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Impact of Irrigation Systems on Seasonal Occurrence of *Brevicoryne brassicae* and Its Parasitism by *Diaeretiella rapae* on Canola

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ABSTRACT

Seasonal population dynamics of *Brevicoryne brassicae* (L.) and *Diaeretiella rapae* (McIntosh) were studied in two canola (*Brassica napus*) fields with two different irrigation methods (flooding and sprinkler irrigations) from April to June 2010. Aphid and parasitoid (mummies) populations were sampled once a week. The highest aphid density was recorded on May 12th (792.5 ± 55.5) and on May 18th (1701.1 ± 195) in the sprinkler and flood irrigated fields, respectively. Parasitism rate recorded ranged from 0.4 to 19.5% and from 1.0 to 11.9% in sprinkler and flood irrigated fields, respectively. During the course of the study, there was no significant relationship between the weather values recorded and aphid populations observed in the field. However relationship with weather values was found in the case of *D. rapae* populations. Secondary parasitism was higher at the end of growing season and two hyperparasitoid species were dominant: *Pachyneuron aphidis* (Bouche) and *Alloxysta fuscicornis* (Ashmead). We discuss role of seasonal parasitism and irrigation systems for management of *B. brassicae*.

Key words: Aphid parasitoid, aphid, population dynamic, hyperparasitoid, flooding and sprinkler irrigations.

INTRODUCTION

The cabbage aphid, *Brevicoryne brassicae* L. (Hem.: Aphididae) has a worldwide distribution damaging many cruciferous crops like canola, cabbage, cauliflower, radish and mustard (Blackman and Eastop, 2000). The pest can induce plant deformations (Oatman and Platner, 1969) and high densities can be very destructive in early stages of plant growth (Bonnemaison 1965; Ulusoy and Bayhan, 2006). It can also induce indirect damages through transmitting more than 20 different viruses such as yellow mosaic, cucumber mosaic, onion yellow dwarf and turnip mosaic viruses (Blackman and Eastop, 2000). In Iran, canola, *Brassica napus* is one of the most important oil seed crops in Iran (185,000 Ha) and the management of *B. brassicae* relies mainly on extensive use of chemical pesticides, however such control methods can induce multiple side effects on human health and non-target organisms (Weisenburger, 1993; Desneux *et al.,* 2007; Biondi *et al.,* 2012). Moreover, repeated application of insecticides can ultimately lead to the development of resistances in pests (Casida and Quistad, 1998; Roy *et al.,* 2011; Liang *et al.,* 2012) as already reported for *B. brassicae* in Kenya (Oduor *et al.,* 1997). The development of environmentally-sound methods such as biological control (Parolin *et al.,* 2012; Pizzol *et al.,* 2012) is needed to help reducing applications of insecticides (e. g. see Desneux *et al.,* 2010; Lu *et al.,* 2012).

Aphid parasitoids within the braconid subfamily Aphidiinae are well known natural enemies of various aphids in many crops (Desneux *et al.,* 2009; Ahmadabadi *et al.,* 2011; Mejias *et al.,* 2011; Pons *et al.,* 2011; Stara *et al.,* 2011). The endoparasitoid *Diaeretiella rapae* M'Intosh (Hymenoptera: Braconidae) has been reported as a major natural enemy of *B. brassicae* in various crops (Hagvar and Hofsvang, 1991; Souza and Bueno, 1992; Desneux *et al.,* 2006a). *D. rapae* is a polyphagous and cosmopolitan parasitoid that is known to attack other aphids such as *Aphis gossypii* Glover, *Aulacorthom solani* Kaltenbach, *Lipaphis erysimi* Kaltenbach, *Myzus persicae* Sulzer (Desneux *et al.,* 2005; 2006a; Akhtar *et al.,* 2010; Kavallieratos *et al.,* 2010) and various cereal aphids (Bernal and Gonzalez, 1997; Tazerouni *et al.,* 2012). *D. rapae* is reported from southern Iran (Farsi *et al.,* 2010), but its population dynamic has not been studied in western Iranian provinces. The parasitoid is known to attack and develop in all stages of the cabbage aphids (Zhang and Hassan, 2003). However, hyperparasitoids may reduce its ability to limit *B. brasicae* population growth in canola fields as reported on cabbage (Oatman and Platner, 1973).

