Comparative Biology and Life Cycle of The Barley Stem Gall Midge and Hessian fly (Diptera: Cecidomyiidae) in Morocco

Comparaison de la Biologie et du cycle de vie de la cécidomyie à galle de l'orge et de la mouche de Hesse (Diptères: Cecidomyiidae) au Maroc

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ABSTRACT

The barley stem gall midge, *Mayetiola hordei* (Keiffer) is the most serious pest of barley in Morocco. The biology and life cycle of this insect were studied in a laboratory and under natural weather conditions. The results showed that similarly to Hessian fly, barley stem gall midge has two feeding instars and a third non-feeding instar. The generation time was longer for barley stem gall midge than for Hessian fly (45 vs 32 days at 18 ± 1°C, and a 12:12 (L: D) h photoperiod). The eggs of barley stem gall midge hatched in 7 days compared to 4 days for Hessian fly. The largest discrepancy in developmental time was for second instar and pupa. Second instars and pupae of barley stem gall midge required twice as long as those of Hessian fly to develop and molt into next stage (12 vs. 6 days). The first and third instars of barley stem gall midge also required a little bit longer to complete development (9 and 10 days vs. 7 and 8 days for Hessian fly). Like for Hessian fly, barley stem gall midge reproduces mostly by unisexual progenies. Four progeny classes were observed; unisexual female progenies, unisexual male progenies, predominantly female progenies, and predominantly male progenies. The proportion of unisexual female and male progenies and the proportion of predominantly female and predominantly male progenies were similar. Overall, the sex ratio of both species was about 1:1. Under field weather conditions that prevail in the Chaouia region of Morocco, barley stem gall midge has two complete generations and a third partial one. The first generation starts late October, and ends late December. The second generation develops from January until early March. A high proportion of third instars of this generation fail to pupate (35%). The third generation is only partial; adults of the second generation emerge during March, oviposit and larvae develop to third instars but all go into summer diapause.

Key words: Barley stem gall midge, Hessian fly, biology, life cycle.

RESUME

La cécidomyie à galle de l'orge, *Mayetiola hordei* (Keiffer), est le ravageur le plus destructif de l'orge au Maroc. La biologie et le cycle de développement de l'insecte ont été étudiés au laboratoire et sous des conditions climatiques naturelles. Les résultats ont montré

que similairement à la mouche de Hesse, la cécidomyie à galle de l'orge possède deux stades larvaires qui s'alimentent et un troisième ne s'alimentant pas. Cependant, la durée de génération était plus longue que celle de la mouche de Hesse (45 contre 32 jours à une température de 18 ± 1°C et une photopériode de 12:12 heures (L:O)). Le stade œuf de la cécidomyie de l'orge s'est développé en 7 jours au lieu de 4 pour la mouche de Hesse. La plus grande différence dans la durée de développement fut enregistrée pour le deuxième stade larvaire et le stade pupe. Ils ont nécessité deux fois plus de temps pour compléter leur développement que pour la mouche de Hesse (12 contre 6 jours). Les premier et troisième stades larvaires ont aussi nécessité un peu plus de temps; 9 et 10 jours pour la cécidomyie de l'orge contre 7 et 8 chez la mouche du blé.

De même que pour la mouche de Hesse, la cécidomyie à galle de l'orge se reproduit principalement par progénitures unisexuées. Quatre classes de progénitures ont été observées; progénitures unisexuées femelles, progénitures unisexuées mâles, progénitures à prédominance femelles, et progénitures à prédominances mâles. La proportion des progénitures unisexuées femelles et mâles était similaire à celle des progénitures à prédominance femelles et mâles, donnant ainsi un sexe ratio de 50:50.

Sous les conditions climatiques de la région de Chaouia, la cécidomyie à galle de l'orge passe chaque année par 2 générations complètes et une troisième partielle. La première génération commence en automne, vers fin octobre et s'achève vers fin décembre. La deuxième s'étale entre janvier et mars. Une proportion pouvant arriver à 35% des individus de troisième stade larvaire de cette deuxième génération ne passe pas au stade pupe, et rentre immédiatement en diapause. La troisième génération est partielle; les adultes de la deuxième génération émergent et procèdent à la ponte, mais les larves qui en sont issues se développent jusqu'au 3eme stade larvaire, et rentrent toutes en diapause estivale, bouclant ainsi le cycle de développement.

Mots Clés: Cécidomyie à galle de l'orge, mouche de Hesse, biologie, cycle de vie.

INTRODUCTION

Cereals are the major food and feed crops in Morocco. They occupy more than 5 million hectares annually. Bread wheat is grown over 2 million ha, durum wheat over 1 million ha, and barley over 2 million ha. However, these crops are far from nearing their yield potential because of adverse weather conditions and negative effects of biotic stress resulting from attacks of *Mayetiola* species.

Mayetiola (Cecidomyiidae) is a paleartic genus of gall midges that contains 29 species, all of which evolved on and are restricted to particular genera and tribes of the grass family Poaceae (Gramineae) (Skuhrava 1986, Gagné & Jaschhof, 2014). *Mayetiola* species are mono-phagous, restricted to one host, or oligophagous, restricted to a few closely related hosts. Adults do not feed, are short-lived, and are narrowly adapted to and synchronized with their hosts (Gagné 1989). Females spend most of their short life-span searching for host plants and laying eggs. Like some other plant-feeding cecidomyiid groups, *Mayetiola* females have preferences for hosts that are suitable food for their offspring (Harris & Rose 1989, Lhaloui 1995).

