetles (Chrysomelidae). According to Riechert and Lawrence (1997), Hunting spiders reduced phytophagous populations Coleoptera in Tennessee. Also, I think that the second reason is the significant numbers of linyphiid spider in organic fertilizers and flowering plants treatment may suppress the numbers 28-spotted ladybird beetle because they can move and spread easily by the wind (Luczak, 1979; Weyman et al., 2002). Nentwig (1983) showed that Coleoptera was seldom captured by linyphiid spiders. Also, Linyphiid spiders capture prey on their web or stopping periodically to hunt prey (Alderweireldt, 1994).

In conclusion, the use of organic fertilizers and predatory-attracting plants enhance the density of lycosid and linyphiid spiders and their alternative prey in Japan. And these results suggest that high densities of these spiders suppress some of the insect pests in eggplant fields.

CHAPTER 3:

Evaluation of a ground dwelling spider, Pardosa sp. (Lycosidae) as an effective predator against cotton aphid by PCR-based gut contents analysis

CHAPTER 3- Evaluation of a ground dwelling spider, *Pardosa* sp. (Lycosidae) as an effective predator against cotton aphid by PCR-based gut contents analysis

3.1- INTRODUCTION

Spiders are a large group with more than 30000 species in the world and they inhabit every terrestrial habitat in the ecosystems. They are very important predators because they can suppress the numbers of insect pests. Many species of genus *Pardosa* (Lycosidae) are regularly found in high numbers in the agroecosystems (Schmidt et al., 2005). On the other hand, the cotton aphid (*A. gossypii* Glover) is a serious insect pest because it has a wide range hosts and because of the direct and the indirect effects. The direct damage is feeding on the host which can destroy the plant, also the production of the plant will decrease before the death of the plant (Cartwright, 1992). The other kind of damage includes the indirect damage through aphid honeydew and transmission of many plant viruses. Moreover, honeydew is very harmful to the plants because it increases the nutrients for fungi that reduce photosynthesis by blocking sunlight. *Pardosa* spider might be an essential predator in controlling *Rhopalosiphum padi* populations below the economic threshold (Öberg and Ekbom, 2006).

Spider predation is too hard to see and to evaluate directly because of: the small body size of spiders and aphids, the mobility of spiders from place to place or inhabiting under vegetation or mulch. Generally, gut investigations have fundamentally been performed by analyzation or identification of prey protein remains with serological and electrophoretic strategies (Harwood and Obrycki, 2005). Recently, polymerase chain reaction (PCR) has become a widely used tool for detecting prey DNA remains in the guts of predators to understand the trophic interactions in the agroecosystems (Sunderland et al., 2005; King et al., 2008; Pompanon et al., 2012; Traugott et al., 2013; Gariépy et al., 2007). More than hundred previous studies have used some of the gut content investigation for different predators to evaluate predation of aphids (Harwood and Obrycki, 2005). The main purpose of this study is to use the polymerase chain reaction (PCR) to detect DNA of *A. gossypii* from spider guts to evaluate the ground dwelling spider as an effective predator of cotton aphid under field conditions.

3.2- MATERIALS AND METHODS

Spiders and aphids

In the present study, adult males and females of *Pardosa* sp. (Araneae: Lycosidae) and *Aphis* gossypii (Hemiptera: Aphididae) were collected from the eggplant field located in Kiire, Kagoshima City, Japan during June and July 2015. An open Petri dish was thrown over the running spider (Lycosidae), which was carefully caught in the Petri dish and then it was closed carefully. Of them, 80 individuals of the spider were preserved directly in absolute ethanol (99.5%), under 20 °C until DNA extraction for field samples. The aphid colony was kept under laboratory conditions at 25 °C and photoperiod 16L:8 D and used within 3 days after collecting from the field. Then living spiders for the primer choice experiment were enclosed in aerated Petri dishes with moistened filter paper and starved for four days.

DNA extraction

Before extracting DNA, the spider samples were dissected and picked up just their guts to avoid contamination from material attached to the outer surface of their body. The extraction of DNA from these guts of spiders was conducted by the procedure of Cruickshank et al. (2001) with DNA Blood &Tissue Kit (Qiagen).