Growing practices and climatic conditions that affect the physiological and morphological status of plant may also affect aphid population growth in agricultural ecosystems. For example, water stress is known to impact aphid abundance in various crops (Kindler and Staples, 1981; Michels and Undersander, 1986; Michels *et al.,* 2002). For example, the peak abundance of aphid *Schizaphis graminum* Rondani increased with irrigation levels on grain sorghum (Michels *et al.,* 2002). Cabrera *et al.,* (2000) also reported that *S. graminum* population growth reared on water-stressed barley was reduced by 77-89% when compared with regularly watered barley. In addition, a recent study reported that the ability of parasitoids to parasitize aphids on canola can be function of plant characteristics (e.g. leaf epicuticular waxes) mediated by growing conditions (Desneux and Ramirez-Romero, 2009).

Due to the rapid increase of canola acreage in Iran and the spread and damage due to *B. brassicae*, there is a need to identify potential cultural and biological control practices that may limit its damage. The goals of present study were to evaluate the parasitism rate and the effect of *D. rapae* on cabbage aphid population on canola and also to document aphid, parasitoid and hyperparasitoid population dynamics on canola crop when irrigated with sprinkler and flood irrigation systems.

MATERIAL AND METHODS

Experimental sites

The research was carried out in two 1-ha fields of Bu-Ali Sina University research farm at Dastjerd, Iran (N 35° 1´, E 48° 31´), from April to June of 2010. The fields were sown on September 15^{th} 2009 and irrigated by either traditional flood irrigation (van Steenbergen 1997) or overhead sprinkler irrigation. Each field was divided in four 25×20 m plots, and separated from adjacent plots by 70 cm bare soil space. Sprinkler irrigation occurred every 10 days and duration of irrigation was 8 hours. The type of sprinkler system used was solid set sprinkler system, having Ambo ®valves and nozzles (diameters: 5.3 mm) spaced by 12 meters from each other (i.e. 8 nozzles to cover all parts of the field studied). No pesticides or chemical fertilizers were applied but all other standard farming practices were followed according to regular cropping practices for canola in Iran.

Sampling procedures

Sampling in the field was carried out on a weekly basis and started from the first appearance of cabbage aphids in the fields (April 2^{nd} 2010) till the harvest of plants (June 30^{th} 2010). Thirty plants from each field were randomly sampled per sampling date and the terminal 20 cm of the main shoots infested with cabbage aphid were cut, kept separately in a plastic bags and brought back to the laboratory. Numbers of live aphids; mummies and adult parasitoids were counted in each sample. All samples were placed individually in a growth chamber (25±1°C, 65 ± 10% RH and 16L: 8D) for ten days to allow primary parasitoids and hyperparasitoids to emerge. The species were identified using the key developed by Lotfalizadeh (2002). The rate of parasitism was calculated as the percentage of un-emerged mummies (i.e. before parasitoids started emerging in the laboratory) to total aphids on each sample (Kavallieratos *et al.,* 2002a; Kavallieratos, Stathas, *et al.,* 2002b; Kavallieratos *et al.,* 2004). Sex ratio of *D. rapae* was calculated and compared between the irrigation treatments. Meteorological data (i.e. temperature, relative humidity, wind speed and total rainfall) were collected at the Bu-Ali Sina University research farm.

Number of aphids were first tested for normality using Shapiro-Wilcoxon test, and transformed if necessary. Nonparametric tests (e.g. Wilcoxon test) were used when the assumptions of parametric tests were not met and could not be achieved using transformations. We compared the number of aphids, parasitoids and parasitism percentage among sampling dates (SAS Institute Inc. 1988). Stepwise multiple regression analysis and correlation tests were made using aphid population density as dependent variable and parasitoid density and weather data as independent variables.

RESULTS

In the sprinkler irrigated field, *B. brassicae* density was very low until early May (Fig. 1). After that date, aphid populations gradually increased up to a peak of 792.5 ± 55.4 on May 12th (Chi-square= 194.6, df = 9, $P < 0.0001$).

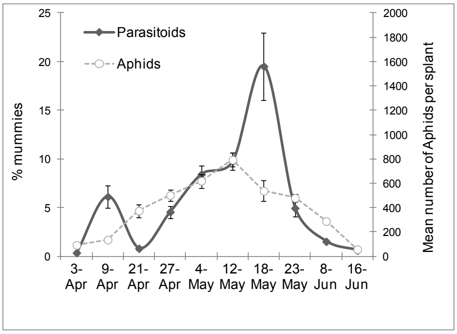

Fig. 1. Average number of *B. brassicae* (mean ± standard error) per plant on 20 cm terminal main shoot and parasitism percentage by *D. rapae* of total numbers of aphids collected for each sampling date in sprinkler irrigated field.