Among *Mayetiola* species, the Hessian fly, *M. destructor* (Say) (H. fly) and the barley stem gall midge, *M. hordei* (Keiffer) (BSGM), are the most serious pests of cereal crops. The H. fly has been

recognized for many years as a major pest of wheat in the United States and Morocco, and in many other wheat-growing regions of the world (Jourdan 1938a, Barnes 1956, Miller & *al*., 1989, Ratcliffe & Hatchett 1997, Lhaloui & *al*., 2005). Originally from West Asia, the Hessian fly has spread to several countries of Europe, North Africa and North America. The BSGM is indigenous to Mediterranean countries. In Morocco, losses caused by these pests have been estimated to an annual average of more than a third of the wheat and barley yields (Lhaloui & *al.* 1992a), and could amount to total crop loss when heavy infestations coincide with young plant tillers such as in the case of late seeding. Studies conducted using Carbofuran applications to keep H. fly and BSGM free plots and compare their yield to that obtained from infested plots, showed an estimated 42% loss for bread wheat, 32% for durum wheat, and 45 % for barley (Lhaloui & *al.*, 1992b). Similar studies using near-isogenic H. fly resistant/susceptible bread wheat lines indicated an average 36% yield loss (Amri & *al.*, 1992).

On the other hand, surveys conducted in Morocco between 1986 and 1990 showed average infestation levels of 88, 85 and 80% of bread wheat, durum wheat, and barley, respectively (Lhaloui & *al.* 1992b, Lhaloui & *al.* 2005). Recent studies indicated that these pests are more severe in drought prone areas, but are omnipresent all over the cereal growing regions of the country (Lhaloui & *al.*, 2014). Economically, losses dues to H. fly were estimated at a value of 200 million DH per year on bread wheat in 5 major regions of Morocco. This indicates that losses are much heavier when all cereals and regions are included. The same study revealed that investing in research for developing resistant varieties has an internal rate of return of 39% (Azzam & *al*., 1997).

Because of the great economic importance of H. fly, much is known about the insect's biology, life cycle, and bionomics relative to its hosts, and several control methods have been identified. The mechanisms of resistance have largely been investigated. They are mostly expressed as larval antibiosis controlled by single partially or completely dominant genes. So far, 34 Hessian fly resistance genes have been identified (Li & *al.*, 2013), and a large number of wheat cultivars carrying resistance to this pest have been released. However, research on the BSGM is very limited, although 3 barley cultivars (Fleet, WPBS, and Gwylan) have been identified as carrying antibiosis to H. fly (Lhaloui *&* *al*., 1996). No resistance has so far been identified in cultivated barley for the BSGM, but two wild accessions of *H. bublbosum* were reported as carrying antibiosis to this pest (Lhaloui, 1995).

The BSGM has long prevailed as a destructive pest of barley in the barley-growing regions adjacent to the Mediterranean Sea, particularly in North Africa (Roberti 1953, Alfaro 1955, Lhaloui & *al.*, 1992b, Lhaloui & *al.*, 2000, Lhaloui & *al.*, 2005). Although now recognized as a distinct species, *M. hordei [=M. mimeuri* (Mesnil)], the taxonomic status of the BSGM had been in question since it was described as *M. mimeuri* (Mesnil 1934). Mesnil believed that *M. mimeuri* was a new species different than H. fly that attacks not only barley but also wheat and oats. Mesnil also believed that H. fly did not occur in Morocco. Later, Balachowsky and Mesnil (1935) using illustrations to describe the differences between *M. mimeuri* and *M. destructor*, concluded

that *M. mimeuri* was a pest of both wheat and barley in Morocco and Algeria, but caused galls only on barley. Jourdan (1937, 1938b) compared the biology of *Mayetiola* specimens combined from wheat and barley in Morocco to *Mayetiola* in Europe and found no difference between the two populations. In another paper, Jourdan (1938a) compared morphological characters of larvae and adults taken from various fields of wheat and barley in Morocco. Based on illustrations of male genitalia and posterior segments of larvae, he concluded that the variations observed were too small to give any clear-cut differences between the descriptions of *M. mimeuri* and *M. destructor* provided by Mesnil (1934). He rejected the notion that *M. mimeuri* was a distinct species and concluded that *M. destructor* was a largely variable species that occurred in Morocco as well as in Europe. The results of Jourdan (1938a) were later supported by Hudault & Zelensky (1939), who stated that *M. destructor* was a pest of both wheat and barley, but galls were only formed on barley.

To check their hypothesis, Hudault & Jourdan (1954) made crosses between *Mayetiola* adults reared from wheat and barley and found that females reared from wheat mated with males reared from barley, and vice versa, and produced viable offspring. They concluded that since flies were interfertile, they may be the same species. Later, Coutin & *al.*, (1974) found that *Mayetiola* adults from wheat and barley were not interfertile. Moreover, they reported that the puparia from barley were more variable and corresponded to either *M. destructor* or *M. mimeuri*, while puparia from wheat always corresponded to *M. destructor*. They concluded that *Mayetiola* on wheat and barley represented at least two well isolated biotypes.