Primer choice experiment

The DNA of cotton aphid was used to evaluate the three primer sets (Table 4) and to determine which one can detect clear bands of cotton aphid.Also, after four days starvation for lycosid spiders, ten individuals were transferred to clean Petri dishes with moistened filter paper. Then, each individual was offered one individual of cotton aphid (4^{th} instar and adult). Spiders were observed until the feeding started (as the positive control). On the other hand, ten individuals of the spider after four days starvation were prepared as the negative control. The guts of these spiders were picked up and used for DNA extraction of the primer choice experiment.

The three primer sets (Table 4), which were specific for cotton aphid, were designated based on the DNA sequences of CO1 on GenBank (accession No. GU591547) with primer 3 (Rozen and Skaletsky, 1996-1998) to amplify target fragments. These primers were tested to determine the suitable one to detect the DNA of cotton aphid effectively.

PCR-based gut content analysis of the field samples

Eighty field samples of the spider, *Pardosa* sp. were preserved in absolute ethanol (99.5%) and they were used to detect aphid DNA remains (*mt* CO1: 132bp) in spider gut content with the used primer set, AphF1 and AphR1(Table 4).

When investigating the *Pardosa* spider samples for the presence of *A. gossypii* DNA remains, the PCR was used according to Chen et al.

(2000) with some modifications. The PCR amplifications were done in total volumes (25 µl) containing 1.5 mM of dNTP, 16.8 distilled water 1.5µM of each primer (forward and reverse) and 0.2 of TaqTM polymerase (Takara) in 10× 2.5 buffer and 1µl of DNA extract. The optimal annealing temperatures for the three primers were assessed by PCR. Amplification conditions were: initial denaturation at 94°C for 1 min; 25 cycles of 94°C for 30s, 50°C for 30s, 72°C for 1 min; and a final elongation step at 72°C for 10min.The products of PCR were visualized with UV light on 2% agarose gels stained with ethidium bromide after electrophoresis in the TBE buffer (abuffer solutioncontaining a mixture of: Tris base, boric acidandEDTA).

Table 4: Primer sequences and expected product sizes (bp) from the targeted gene, mitochondrial CO1.

Primer name	Target	Position	Sequences
AphF1 (forward)	132-bp	353-373	TCA GTA GAC TTA ACT ATT TTT
AphR1 (reverse)		484-465	TGG AAA TAG AGG AAT TTG AT
AphF2 (forward)	87-bp	397-416	ATC AAT TTT AGG AGC AAT TA
AphR2 (reverse)		483-464	GGA AAT AGA GGA ATT TGA TT
AphF3 (forward)	228-bp	353-372	TCA GTA GAC TTA ACT ATT TT
AphR3 (reverse)		580-561	TGT ATT TAA ATT TCG ATC TG

3.3- RESULTS

Primer choice experiment

The DNA of cotton aphid was amplified and detected with clear bands(Fig. 7), lane 1, 2 and 3 by using the three previous primers Table 4. While lane 1 and 3 (132 bp and 228 bp) were clearer than lane 2 (87-bp). On the other hand, as shown in Fig. 8 it was possible to detect the DNA remains (132-bp) of cotton aphid from *Pardosa* spider after feeding on one individual of cotton aphid.

PCR-based gut content analysis of the field samples

As shown in Figure 9, the nine tested field samples of spiders, lane 1 to 9. (Fig. 9a), lane 2 and 6 are positive for cotton aphid; (Fig. 9b), lane 13 is positive, and (Fig. 9c), lane 22, 26 and 27 are positive samples (contained DNA of cotton aphid) while other samples were negative for the DNA remains of cotton aphid. And the present study, by using polymerase chain reaction, the total tested spiders were 80 individuals (they were collected from the eggplant field) and 13 samples of them were positives for aphid DNA remains from spider guts.

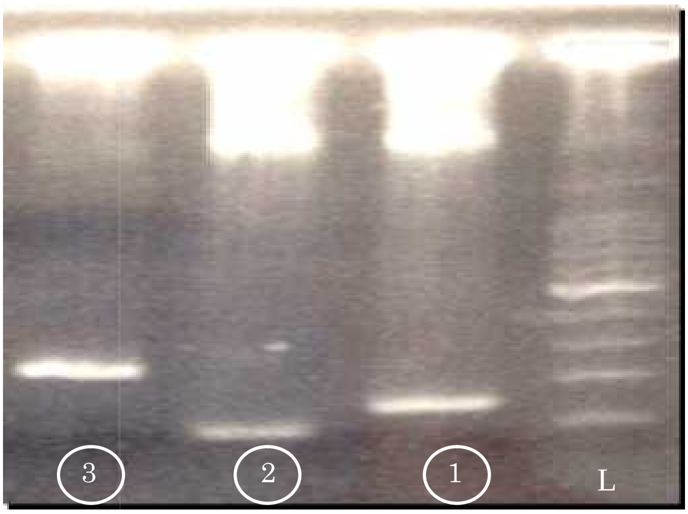

Fig. 7: Electrophoresis for primers evaluation. Lane L, ladder; lane 1, Aph1 primer set (132-bp) of *A. gossypii*; lane 2, Aph2 (87-bp);lane 3, Aph3 (228-bp).