At the beginning of aphid population growth (in April), levels of parasitoid activity were very low, but as aphid populations built up, the parasitoid *D. rapae* became abundant and its population dynamics followed aphid population trend in a density dependent manner (Fig. 1). The peak of percent parasitism appeared one week after the highest aphid population level was reached. Highest parasitism rate recorded was 19.5 ± 3.48% on May18th (Chi-square = 188.5, df =9, $P < 0.0001$; Fig. 1).

The aphid density in the flood irrigated field varied significantly during season (1701 ± 195, Chi-square= 217.7, df = 9, $P < 0.0001$) and the aphid population peaked in flood irrigated field one week later than in the sprinkler irrigated one. Furthermore, the aphid density peak in flood irrigated field was two-fold higher than in sprinkler irrigated field (Fig.1 and Fig.2). Percentage parasitism increased rapidly and reached a peak on April 27th (11.9 ± 3.87), (Chi-square = 164.65, df = 9, $P < 0.0001$; Fig. 2).

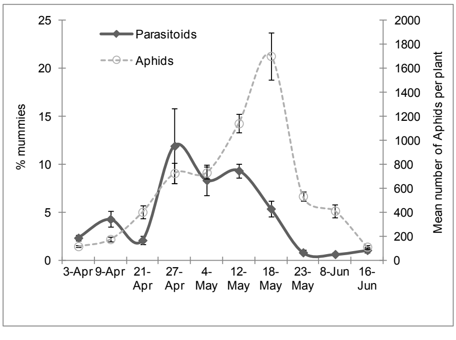

Fig. 2. Average number of *B. brassicae* (mean ± standard error) per plant on 20 cm terminal main shoot and parasitism percentage by *D. rapae* of total numbers of aphids collected for each sampling date in flood irrigated field.

Stepwise multiple regression analysis on weather factors i.e. temperature, relative humidity and air movement speed showed that these factors did not have a significant relationship with cabbage aphid or *D. rapae* population growth during the course of the experiment in the fields. However, *D. rapae* were positively correlated with aphid populations (r = 0.88, *P*= 0.006) and notably in the flood irrigated field (Fig. 2). In addition, based on elimination of non significant variables with stepwise regression analysis, the best fitted regression equation was Y = 201.3 + 7.75x (R^2=0.76, *P* = 0.001) and Y = 316.88+ 11.67x (R^2=0.65, *P* = 0.005) (Y: aphid number, x: parasitoid number) for sprinkler and flood irrigated fields respectively.

Two hyperparasitoid species *Pachyneuron aphidis* (Bouche) and *Alloxysta fuscicornis* (Ashmead) were present in the samples (identified by Dr. H. A. Lotfalizadeh) with *P. aphidis* being dominant in both irrigation systems (notbly in the end of the season.) These hyperparasitoids appeared mostly at the end of growing season. Highest hyperparasitism rates were recorded on June 16th in both sprinkler irrigated and flood irrigated fields (Fig. 3).

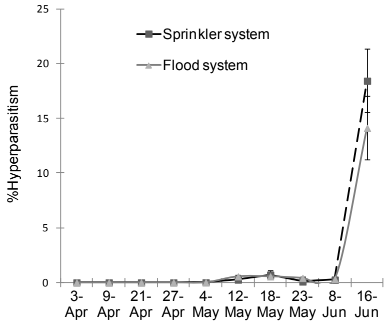

Fig. 3. Percentage of hyperparasitism (± standard error) by *P. aphidis*.

Hyperparasitism rate of *A. fuscicornis* varied largely among the sampling dates in the sprinkler irrigated field (Chi-square= 68.73, df= 9, *P*= 0.0001) as well as in the flood irrigated field (Chi-square= 77.66, df= 9, *P* < 0.0001) (Fig. 4). Sex ratio of *D. rapae* was female biased with 0.77 and 0.79 in the sprinkler and flood irrigated fields respectively (Fig. 5). At the end of the season, the sex ratio dropped (i.e. more males were observed), notably in the sprinkler irrigated field.