Outside of Morocco, Roberti (1953) presented accurate illustrations of Italian *Mayetiola* spp. from wheat and barley. He conclusively demonstrated that specimens from wheat (as *M. destructor*), and from barley (as *M. mimeuri*) were distinct. Alfaro (1955) also demonstrated the existence of two distinct species, *M. destructor* on wheat, and *M. mimeuri* on barley in Spain. Barnes (1956) treated *M. mimeuri* as a separate species that infests wheat and barley in North Africa, but doubted its validity. He also questioned the findings of Roberti (1953). Finally, Ertel (1975) classified *M. mimeuri* among species of uncertain origin; and Shuhrava (1986) listed it as a synonym of *M. destructor*.

In their study, Gagné & *al.*, (1991) redescribed the Moroccan *Mayetiola* species from wheat and barley with fine illustrations of the spicules of the posterior abdominal sternites of puparia, the shape of the female postabdominal tergites, and the shape of male genitalia. They demonstrated conclusively that *Mayetiola* populations on wheat and barley in Morocco are two distinct species that are sympatric in the major cereal growing regions. The BSGM infests barley but not wheat, whereas the H. fly infests wheat and, to a lesser extent, barley. Gagné *& al.*, (1991) also noticed that *M. hordei* puparia were always embedded in galls and associated with plant stunting, whereas H. fly puparia were associated with plant stunting but caused no stem swellings. No intermediates were found; all specimens collected clearly belonged to one or the other species. Gagné & *al.* (1991) concluded that BSGM, the dominant species on barley, rarely reproduces on wheat and may be host specific to barley and other *Hordeum*

species, while H. fly has a distinct preference for wheat, but can also reproduce on barley. Because the *Mayetiola* species found on barley was first described by Keiffer (1909) and given the name of *M. hordei* (Keiffer), Gagné & *al.*,(1991) resurrected the name *M. hordei* and considered *mimeuri* as a junior synonym of *hordei.*

Gagné & *al*. (1991) stated that the taxonomic confusion of *Mayetiola* species was created by failure of earlier authors to correctly identify the hosts of specimens or isolate specimens from individual host plants. Also, failure to recognize that the BSGM and the H. fly may occur together on barley led to erroneous conclusions concerning species variability and host preference. Therefore, much of the information previously published on the biology and life cycle of the two species on barley in North Africa may be invalid (Gagné & *al.*, 1991).

The objectives of this study were 1) to redefine the biology and life cycle of BSGM in the field and under controlled environmental conditions, 2) compare the developmental time of life stages of the BSGM and H. fly, 3) determine the sex ratios of BSGM and H. fly, and 4) determine the number and durations of the generations.

MATERIALS AND METHODS

The experiments were conducted at the National Institute of Agronomic Research, Regional Center of Settat, INRA-CRRA-Settat, Morocco. BSGM females used in the tests conducted under controlled environmental conditions were obtained from a pure culture that had been reared in isolation on 'Tamelalt' barley in a greenhouse. The culture had been purified by rearing individual progenies of single females on barley for three generations and selecting only individuals that produced galls. Females used for the tests conducted under natural weather conditions emerged from infested barley stubble and plants collected from the field at the Jemaa Shaim Experiment Domain. H. fly females were also obtained from a pure culture that had been reared in isolation for two generations on 'Nesma' wheat in a greenhouse.

Description of Life Stages and Generation Time of Barley Stem Gall Midge under Controlled Environmental Conditions.

To study the life stages of the BSGM, 'Kanby', a susceptible barley, was seeded in a standard wooden flat (54 x 36 x 8 cm) containing a mixture of 1/3 peat and 2/3 soil, at a rate of 10 rows per flat and 50 seeds per row. The flat was kept in a greenhouse, and when the plants were at the one or two-leaf stage, the flat was moved to an environmental chamber programmed for 18 ± 1°C and a 12:12 (L:D) h photoperiod, with the photophase from 0600 to 1800 h. The flat was caged with a cheesecloth tent and about 200 newly emerged and mated females were released under the tent. Releases were made between 0830 and 0900 h. To obtain offspring of similar age, females were allowed to oviposit for 8 h then removed. Relative humidity was not controlled in the environmental chamber, but the flat was watered regularly to provide adequate moisture for the plants and maintain high humidity.

Starting the third day after infestation, 10 plants were randomly selected and removed daily from the flat. Plants were examined under a microscope (10x) to determine the number of eggs laid on the adaxial and the abaxial leaf surfaces and

the stems. For the first and second instars, plants were dissected and the number of larvae on each plant was recorded. The size (length) of larvae was measured using an ocular micrometer. For third instars and pupae, a dissecting needle was used to remove the puparia and the number and development stage of each stadium were recorded. When adults began to emerge, they were removed daily from the flat. When adult emergence ceased, plants that remained in the flat were removed, and the puparia were dissected to determine the number of third instars that had not pupated.

Comparative Developmental Time of Life Stages of Barley Stem Gall Midge and Hessian fly.

