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Bio-ecology of *Cassida rubiginosa* fed on *Silybum marianum* and *Onopordum boissieri* in laboratory conditions

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Abstract
Cassida rubiginosa Muller (Coleoptera: Chrysomelidae) is a polyphagous pest and is commonly distributed across the world. This species is regarded as natural enemy of weeds by many researchers, while it is also pest of some cultivated plants. In this study, the biological parameters of *C. rubiginosa* fed on *S. marianum and O. boissieri* (Asteraceae) were investigated.
Average periods of egg and 5 larval stages, pre-pupa, pupa and total development of *C. rubiginosa* fed on *S. marianum* and *O. boissieri* were 6.67, 7.71; 2.04, 2.19; 2.05, 2.45; 2.01, 2.74; 2.58, 3.26; 4.41, 4.88; 1.63, 2.12; 5.64, 5.35 and 26.99, 30.35 days, respectively. There were significant differences between all biological stages, except pupa stage between *S. marianum* and *C. scolymus*. Biological parameters were calculated as Intrinsic rate of increase: 0.018, 0.017; Net reproductive rate: 214.16, 357.71 and Mean generation time: 297.41, 337.19 for the populations which used *S. marianum and O. boissieri* as the host, respectively.

Keywords
Weed, Biological control, Thistle tortoise beetle, Milk thistle, Cotton thistle

Introduction
Some species of the Chrysomelidae family are used as biological control agents agains weeds (Batra et al., 1981; Kısmalı and Madanlar, 1990; Kısmalı and Sassi, 1994; Ang et al., 1995; Kok et al., 2000; Van Driesche et al., 2002; Aslan and Özbek, 2002; Koji and Nakamura, 2006; Majka and Lesage, 2008). *Cassida rubiginosa* Muller (Thistle tortoise beetle) is a polyphagous species spreading all over the world (Zwölfer and Eichhorn, 1966; Majka and Lesage, 2008; Cripps, 2013). This species known as natural enemy of weeds also feed on weeds belonging to Asteraceae (=Compositae) family causing yield reduction in many pastures and harm to many animals feeding on these pastures (Batra et al., 1981). Weeds named *Silybum marianum* (milk thistle) and *Onopordum boissieri* (Cotton thistle), which belong to the Astracae family, cause physical damage to the animals fed in these areas with their thorny structures as well as yield losses due to the area they cover in the pasture areas (Batra et al., 1981).

Studies have also reported its feeding activity on cultivated plants (Zwölfer and Eichhorn, 1966; Kısmalı and Sassi, 1994; Jacob et al., 2006).

In this study, the effects of *Silybum marianum* and *Onopordum boissieri* used as hosts on the bio-ecology of *C. rubiginosa* were investigated.

Materials and Methods
Cassida rubiginosa Muller (Coleoptera: Chrysomelidae) individuals used in this experiment were collected from *Silybum marianum* (L.) Gaertner and *Onopordum boissieri* Willk plants in the crop production fields of Çukurova University, Agricultural Faculty in Adana, Turkey. Mating males and females of

C. rubiginosa collected separately from these plants were brought to the laboratory and placed in cages (12 × 8 × 7 cm) covered with gauze to lay eggs. Wetted sponges were placed in the cages to provide sufficient moisture. Young foliated plants about 10 cm in height were placed in the cages and their roots were continuously immersed in water.

Females transferred to a new cage with food to obtain eggs. Development time of all pre adult stages was recorded. The first stage larvae emerging from the eggs were transferred to new culture cages with a soft brush and plastic culture cage labeled by marker. The larvae were fed on fresh plants every two days until pupation. Larval stages, and pre-pupal and pupal periods of these individuals were recorded daily.

Newly hatched adult females were removed and transferred individually into another cage containing test plants. The cages were formed in two parts. Pods (10 × 20 cm) were placed on the bottom part of the cage and a plastic culture box (25 cm × 8 cm) was placed on it. The holes (10 cm x 8 cm) were opened on both sides of the plastic box and covered with gauze. Finally, these cages including adult *C. rubiginosa* individuals were placed in the field. Depending on temperature, feeding adults moved to plant roots for aestivation and wintering periods. After this period, adults came up to soil surface and started to feed on plants. When necessary, each cage was supplied with a new plant. For mating of these females, male individuals were collected from the field and placed in cages after marking of their elytra. Leaves with eggs deposited by female were removed daily from the plants and egg numbers were counted.