In addition to aphid parasitoids and hyperparasitoids recorded, other natural enemies were also observed. They were collected and identified: *Episyrphus balteatus* (De Geer 1776), *Eupeodes nuba* (Widemann 1830), *Eupeodes corollae* (Fabricius 1794) and *Scaeva albomaculata* Stone (Dip.: Syrphidae:), *Hippodamia variegata* (Goeze) and *Coccinella septumpunctata* (Linnaeus 1758) (Coleoptera: Coccinellidae:) and *Chrysoperla carnea* (Stephens 1836) (Neu.: Chrysopidae) (Amini *et al.,*, 2011). Voucher specimens were kept in Insect Ecology Laboratory, Dept. of Plant Protection, Bu-Ali Sina Univ. (Iran). Some of these natural enemies showed high population densities during the course of our study, especially at the end of growing season.

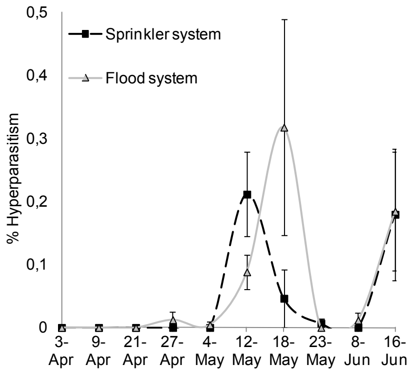

Fig. 4. Percentage of hyperparasitism by *A. fuscicornis* (mean ± standard error).

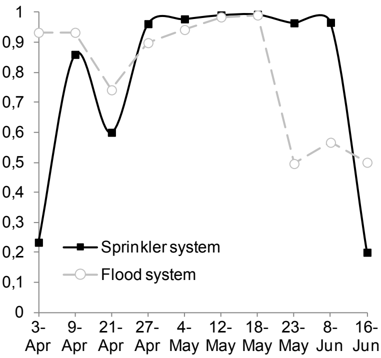

Fig. 5. Sex ratio of *D. rapae* on *B. brassicae* on canola.

DISCUSSION

The sprinkler irrigation system is more versatile than traditional flood irrigation; in addition, farmers can still apply fertilizers and pesticides effectively (when needed). Moreover, uniform water application is most easily achieved with sprinkler systems. The present study showed that this system can have an impact on population dynamic of sucking pests like aphids (as already demonstrated for other arthropods, e. g. thrips, leafhoppers and spider mites, Kinn, *et al*., 1972; Zare Abyaneh *et al*., 2009) with fewer aphid in the canola field irrigated with the sprinkler system. Very few studies have documented the role of irrigation systems in pest management. Our study demonstrated that a given type of irrigation system could be selected preferentially to slow down pest population growth. The type of irrigation influences aphid population density and also levels of parasitism by Aphidiinae parasitoids. Aphid populations were higher in field having flood system irrigation than in the field with sprinkler system. The water drops fall like rainfall in the sprinkler irrigated fields and it likely causes some aphids to fall off the plants, thus killing many of them before they could actually reproduce (when they can not climb back on the canola plants).

Previous field studies reported only one single peak for *B. brassicae* population on canola per season in Netherland (Geiger 2005) and in Iran (Modares Najafabadi *et al.,* 2005) but, Chua (1977) observed two peaks for population dynamics of the cabbage aphid in UK.

Population of *D. rapae* followed changes in cabbage aphid density (Figs.1-2) with a short delay. This was obvious, especially in sprinkler irrigated field. However, there was an asynchrony between aphid and aphid parasitoid populations in the flood irrigated field. In addition, in our study, the most influencing factor on aphid population dynamic was *D. rapae* population density for both irrigation systems.

Various parasitism rates of *B. brassicae* by *D. rapae* have been reported in previous studies carried out on *Brassica* plants. Costello and Altieri (1995) reported 3.3-17.8% *B. brassicae* parasitism on broccoli and Oduor *et al.,* (1997) reported only 1.2% of parasitism on cabbage in Kenya. Mussury and Fernandes (2002) reported unusual high rate 89.7% for cabbage and mustard aphids during the canola flowering phase, although they did not estimate parasitism levels for each aphid species separately. In contrast Zhang and Hassan (2003) reported low parasitism rate, 1.4% on broccoli control plots versus 6.7% in plots where *D. rapae* was released against the cabbage aphid. Nieto *et al.,* (2006) studied population dynamics of *B. brassicae* on organic broccoli and reported a parasitism rates of up to 30 and 15 % during 2002 and 2003, respectively. Kakakhel (2006) and Desneux *et al.,* (2006a) reported 37% and 22% parasitism for mustard aphid, (*L. erysimi)* and *B. brassicae on winter canola, respectively.* Bayhan *et al.,* (2007) studied the effect of various cruciferous crops on parasitism rate of *B. brassicae* and reported highest rate (40.2%) on cabbage followed on cauliflower (40%) and the lowest on turnip (32.6%). Similar to our results, Duchovskienė and Raudonis (2008) reported that the parasitism rate of canola aphid at the beginning of the season was low (on average 8%) but increased gradually to reach 23.9-26.2% aphids parazitised midseason. All these results suggested that aphid parasitoids can play a key role in suppressing cabbage aphid populations although efficacy of the parasitoids likely depends of multiple factors (e.g. irrigation system). For example, higher fertilization could positively influence parasitism rate (through increased plant nutritional quality) (Duchovskienė and Raudonis, 2008).