From earlier field observations (Lhaloui & *al.* 1992b), it was noted that the life cycle of BSGM was somewhat longer than that of H. fly. The objective of this study was to compare the developmental time of the life stages of both species. 'Nesma', a wheat cultivar susceptible to H. fly, and 'Kanby', a barley cultivar susceptible to BSGM, were seeded in separate standard greenhouse flats at a rate of ten rows per flat and fifty seeds per row. Flats were kept in a greenhouse until plants were at the two-leaf stage. Flats were then moved to an environmental chamber programmed for 18 ± 1°C and a 12:12 (L:D) h photo-period, with the photophase from 0600 to 1800 hours. Flats were caged with cheesecloth tents, and about 200 newly mated H. fly females were confined for oviposition on wheat, and 200 BSGM females were confined for oviposition on barley. Females were allowed to oviposit for only eight hours to obtain eggs of similar age. Starting on the third day after infestation, random samples of 10 plants were taken daily from each flat. The plants were examined under a stereoscopic microscope (10x) and the number of eggs on the leaves and the stems and the number of larvae and their developmental stages were recorded. At the end of the experiment, data were interpreted to estimate the developmental time of the egg stage, feeding-stage larvae (first and second instars), and the non-feeding or puparial stage (third instar and pupa).

Estimation of maximum potential fecundity and achieved fecundity

To estimate the maximum potential fecundity of the two species, 50 females each of H. fly and BSGM were randomly collected 15 to 20 minutes after eclosion, placed individually in petri dishes containing moistened filter paper, then dissected under a stereoscopic microscope (10x). The number of eggs in the ovaries of each female was counted. The eggs were crushed as they were counted to avoid errors.

To estimate the achieved fecundity, post reproductive females were dissected and examined for eggs that remained in their ovaries. The difference between the maximum potential fecundity and the number of eggs remaining in the ovaries of spent females was used to estimate the achieved fecundity.

Determination of Sex Ratios of Barley Stem Gall Midge and Hessian fly.

The sex ratio of H. fly has been extensively investigated in the United States (Painter 1930, Gallun & *al.* 1961, Stuart & Hatchett 1991). The H. fly reproduces by unisexual families; most progenies are either all males or all females. Families of bisexual predominantly female or predominantly male progenies are produced at low frequencies. However, the sex ratio of BSGM has not been studied. Although the sex ratio is species specific and the sex ratio of H. fly in Morocco should be

similar to that of H. fly in the United States, where it has already been studied, the sex ratio of the Moroccan H. fly was investigated for comparison with that of BSGM.

To determine the sex ratio of BSGM, single newly mated females were confined individually on seedlings of 'Kanby' barley grown in small plastic pots. Pots were covered with clear plastic cages having a cheesecloth top for ventilation and placed in an environmental chamber programmed for 18 ± 1°C and a 12:12 (L:D) h photoperiod with the photophase from 0600 to 1800 hours. The females were allowed to oviposit until they died. The pots were caged throughout the experiment. When adults began to emerge, they were removed from the cages daily, counted, and sexed. The progenies of 200 randomly mated females were examined. Data were analyzed to determine the sex ratios for each progeny and the proportion of the progenies that were unisexual or bisexual.

To determine the sex ratio of H. fly, the same experiment was repeated confining single mated females of H. fly on 'Nesma' wheat seedlings for oviposition. Data were recorded and the progenies were classified in the same way as described for BSGM.

Duration of Life Cycle and Number of Generations of BSGM under Field Weather Conditions.

This experiment was conducted to determine the duration of the life cycle and the number of BSGM generations per year under natural weather conditions. Females used in this experiment emerged from infested barley stubble and plants collected from the field. Barley stubble infested with puparia was collected from the field in early October, taken to the laboratory and examined for BSGM puparia. Puparia not embedded in galls were removed from the stems. Afterwards, the infested stems were placed in empty wooden flats and covered with cheesecloth. The flats were placed outdoors and exposed to natural weather conditions. The stubble was sprinkled daily with water to prevent puparia from desiccating.

After the first significant rainfall in mid-October, adult emergence of the oversummering generation was monitored daily in the collected stubble and by examining newly emerged barley seedlings in the field for eggs. At the same time, 'Kanby' barley was seeded in standard wooden flats and grown in a greenhouse. When first eggs were observed on field plants, three flats of barley in the one-or two-leaf stage were placed outdoors, near the greenhouse. The flats were caged with a cheesecloth tent, and the newly mated females that emerged from the stubble were collected and confined for oviposition on the plants. To simulate natural conditions, the flats were infested over 5 days and females were allowed to oviposit until they die. A total of about 200 females were confined for oviposition on each flat. The flats were kept caged throughout the experiment to prevent infestation from field populations of *Mayetiola* species. Plants were exposed to all natural weather conditions, although flats were watered as needed to maintain healthy plants.

Starting on the third day after infestation, plants were sampled for eggs and larvae. When adults of this generation began to emerge, they were removed from the flats each morning to prevent new oviposition. At the same time, a new set of three flats

seeded to barley were again taken out of the greenhouse and placed close to the first set. Also infested barley plants were again collected from the field and examined to eliminate all puparia that were not associated with galls. Plants were placed in flats and observed for adult emergence in the same way as described for the oversummering generation. Females that emerged from these plants were used to infest the new set of flats. Plants were infested, sampled, and observed in the same way as described for the first generation. Data were taken in the same way as described previously.