The pots were watered every 2 days. The period of *C. rubiginosa* on the plant roots was determined under field conditions; the other periods were determined in laboratory (25±1 °C, 65% ±5 RH and 16 hours light (4000 lux) and 8 hours dark). The experiments were checked every 8 hours (three times per day) to obtain the life table parameters

Life Tables

The life table parameters of *C. rubiginosa* on two host plants were calculated according to the Euler-Lotka equation (Birch, 1948; Kairo and Murphy, 1995; Imura, 1987; Southwood, 1978; Carey, 1993). RmStat-3 was used to calculate all parameters (Özgökçe and Karaca, 2010).

Life table statistics were calculated for the populations on different host plants. The differences of life table parameters (r_m, R_0 and T_0) were tested. For this, jack-knife method was used (Meyer et al., 1986; Sokal and Rohlf, 1995; Özgökçe and Atlıhan, 2005). The mean growth rates in each treatment were subjected to analysis of variance followed by SPSS, (ver. 17; P<0.01) and JMP (ver. 8).

The survivorship data of all individuals of *C. rubiginosa* on different host plants were calculated by using the Weibull frequency distribution

$$S_p(t) = e^{-(\frac{t}{b})^c}$$

(Deevey, 1947; Pinder et al., 1978).

Statistical analyses were done with CurveExpert pro (ver. 1.6.8), SPSS (ver. 17).

Enkegaard equation was used for the number of age-specific eggs laid by a female:

$F(x) = a.x.e^{(-b.x)}$ (Enkegaard, 1993; Hansen et al., 1999; Enkegaard and Broodsgard, 2001)

Analyses were done with CurveExpertPro (ver., 1.6.8), JMP (ver. 9).

Results and Discussion

Development time, preoviposition, oviposition and post oviposition periods of *C. rubiginosa* fed on different host plants are shown in Table 1.

There were some significant differences ($P < 0.05$) in developmental times of *C. rubiginosa* on *S. marianum and O. boissieri* for all individual life stages (Table 1).

In field studies in Erzurum Province (Turkey) by Aslan and Özbek (2002) conducted under temperatures of 20-28 °C, the egg period of *C. rubiginosa* on *Circium arvense was* 7-8 days. In the same study, larval stages and pupal period were 3-4, 5-6, 5-7, 7-8, 7-8 and 5-7 days. *C. rubiginosa* under field conditions and with two hosts (Canada thistle and Cotton thistle) had egg, 1st, 2nd, 3rd, 4th, 5th instar, pre pupa and pupal development times of 5.7, 5.9; 3.2, 3.1; 2.9, 3.1; 2.6 and 2.9; and 3.1, 3.1; 2.8, 2.7; 1.9, 1.8; 6.1, 6.5 days, respectively (Spring and Kok, 1997). Development times of *C. rubiginosa* were found very close each other in all research. The total developmental time of pre adult stage was statistically similar between the 2 hosts.

The total developmental times on *S. marianum* and *O. boissieri* were 26.99 days and 30.35 days approximately. Spring and Kok (1997) reported that the total developmental time of *C. rubiginosa* was 27.4 and 28.5 days on musk and Canada thistle under field conditions, respectively. *Cassida rubiginosa* in the laboratory at constant temperatures had development times of 20, 26, 41 and 60 days at 32.5, 26.6, 21.1 and 17.8 °C, respectively (Ward and Pienkowski, 1978). All study results for the total developmental time were very similar.

Finally, these cages including adult *C. rubiginosa* individuals were placed in the field. Depending on temperature, feeding adults moved to plant roots for aestivation and wintering periods. After this period, adults came up to soil surface and started to feed on plants. Three male released with each female for mating. Preoviposition, oviposition and postoviposition periods of *C. rubiginosa* fed on *O. boissieri* were longer than on *S. marianum.* In addition, longevity of the shield beetle fed on *S. marianum* and *O. boissieri* was 360.40 and 439.50 days, respectively (Table 1).