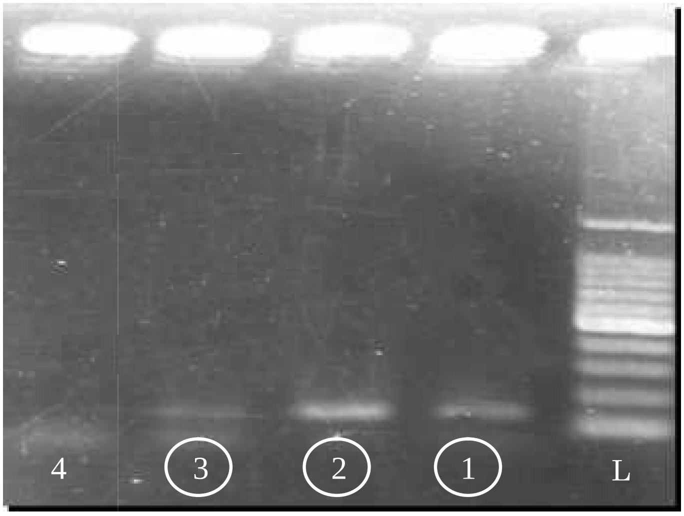

Fig. 8: Electrophoretic (2% agarose gel) comparison of PCR amplicons of *A. gossypii* and gut contents of *Pardosa* sp. amplified by a primer set Apf1 (132bp), lane L, ladder; lane 1, one individual of spider mixed with one individual of *A. gossypii*; lane 2, DNA of *A. gossypii*; lane 3, spider gut content after feeding on one individual of *A. gossypii*; lane 4, starved spider without feeding for four days.