At the end of the second generation, three other flats seeded to barley were again taken out of the greenhouse and placed close to the first flats. Plants of the new flats were infested and sampled and data were recorded in the same way as described for the second generation. These data were used to describe the third generation.

For all three generations, after the peak of adult emergence, the plants remaining in the flat were removed and dissected in the laboratory to estimate the number of third instars that did not emerge (went into diapause). This experiment was repeated over 3 seasons, representing average weather conditions of the Chaouia region. Data is summarized in this work.

Statistical analysis

All Data were analysed using proc GLM (SAS Institute). Angular transformation was performed on all the percentages, pre-transformed data is reported. Means were separated using Fisher's Least Significant Difference (LSD) test at P=.05.

RESULTS AND DISCUSSION

Description of Life Stages and Generation Time of Barley Stem Gall Midge under Controlled Environmental Conditions.

Description of Egg Stage

Eggs of BSGM are very similar in appearance to H. fly eggs. The egg is approximately 0.5 mm in length, slender, glossy, and pale red in color, becoming deeper red near eclosion. Eggs are laid in the grooves between the longitudinal veins of leaves. Larvae that eclose from eggs laid on the adaxial leaf surface migrate down behind the leaf sheath to the base of the stem where they feed. Under the environmental conditions of this experiment, a few eggs hatched 7 days after oviposition and neonate larvae were observed migrating down the leaves and stems, but none had reached the base of the stem. Most of the eggs hatched 8 days after oviposition (Table 1).

Description of Feeding-Stage Larvae

First instar.

At eclosion, first instars are the same size and color as the eggs. After eclosion, neonates were observed crawling down the leaves, guided by the grooves between the leaf veins. When larvae reached the leaf sheath, they moved over or around the sheath collar and crawled behind and downward between the leaves until they reached the base of the stem where they began to feed. Some first instars were not able to get beyond the leaf ligule and were found dead at this site. Also, many larvae were found dead on the outside surface of the stem. These larvae eclosed from eggs that were either laid on the stem itself or on the abaxial leaf surface. The first instar required about 9 days to complete development (Table 1) and most larvae molted to

second instars 10 days after they eclosed from the eggs. After the first 3 days of feeding, first instars turned completely white and were about 1.0 mm in length (Fig. 1). The larvae gradually increased in size and reached about 1.4 mm in length as full-grown first instars. At this time, a small depression and a discoloration of plant cells were observed at the feeding sites, but larvae were not firmly attached to the plant tissue.

Second instar.

Newly molted second-instar larvae measured 1.6 to 1.8 mm, but soon after molting, they increased in size to about 2.0 mm. Also, these larvae had deep, well defined metameres, but became completely smooth and cylindrical after 3 to 4 days of feeding. Depending on larval density, second instars gradually increased in size and were 2.5 to 4.0 mm in length. The second instars completed development in 11 to 12 days. During this stadium, gall tissue gradually formed around the larvae and each larva became completely embedded in a pea-sized gall. Second instars were also able to survive on leaves. Apparently, some first instars initiate feeding on leaves while still enclosed inside the whorl. As these leaves grow, larvae are carried upward with the emerging leaf and continue to feed and develop normally. These larvae also formed galls on leaves. The gall tissue may protect the exposed larvae from desiccation which allows the larvae to survive and develop on the leaves. H. fly larvae have never been reported to survive on leaves.

Second instars of BSGM are also morphologically similar to the second instars of H. fly when observed with the naked eye. But microscopic examination revealed that the second instar of BSGM has no spicules on the anterior abdominal sternites, whereas the second instar of H. fly has dense spicules. These findings suggest that the almost complete lack of spicules on BSGM larvae may explain why larvae adhere tightly to the plant tissue (Gagné & *al.*, 1991).

Description of Non-feeding Stage.

This stage contains the third instar and the pupa, both of which are enclosed in a puparium (the hardened cuticle of the second instar), thus the name flaxseed given to this stage.

Third instar.

This instar had well defined segments and was shorter than the second instar, measuring 2.5 to 3.5 mm (Fig. 1). Similar to that observed for H. fly third instar, the larva reverses its position in the puparium so the adult will be oriented head upward for emergence and escape from the plant.

Most nondiapausing third instars developed into pupae 10 days after they molted (Table 1). About 7% of the larvae failed to pupate. This suggests that even in a favorable environment, a small proportion of third instars enter diapause. This behavior may be a genetic trait that prevents all individuals of a specific brood from emerging. Diapausing third instars may survive more than a year and pupate only during the next favorable cropping season. The same behavior has also been observed for Hessian fly (McColloch 1930, Painter 1930). The termination of diapause in cecidomyiids is usually well timed to the host's biology (Gagné 1989).

Pupa

The developmental period for the pupa was about 12 days. During this time, the pupa gradually transforms into an adult. The puparium of the BSGM (being the second instar cuticle) also differs from that of H. fly by the absence of spicules at the posterior ventral end (Gagné & *al.*

1991). The puparia of H. fly are covered entirely with spicules. The lack of spicules allows the puparia to adhere tightly to the plant tissue, in contrast to puparia of H. fly which readily come loose from plant tissue (Gagné & *al.*, 1991). This difference in puparia attachment to plant tissue is very reliable in distinguishing the two species without having to utilize a microscope.