Kosior (1975) reported that it started to lay eggs between three and seven days after mating, depending on temperature, photoperiod, rain and wind, and the duration of oviposition was 12 weeks. *C. rubiginosa* laid from 36.0 to 61.4 eggs per individual (Koji et al., 2012)

Table 1. Mean development time (days ±SE) of *Cassida rubiginosa* fed on *Silybum marianum* and *Onopordum boissieri*

Stages	n	*Silybum marianum*	n	*Onopordum boissieri*
Eggs	30	6.67±0.09 b*	30	7.71±0.08 a
1st Larvae	30	2.04±0.06 b	30	2.19±0.07 b
2nd Larvae	30	2.05±0.08 b	30	2.45±0.13 a
3rd Larvae	29	2.01±0.06 b	30	2.74±0.17 a
4th Larvae	28	2.58±0.06 a	27	3.26±0.09 a
5th Larvae	28	4.41±0.07 b	25	4.88±0.14 a
Prepupae	28	1.63±0.07 b	25	2.12±0.08 a
Pupae	28	5.64±0.20 a	23	5.35±0.09 a
Total development times	28	26.99±0.22 a	23	30.35±0.27 a
Preoviposition	12	246.42 ± 2.59 b	16	285.19 ± 1.24 a
Oviposition	12	58.08 ± 4.71 b	16	67.69 ± 6.80 a
Postoviposition	12	28.92 ± 4.69 b	16	56.38 ± 10.07 a
Longevity	12	360.40	16	439.50

*Means in the same row followed by a different letter are significantly different (Tukey-Kramer HSD test, $P \leq 0.05$).

Life table parameters of *C. rubiginosa* fed on *S. marianum* and *O. boissieri* were calculated with RmStat3 (Özgökçe and Karaca, 2010) and are presented in Table 2.

(r_m), which is a basic parameter for an insect population (Birch, 1948), was significantly different for the two host plants. Other two ecological parameters affected r_m; R_o and T_o of *C. rubiginosa* fed on S. *marianum, O. boissieri* and *C. scolymus* were also significantly different (Table 2).

Table 2. Life table parameters of *Cassida rubiginosa* on *Silybum marianum, Onopordum boissieri* and *Cynara scolymus*

Parameters	*Silybum marianum*	*Onopordum boissieri*
Intrinsic rate of increase, r_m	0,01804±0.0000262 a*	0,01743±0.0000153 b
Net reproductive rate, R_o	214,161±1.413 b	357,708±1.935 a
Mean generation time, T_o	297,406±0.354 b	337,198±0.110 a
Gross reproduction rate, ***GRR***	225,545	383,394
Doubling time, T_2	38,412	39,752
Finite rate of increase, λ	1,0182	1,0176

*Within the first three rows, means with different letters are significantly different (Tukey)

Net reproductive rate (R_0) of *C. rubiginosa*, fed on *O. boissieri* was greater than that fed on *S. marianum*. In contrast, intrinsic rate of increase (r_m) was greater when it was fed on S. *marianum*. Because mean generation time of *C. rubiginosa* was shorter on *S. marianum*.

Indeed, Levontin (1965) stated that r_m depended on the beginning of reproduction time and distribution of the age specific eggs rather than the number of eggs.

The survivorship curve (l_x) and age-specific fecundity rate (m_x) of *S. marianum* and *O. boissieri* are shown in Figure 1. The survivorship curve showed that the mortality rates of *C. rubiginosa* fed on *S. marianum* and *O. boissieri* were zero up to 307th and 331th day, respectively, and then the survival rate started to decrease dramatically, reaching zero by the 406 th and 502nd days, respectively. Similarly, age specific fecundity rates (r_m) of *C. rubiginosa* fed on *S. marianum and O. boissieri* started to increase by the 248 th and 308 th days respectively, and peaked at the 295 th and 321 th days, respectively, and declined in a gradually decreasing trend (Figure 1).

Weibull frequency distribution and the curve shape parameters were shown on Figure 2 and Table 3. Survivorship data can be effectively summarized by using the Weibull frequency distribution (Pinder et al., 1978). In two host plants it is a type I survival curve, as parameter c, is >1. Depend on this results, populations of *C. rubiginosa* fed on *S. marianum* and *O. boissieri* had an increased population.