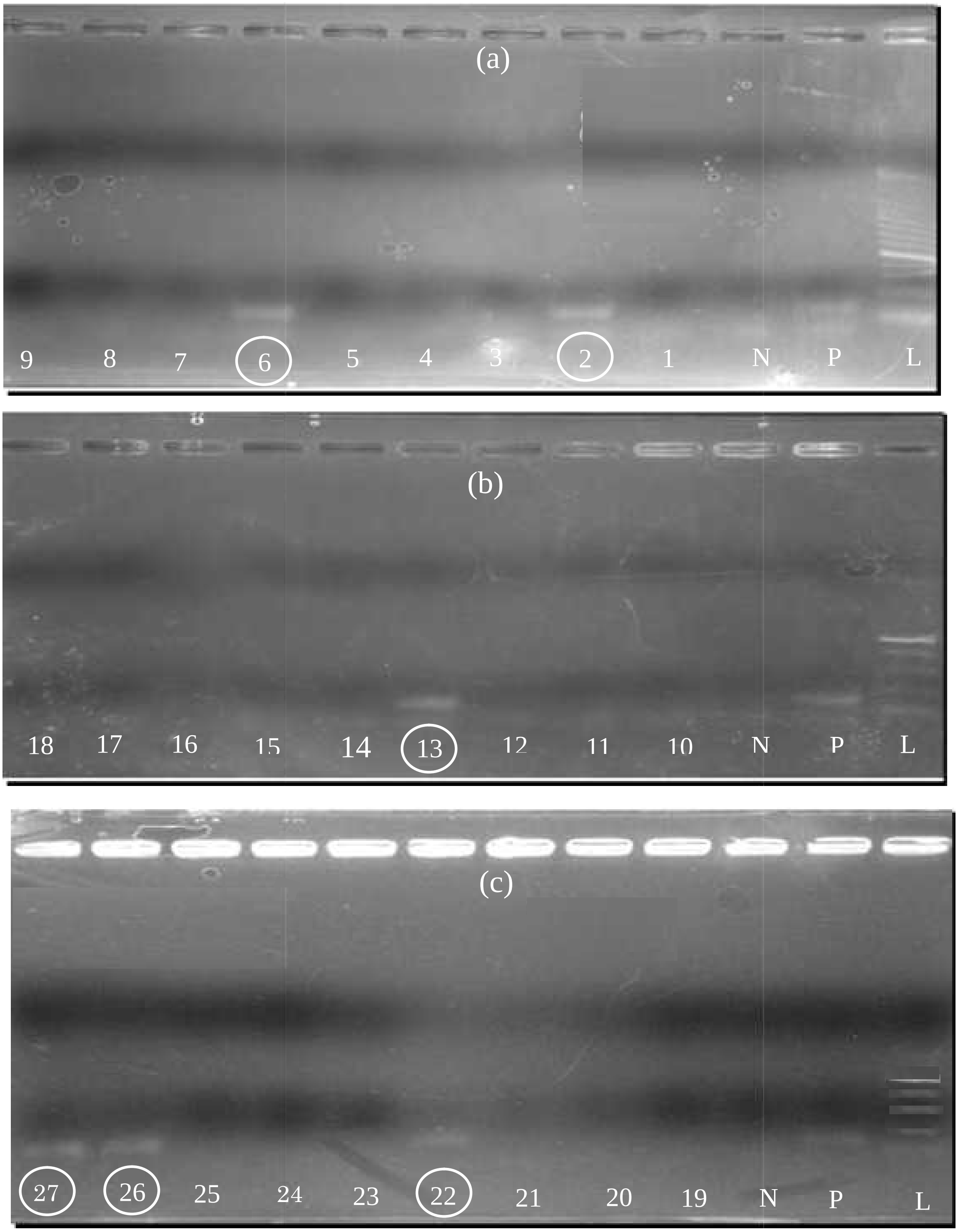

Fig. 9: Three electrophoretic gels (2% agarose gel) of *A. gossypii* specific fragment (132 bp) amplified from the gut contents of the field sample of *Pardosa* sp. (a), (b) and (c), lane L, ladder; lane P, positive control (one

individual of spider after feeding on one individual of aphids); lane N, negative control (starvation for 4-days); from lane 1 to 27, the tested field samples. Lanes2, 6, 13, 22, 26 and 27 are positive for cotton aphid remains.

3.4- DISCUSSION

The present study showed that it is able to be achieved to track field predation of *A. gossypii*, by using PCR-based analysis of the gut-content of the *Pardosa* spiders. In spite of cotton aphid was small, 13 individuals (16.25 %) of the 80 collected spiders had eaten at least one individual of *A. gossypii* within four days before collecting them. Kuusk et al. (2008) indicated that the field- collected *Pardosa* spiders were analyzed by using PCR and the percentages of positive samples for *R. padi* were 26% and 19% in 2004 and 2005 respectively. Öberg and Ekbom (2006) showed that *Pardosa* spider may be an important predator in decreasing *R. padi* populations below economic threshold levels. The detection prey DNA remains by using PCR was succeeded in 80% of *Lycosa* sp. up to 72 hours after eating an individual larva of the fourth instar of *Plutella xylostella*(Ma et al., 2005). Agustí et al. (2003) detected springtail DNA remains in total 100% of the linyphiid fed on one individual springtail 24 h after ingestion and Sheppard et al. (2005) amplified DNA remains of the grain aphid (*Sitobion avenae*) up to 50 h after ingestion in total 50% of the linyphiid spiders. On the other hand, the body sizes of Linyphiids are generally less than half of that of *Pardosa* spiders. Hagler and Naranjo (1997) demonstrated that predator size can be an important role affecting detection of prey DNA remains. Sunderland and Samu (2000) showed that spiders perform best as a biological control agent when their numbers are high. And PCR technique is also important to know if the increased predator densities result in improved biological control and decreased crop damage or not. Kuusk et al. (2008) recommended that spiders of *Pardosa* are very important for conservation biological control of *R. padi* and deserve specific attention. At the end, this results showed that the increased spider densities result in enhanced biological control for *A. gossypii*.