Adult

Adults of the BSGM resemble adults of H. fly in many ways. They are grey and have a mosquito-like form. The adults do not feed and are short-lived. Mated females live for 2 to 3 days, although unmated females may live for 5 to 7 days. Males also live for 4 to 7 days. Males have two peaks of eclosion; some eclose in the afternoon between 1600 and 1800 h and some eclose the next morning at the same time as the females. Females eclose in the morning between 0700 and 0930 h. If males are present, mating may occur within 30 to 60 minutes after eclosion. After mating, females sit for two to four hours before they start ovipositing. The oviposition period usually lasts over two days (Lhaloui 1995). BSGM adults can be readily distinguished from H. fly adults in the morphology of the gonostyli in males and the morphology of the sixth through eighth abdominal tergites in females (Roberti 1953, Alfaro 1955, Gagné *& al.*, 1991).

Results indicated that the generation time for non-diapausing BSGM (from egg to adult) is about 45 days at 18 ± 1°C, and 12:12 (L:D) h photoperiod. However, adult emergence occurred over an extended period after peak emergence. Adults continued to emerge for more than one month after peak emergence, which indicates that third instars vary considerably in their developmental time. The condition of the plant and the site on the plant where the second instar feeds may play a role in the duration of third instar development. It was noted that when larvae were present at high densities (10 to 15 larvae per plant) and plants were in poor condition, the larvae developed into third instars and pupae much faster than when larvae were at low densities and plants were in good condition. Similar results were described by El Bouhssini & *al.* (1996) who reported that growth and development of H.fly larvae are density dependant.

Table 1. Duration of Developmental Stages of Barley Stem Gall Midge.

Stage or instar	**No. days to complete development**
Egg	7.2 ± 0.5 d*
First instar	8.7 ± 0.7 c
Second instar	11.7 ± 0.8 a
Third instar	10.1 ± 0.8 b
Pupae	11.6 ± 1.1 a
Adults	
Females	5-7[a]
Males	4-7[a]

*Means ±SD, No. of days for instars (stages) to complete development and molt into next instar (stage). Means within the column followed by the same letter are not significantly different (Fisher's Least Significant Difference (LSD) test. P = 0.05 [SAS Institute]).

[a] Number of days adults lived.

Comparative Developmental Time of Life Stages of BSG Midge and H. Fly.

Egg Stage.

Under the conditions of the experiment, 70% of the eggs of H. fly developed and eclosed into first instars in 4 days, and by the next day, 100% of the fertile eggs had eclosed and first instars had migrated to their feeding position. Eggs of barley stem gall midge took longer to complete their development. Ninety-two percent of the eggs eclosed after 7 days of development and the remaining eggs eclosed on the next day (Fig. 1 & 2).

First instar.

The developmental time of first instars of both species were significantly different (Fig. 1 & 2). A few first instars of H. fly molted to second instars 7 days after egg eclosion, and 100% of the larvae had molted 8 days after egg eclosion. Gagné & Hatchett (1989) reported that at 20 ± 1°C the developmental period of the first instar was 6 to 7 days. For BSGM, 92.5% of the first instars reached second instars 10 days after egg hatch and about 10% after 9 days (Fig. 1 & 2).

However, first instars of both species increased in size to 1.5 mm before they molted into second instars. Neonates of both species grew to almost twice their size after feeding for 3 days. At this time, they became translucent white and lost their reddish color. Larvae became more cylindrical and their body segments were less distinct.

Second instar.

Second instars of H. fly developed over 6 days and the cuticles became brown on the seventh day (puparia).

At 20°C, Gagné & Hatchett (1989) reported that the second instar developed over 4 to 5 days. Second instars of BSGM developed over 12 days (twice as long as for H. fly). These results indicate that under the environmental conditions of this experiment, the feeding stage of BSGM lasts from 20 to 21 days, while that of H. fly lasts for about 14 days (Fig. 1 & 2).

The Non-feeding Stage.

Third instar.

H. fly third instars developed over a period of 8 days. BSGM third instars developed over a period of 10 days. Larvae of both species reversed their position inside the puparia, so the emerging adults would be oriented with their heads upwards (Fig. 1 & 2).

Pupa.

The pupal stage took 6 days to complete development for the H. fly, while the pupal stage of the BSGM required 12 days (Table 4). These results indicate that like for the feeding stage, the non-feeding stage of BSGM develops over a longer period than that of H. fly (22 vs. 14 days). (Fig. 1 & 2).

In summary, at a constant temperature of 18 ± 1°C, 12:12 (L:D) h photoperiod, BSGM requires 45 days to develop from egg to adult, but the H. fly requires only 32 days. The largest difference in the developmental time was recorded for the egg stage, the second instar, and pupa.