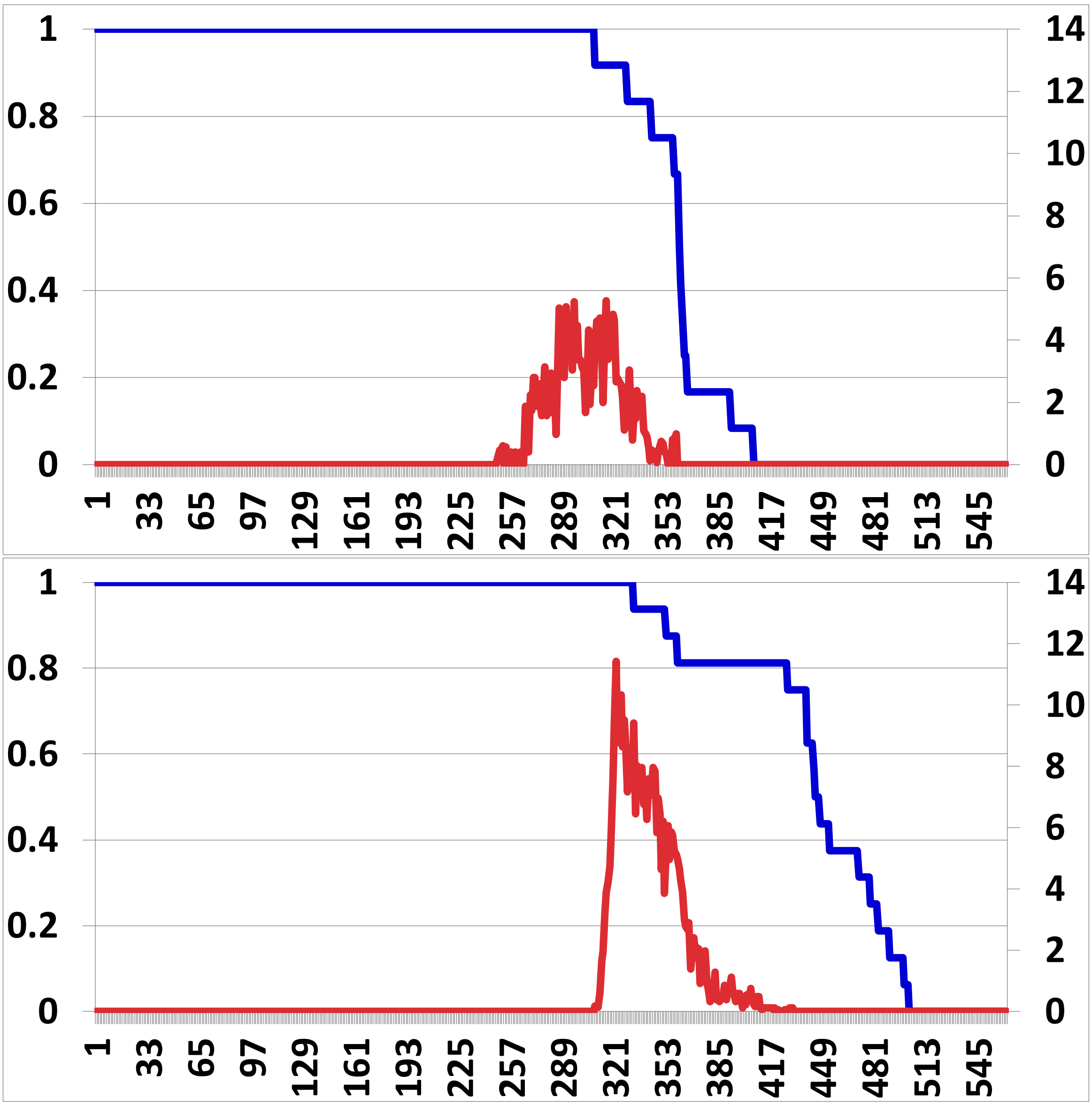

Figure 1. Survival rate (l_x) and fecundity (m_x) of *Cassida rubiginosa* on *Silybum marianum* and *Onopordum boissieri*.