GENERAL DISCUSSION

GENERAL DISCUSSION

The present results indicated that the crab spiders (Thomisidae), predatory bugs (Anthocoridae) are attracted to the flowering plant *Salvia farinacea* Benth and the theridiid spiders were attracted to *Mentha spicata* and these plants could be used to provide a food resource, suitable habitats for these beneficial natural enemies. The manipulation of habitat is an important role of insect pest management and the main way to create a suitable habitat is plant flowers that can provide the natural enemies with food resources in the form of pollen or nectar or both together. Also, it is essential to consider not only the attractiveness of flowering plants to beneficial natural enemies, but also its potential as a reservoir for alternative prey that can enhance the numbers of natural enemies. Hogg et al. (2011) indicated the beneficial insectary plants should have: 1) attractiveness to natural enemies, 2) long and early blooming period, 3) low potential to host plant viruses, 4) the ability to out-compete weeds, 5) low potential to begin weeds, 6) low attractiveness to different pests; and 7) low cost of seeds and establishment. Winkler (2005) showed that the importance of determination of the suitable plant species for natural enemies. Thus, it was concluded that planting of *S. farinacea* can be considered as an effective plant for enhancing the role of biological control in agricultural fields.

The spiders (Araneae) are very important for biological control because all they are obligate predators, and many can feed on insect pests. The enhancement of spider numbers, agricultural ecosystems should be operated in ways according to the needs of the spiders population. The structural complexity of the environment is directly associated with spider numbers. The variation of habitats can provide suitable microhabitats, microclimatic features, alternative food resources, escaping places, and different sites to attach their webs, and all can encourage spiders population (Agnew and Smith, 1989; Rypstra et al., 1999). Moreover, providing refuges and overwintering sites, field borders are very essential for the spiders because they serve as passages for distributing into the main field (Riechert and Lockley, 1984; Marc et al., 1999). Thus, there are many reasons may be responsible for the high numbers of spiders in the organic fertilizers and flowering plants treatment in 2014. 1) the organic fertilization improves the structure of the soil and the microclimate, which are very important for saprophagous insects such as Collembola (Alderweireldt, 1994; Chen and Wise, 1999; Nyffeler, 1999; Axelsen and Kristensen, 2000; Pfiffner and Luka, 2003) and Collembola is considered as an important alternative prey for spiders (Agustí et al., 2003). Also, the organic fertilizers can keep the soil humidity which is very important for ground dwelling spiders and other ground predators. Additionally, Dicks et al. (2013) showed that the richness of the weeds and density and diversity of some invertebrates increased in the organic fertilizer treatment. 2) the flowering plants may increase the lycosid and linyphiid spiders by increasing the suitable refuges and habitats or it may increase the alternative prey and this agreed with Frank (2003) he indicated that the strips of the flowering plants are very important to increase the numbers of alternative prey. 3) flowering plants can enhance some of the spiders by providing the alternative food resources such as pollen.

Many previous studies have indicated that spiders can significantly decrease the density of prey. Lang et al. (1999) found that in a maize crop, spiders suppressed the numbers of aphids. And the present study showed that cotton aphid DNA detection success from the gut of *Pardosa* spiders (field samples). On the other hand, the 28-spotted ladybird beetle numbers were also lower in the organic plots with flowering plants treatment. I think that the increases in lycosid and linyphiid spiders may affect the beetle populations. Although the lycosid spiders usually dwell on the ground they can hunt the individuals of 28 spotted ladybird beetle which drop to land surface and they sometimes climb the plants especially the lower leaves. Since linyphiid and other web spiders live on the plants, they can easily capture the beetles by their nets.

SUMMARY

SUMMARY

Spiders are very important as stabilizing agents and regulators of insect numbers in the agroecosystems because they are obligate predators and they can feed on several species of insect pests. To increase and conserve the populations of spiders, the agricultural ecosystems should be operated according to the needs of the spider populations.

Flowering plants are very important to raise the numbers of predators and their alternative prey (Frank, 2003). Also, Riechert and Lockley (1984) and Rypstra et al. (1999) showed that increasing various habitats may provide the agroecosystem with suitable alternative food resources, microclimates, microhabitats, and web attachment sites, thus they may enhance the numbers of spiders. Landis et al. (2000) and Ambrosino et al. (2006) indicated that many predators and parasitoid species feed on pollen and nectar.

Organic fertilization by manure application improves the soil quality and structure, and it enhanced the populations of saprophagous insects such as springtails (Alderweireldt, 1994; Chen and Wise, 1999; Nyffeler, 1999; Axelsen and Kristensen, 2000; Pfiffner and Luka, 2003). Also, Agustí et al. (2003) showed that the spider numbers enhanced by Isotoma anglicana (Collembola), thus increasing the alternative prey can enhance the spider numbers.