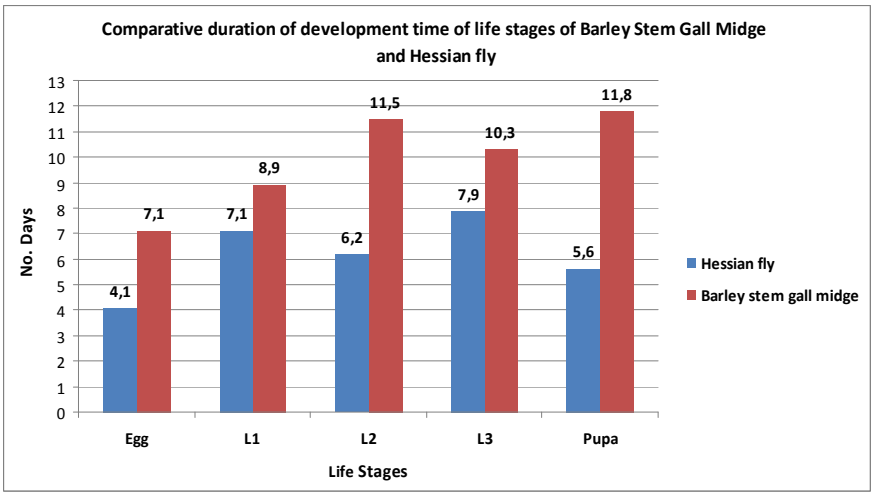

Figure1. Comparative duration of developmental time of life stages of BSGM and H. fly at 18 ± 1°C and 12:12 (L:D) h photoperiod

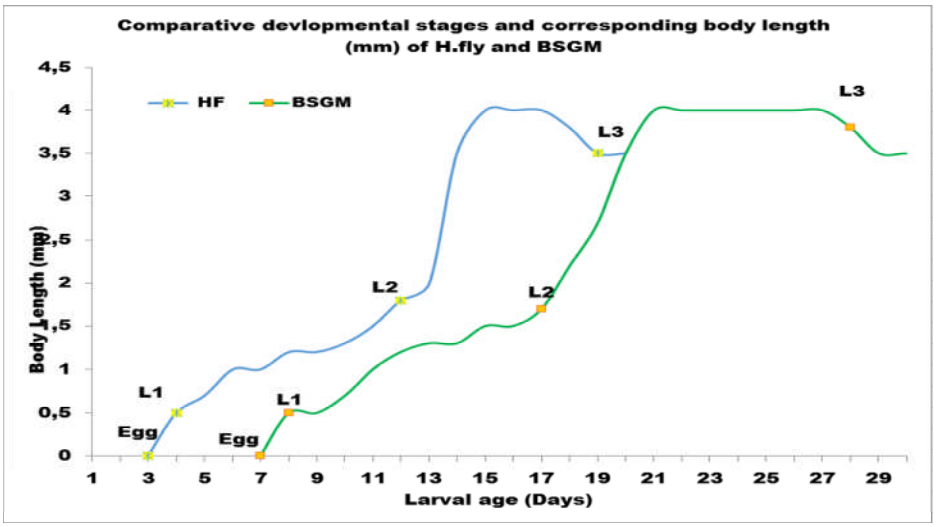

Figure 2. Development of eggs and instars (L1, L2, and L3) and size (length) of instars of BSGM and H. fly.

Distribution of Eggs on Host Plants.

BSGM and H.fly females have very different behaviors in selecting ovipositional sites on their host plants. BSGM females deposited 61.5% of their egg complements on the adaxial leaf surface, 20.8% on the abaxial surface, and 17.7% on the stem. In contrast, H. fly females laid 91.4% of their egg complements on the adaxial leaf surface, 7.1% on the abaxial surface and only 1.5% on the stem (fig. 3). This indicates

that, unlike H. fly females, BSGM females lay a high proportion of their eggs on the abaxial leaf surfaces and stems of their hosts. Thus, BSGM females have a much lower ovipositional efficiency than H.fly because only first instars from eggs laid on the adaxial leaf surface survive. BSGM females are less able to discriminate between the adaxial and the abaxial leaf surface or the stem as ovipositional sites than H. fly females. BSGM females laid about 40% of their total egg complement either on the abaxial leaf surface or on the stem. Larvae emerging from these eggs would crawl down to the ground and perish. These results corroborate those described by Ming *& al.* (2009) that indicate that Barley is a less suitable host for H. fly and that a high proportion of eggs is laid on the abaxial leaf surface and the stem. Tamer *& al.*, (2015) also showed that BSGM populations in Syria lay a high proportion of their egg complement on the abaxial leaf surfaces and stems of barley.

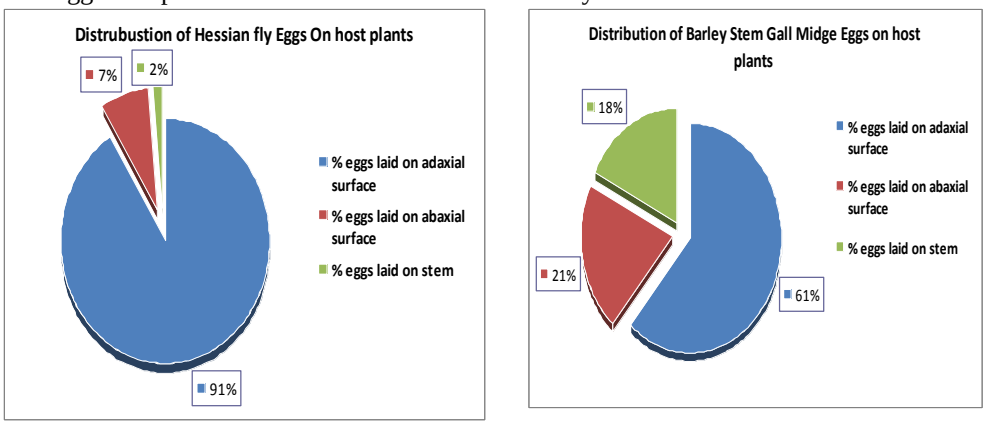

Figure 3. Comparative distributions of Barley stem gall midge and Hessian fly eggs on host plants

Fecundity.