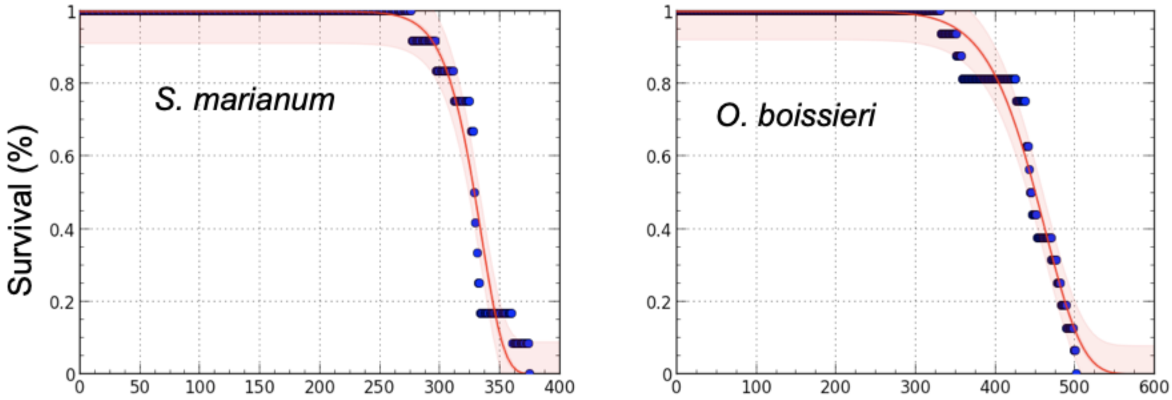

Figure 2. Weibull distribution fitted to survivorship data of *C. rubiginosa* fed on *S. marianum* and *Onopordum boissieri*

Table 3. Weibull parameters for survival curves of *Cassida rubiginosa* fed on *Silybum marianum*, *Onopordum boissieri* and *Cynara scolymus* (mean ±SE)

Parameters	*Silybum marianum*	*Onopordum boissieri*
b	335.00±0.40	465.67±0.59
c	15.59±0.50	10.66±0.18
Type	1	1
R^2	0.97	0.97

The relationship between two host plants regarding fecundity of *C. rubiginosa* was determined via the Enkegaard equation (Figure 3).

The relationship between days and fecundity was well described by the using the model (for *S. marianum* and *O. boissier;* R^2=0.41, a=0.18, b=0.02 and R^2=0.91, a=1.05, b=0.05 respectively). Most of the eggs were laid within the first half of the oviposition period on *O. boissieri* but on *S. marianum* egg laying was spread over the oviposition period (Figure 3).

Besides reports on biological control of weeds with *C. rubiginosa* (Batra et al., 1981; Tipping, 1993; Spring and Kok, 1997; Aslan and Özbek, 2002; Chaboo, 2007; Cripps, 2013), there have been studies of feeding activities on the cultivated plant *Cynara* discussed used in the current study (Zwölfer and Eichhorn, 1966; Kısmalı and Sassi, 1994; Jacob et al., 2006). Furthermore, Kısmalı and Sassi (1994) also observed feeding activity of *C. rubiginosa* on sugar beet (*Beta vulgaris*).

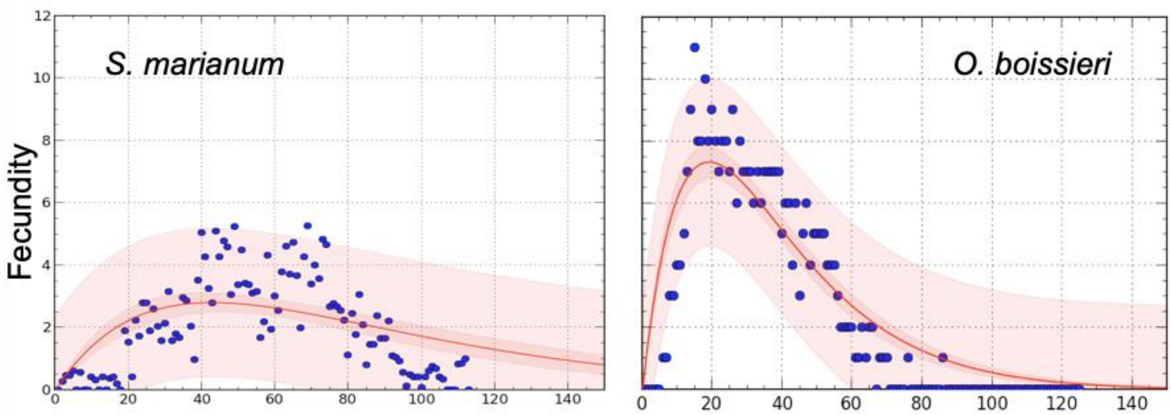

Figure 3. Survival rate (l_x) and fecundity (m_x) of *Cassida rubiginosa* on *Silybum marianum* and *Onopordum boissieri*.

Conclusion

As seen in the present study, *C. rubiginosa* fed on the important meadow pests, *S. marianum*, *O. boissieri*. Insect development and reproduction were similar on the two plants. This insect can be use as a biological control agent for host weeds in grassland areas but it should also be considered that this insect can cause significant looses in agricultural areas, especially in globe artichoke and sugar beet areas.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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