Polymerase chain reaction(PCR) has become an important tool for

identifying prey species in the gut contents of predators to determine the trophic interactions in the open field (Sunderland et al., 2005; King et al., 2008; Pompanon et al., 2012; Traugott et al., 2013; Gariépy et al., 2007). Many previous studies have used some of the guts investigation for different predators to evaluate aphid predation (Harwood and Obrycki, 2005).

The aims of the this study were as follows.

1- To investigate the attractiveness of spiders and insect predators and parasitoids to some flowering plants to determine the suitable plants for natural enemies.
2- To determine the influence of a combination of organic fertilizers and flowering plants on the numbers of spiders and its importance for pest control.
3- To evaluate the spider efficiency as a predator under field conditions by molecular analysis (polymerase chain reaction).

Chapter 1

The first part of the current study was aimed to test some flowering plants and their attractiveness to spiders and insect natural enemies in an eggplant field managed organically at Kiire, Kagoshima City, Japan, in 2013 and 2014. In the first season (2013), five flowering plant species, *Salvia farinacea* (mealy cup sage), *Mentha spicata* (spearmint), *Foeniculum vulgare* (fennel), *Fagopyrum esculentum* (buckwheat), and

Anethum graveolens (dill),were evaluated to their attractiveness to spiders by using a direct inspection in the field from three individual plants for all tested plant species except *Me. spicata*, they were collected from a 30 × 30-cm area and the collections were repeated three times. The results showed that *S. farinacea* attracted high numbers of Thomisidae and *Me. spicata* attracted many individuals of Theridiidae, while the other tested plants attracted fewer numbers of spiders. In the second season (2014), another set of flowering plants, *S. farinacea* (mealy cup sage), *Matricaria recutita* (chamomile), *Achillea millefolium* (yarrow), *Petunia atkinsiana* (petunia), and *Alyssum maritimum* (sweet alyssum) were tested for their attractiveness to some natural enemies; Thomisidae, Ichneumonoidea, Chalcidoidea, Anthocoridae, and Syrphidae. They were collected once a week by a sweeping net using 15 double strokes from each flowering plant species(three double strokes from each replicate). Thomisid spiders and anthocorid bugs clearly preferred *S. farinacea* compared to other plants, whereas chalcidoid wasps increased with *S. farinacea* and *Ac. millefolium*.

Chapter 2

The second object of this study was to determine the treatment that increases the numbers of spiders and their effect on some of the main insect pests in an eggplant field (28 spotted ladybird beetle and cotton aphid). In 2013 and 2014, two treatments, organic fertilizer and chemical fertilizer treatments were applied to the experimental eggplant field and in 2014, the flowering plants *Salvia farinacea* Benth., *Mentha spicata* L., and *Ocimum* *basilicum* L. were planted around the organic fertilizer plots. Eight pitfall traps (four pitfall traps for each treatment) were used to collect ground-dwelling spiders and Collembola. While ten eggplant leaves were randomly collected from each plot and they were kept individually in plastic bags, and they were examined microscopically in the laboratory and the cotton aphid numbers were counted and recorded. The numbers of *Henosepilachna vigintioctomaculata* (28-spotted ladybird beetle), were counted by a direct inspection in the field from a total of 20 leaves per each replicate. Other insect pests were collected by ten double strokes of the sweeping net from each plot. All samples were taken weekly and analyzed by repeated measures ANOVA. The results indicated that there were significant impacts of fertilizer type on the numbers of linyphiid spiders and Collembola in 2013. In 2014, the numbers of Collembola, thrips, and lycosid and linyphiid spiders were higher in organic fertilizer with flowering plants treatment comparing with the chemical fertilizer treatment. Moreover, the numbers of *Henosepilachna vigintioctopunctata* (F.) were significantly fewer in the organic fertilizer with flowering plants treatment than in chemical fertilizer treatment. The enhancement of Thysanoptera and Collembola were essential alternative prey for linyphiid and lycosid spiders and the use of organic fertilizer and flowering plants enhanced the numbers of these spiders and may increase their role in suppressing the populations of *H. vigintioctopunctata* (F.).