Correlations between climatic factors and aphid population dynamic reported in Iran or in other parts of the world do not match our results. Akhtar *et al.,* (2010) showed that relative humidity had a negative correlation with mustard aphid and parasitoid densities while temperature had a positive correlation with *D. rapae* population. Keyhanian and Taghaddosi (2010) showed that in Varamin (Iran), (relatively dry and warm climate) peak of cabbage aphid density occurred on late April or early May in 2002-2003. After peaking, cabbage aphid populations were greatly decreased likely owing to increased temperature and reduced humidity (through reduced aphid fecundity).

Temperature directly influences longevity, mating and oviposition rate of parasitoids

(Pizzol *et al.,* 2010; Andrade *et al.,* 2011). Bernal and Gonzalez (1997) reported that longevity of *D. rapae* females was inversely dependent to temperature (poikilotherm insects). Therefore, it might be expected that temperatures exceeding a given threshold, induce a decrease in longevity and fecundity in *D. rapae*.

Some factors like high aphid density and occurence of hyperparasitoids can slow down the development of *D. rapae* populations relative to aphid populations and the occurrence of hyperparasitoides may reduce parasitism of the cabbage aphid in canola fields. Another that factor can affect parasitism in fields is parasitoid sex ratio. Waage and Hassell (1982) postulated that offspring sex ratio is one of the important factors that potentially influences the success or failure of releases of parasitoids suggesting that a female biased sex ratio may be needed for successful biological control programs. In our study the sex ratio decreased with increasing temperature from May toward the end of the growing season. This is in accordance with other studies that reported different sex ratio of *D. rapae*. Bernal and Gonzalez (1997) reported sex ratios of 0.543, 0.675 and 0.641 at 26.7°C, 21.1°C and 10°C respectively on Russian wheat aphid, *Diuraphis noxia* (Mordwilko). They reported that higher temperatures may have adverse effect on sperm supply and sex ratio of *D. rapae*. Blande *et al.,* (2004) reported a male biased sex ratio of *D. rapae* reared on *M. persicae* when provided with an excessive number of hosts. Other authors reported the effect of temperature on sex ratio of parasitoids (e.g. Zamani *et al.,* 2007) who reported male biased sex ratio of *Aphidius colemani* and *Aphidius matricariae* at extreme temperature tested (i.e., 10 °C and 30°C).

Previous studies have suggested that *D. rapae* may not control aphid populations (e.g. Oatman and Platner, 1973; Chua, 1977) concluding that, owing to many factors like asynchrony between host and parasitoid populations and predation by syrphid larvae, this parasitoid species may not play a dominant role in aphid control. They reported that natural parasitism of the cabbage aphid by *D. rapae* was too low to suppress aphid population growth. Therefore, the use of *D. rapae* as biological control agent against cabbage aphid may have to be combined with other natural enemies and/or control method (pending these do not have adverse effects on natural enemies, e.g. see Desneux *et al*., 2011). Use of selective insecticides associated with release of *D. rapae* may be one possible option. Kahakhel *et al.,* (1998) showed that the insecticide Tamaran (a.i. methamidophos) may be only moderately harmful for *D. rapae*. In the same idea, extensive works by Desneux *et al.,* (2004; 2005; 2006b; 2006c) showed that deltamethrin (pyrethroid) may be used in combination with aphid parasitoids for IPM programs targeting aphids on canola. Further studies would have to identify the safer insecticides for *D. rapae*. This parasitoid has been suggested as a good candidate for releasing in cruciferous IPM programs because it is attracted to volatiles released by cruciferous crops and could be particularly efficient in detecting *Brassica* plants infested by aphids (Reed *et al.,* 1995; Bradburne and Mithen, 2000). An option might be to enhance presence and activity of *D. rapae* by planting attractant crops near the main *Brassica* crops.

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