Chapter 3

The last part of this study was aimed to analyze the gut content of *Pardosa* spiders by polymerase chain reaction (PCR), to detect the DNA of cotton aphid (*A. gossypii* Glover), to test *Pardosa* spiders as a predator under field conditions. The PCR technique played an essential role to evaluate the probability of predation in the field. In this study, adult males and females of *Pardosa* sp. (Araneae: Lycosidae) and *A.* gossypii (Hemiptera: Aphididae) were collected directly from the eggplant field. The DNA of cotton aphid was amplified with clear bands and it was also possible to detect the DNA remains (132-bp) of cotton aphid from the guts of *Pardosa* spiders after feeding on one individual of cotton aphid under laboratory conditions (treated as a positive control). While I could not detect the DNA of cotton aphid from the guts of *Pardosa* spiders after four days of feeding on one individual of cotton aphid (treated as a negative control).Thirteen of total eighty individuals of *Pardosa* spider were positive for DNA of cotton aphid. These results confirmed that *Pardosa* spiders are a very important predator and they can feed on cotton aphid under field conditions and they deserve more attention in control of cotton aphid.

In conclusion, this study showed that *S. farinacea* attracted high numbers of different natural enemies, crab spiders and predatory bugs as predators and chalcid wasps as parasitoids, compared with the other flowering plant species examined here. Also, the use of organic fertilizers and predatory-attracting plants may enhance the density of lycosid and linyphiid spiders and their alternative prey. And the results suggest that high numbers of these spiders can suppress some of the insect pests in the agroecosystems. And the field efficiency of a *Pardosa* spider, which was a representative of lycosid spiders in study field, confirmed by using PCR technique, thirteen of the eighty collected spiders from the eggplant field had attacked *A. gossypii*. Although the confirmed predation rate, 13/80, was not very high, such spiders including *Pardosa* are generalist predators and usually attack many species of insect pests in the agroecosystems. Thus, attracting and conserving them by using organic fertilizers and planting flowering plants must play a very important role to prevent outbreaks of some insect pests, because these spiders attack pests while they are low density.

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JAPANESE SUMMARY

露地野菜栽培における生物的防除素材としての真正クモ類の有効性

エルサイド　モハメド　エルナバウィ

真正クモ類の生物的防除素材としての有効性を評価するために、露地ナス圃場に有機肥料の施用と顕花植物の植付けを行い、これらの処理がクモ類の発生に及ぼす影響を検討した。また、クモ類の捕食能力を評価するために消化管内容物の DNA 解析による識別を試みた。

先ず、2013 年に鹿児島市喜入の圃場に 5 種類の顕花植物を植付けた。それら植物上のクモ類を直接観察したところ、*Salvia farinacea*(ブルーサルビア)はカニグモ科を *Mentha spicata*(スペアミント)はヒメグモ科をそれぞれ有意に誘引した。2014 年には別の 5 種類の顕花植物を植付けてクモ類と他の天敵の発生を直接観察とスイーピング法で調査した。カニグモ科クモ類と捕食性カメムシ類は特にブルーサルビアに誘引された。これに対し、コバチ上科の寄生蜂類はブルーサルビアと *Achillea millefolium*(セイヨウノコギリソウ)の 2 種に誘引された。

喜入露地ナス圃場では2013年と2014年とも圃場を有機肥料区と化学肥料区に分けてクモ類の発生を調査した。また、2014 年にはナス株の周囲にブルーサルビアとスペアミント、*Ocimum basilicum*(バジル)の 3 種を植え付けた。2013 年には有機肥料区においてサラグモ科のクモ類とトビムシ類が有意に多く発生した。2014 年には有機肥料+顕花植物区においてトビムシ、アザミウマ、コモリグモ科とサラグモ科のクモ類の発生が多かった。また、有機肥料+顕花植物区ではニジュウヤホシテントウが有意に少なかった。アザミウマやトビムシ類はサラグモおよびコモリグモにとっては有効な代替餌であり、有機肥料の施用と顕花植物の植付によって増加する。これに伴ってクモ類の密度が増加してニジュウヤホシテントウの発生を抑制していると考えられる。有機あるいは減農薬での露地ナス栽培ではニジュウヤホシテントウの発生が問題となることからニジュヤホシテントウの発生を抑制する天敵としてのクモ類のはたらきは重要である。

捕食性天敵としてのクモ類のはたらきを評価するために、喜入露地ナス圃場で採集したオオアシコモリグモにワタアブラムシを捕食させ、そ

の後の胃内容物内を PCR 法で解析することによって、ワタアブラムシを捕食したことを確認する試験を行った。80 個体中 13 個体の胃内容物からワタアブラムシの DNA を検出されたことから、クモ類の胃内容物から捕食餌を特定することが可能であることが示唆された。