The maximum potential fecundity of BSGM was 190.2 ± 81.3 eggs per female and significantly lower than the fecundity of H. fly, which averaged 230.1 ± 61.4 eggs per female (Table 2). For both species, the actual fecundity was the same as the maximum potential fecundity. Dissection of post-reproductive females (spent females) revealed that no significant numbers of eggs remained in the ovaries. A few females still carried one or two eggs, but most had none. Harris and Rose (1989) also reported that H. fly females lay their full egg complement before they die.

Table 2. Maximum Potential Fecundity of Barley Stem Gall Midge and Hessian fly.

Species of *Mayetiola*	Barley stem gall midge	Hessian fly
Mean no. of eggs	190.2 ± 75.91 b*	230.1 ± 61.4 a

**means ± SD. Means within the row followed by the same letter are not significantly different [Fisher's Least Significant Difference (LSD) Test at P=0.05 (SAS Institute)].*

Sex Ratios of Barley Stem Gall Midge and Hessian fly.

BSGM.

Results revealed that like many other cecidomyiids, the BSGM breeds mostly by unisexual progenies. Four progeny classes were observed. Of the 189 progenies examined, 64 (33.9%) were all female progenies, 60 (31.8%) were all male progenies, 36 (19.1%) were predominantly female progenies, and 29 (15.3%) were predominantly male progenies. Of the unisexual progenies, about half were female (51.6%) and half were male (48.4%). Similarly, 55.4% of the bisexual progenies were predominantly female progenies and 44.6% were predominantly male progenies (Tables 3 & 4). The mean number of females per female progeny was similar to that of males in the male progenies. The average number of males in the predominantly female progenies, and number of females in the predominantly male progenies were also similar (Table 3). In general, females produced an average of 54.2 adults per progeny; and overall, 54.4% of all individuals were females, and 45.6% were males. This indicates that even though BSGM reproduces mostly by unisexual progenies, the sex ratio is about 1:1.

H. fly.

The sex ratio of H. fly was similar to that observed for BSGM. Of the 196 progenies examined, 75 (38.3%) were all female progenies, 62 (31.6%) were all male progenies, 31 (15.8%) were predominantly female progenies, and 28 (14.3%) were predominantly male progenies. Like for BSGM, about half of the unisexual progenies of H. fly were female (54.7%), and half were male (45.3%). Also, 52.5% of the bisexual progenies were predominantly female progenies and 47.5% were predominantly male progenies (Tables 3 & 4). The average number of females per female progeny was similar to that of males per male progeny. The average number of males in the predominantly female progenies and number of females in the predominantly male progenies were also similar (Table 3). Overall, females produced an average of 63.1 adults per progeny; and of the total number of individuals produced by all females, 56.5% were females, and 43.5% were males, indicating that the sex ratio was also about 1:1.

Overall, results showed that the sex ratio of BSGM is similar to that of H. fly. BSGM also breeds mostly by unisexual progenies and has a sex ratio of about 1:1. These results corroborate those described earlier by many authors on H. fly in the United States (Gallun & *al*., 1961; Stuart & Hatchet 1991).

Table 3. Sexual characteristics (numbers and sex of adults) of progenies of randomly mated females of Barley Stem Gall Midge and Hessian fly in Morocco.

	Progeny Class			
Mayetiola Species	Unisexual progenies		Bisexual progenies*	
	Female	Male	No. female > male	No. male > female
Barley Stem Gall Midge	57.1 ± 18.3a	52.8 ± 16.9	47.6 ± 17.5 > 5.8 ± 3.5	44.7 ± 19.4 > 7.1 ± 4.2
Hessian fly	68.1 ± 22.4	58.9 ± 15.1	51.7 ± 15.9 > 7.2 ± 3.1	53.6 ± 19.9 > 9.8 ± 4.8

*Female > male designates predominantly female progenies; male > female designates predominantly male progenies.

[a] Numbers represent mean ± SD of individuals per progeny

Table 4. Frequencies (percentages) of progeny classes of randomly mated females of Barley Stem Gall Midge and Hessian fly progenies in Morocco

	Progeny Class			
Mayetiola Species	Unisexual progenies		Bisexual progenies*	
	% Female progenies	% Male progenies	% progenies with No. female > male	% progenies with No. male > female
Barley Stem Gall Midge	51.6[a]	48.4	55.4	44.6
Hessian fly	54.7	45.3	52.5	47.5

*Female > male designates predominantly female progenies; male > female designates predominantly male progenies.

[a] Numbers represent percentages.

Duration of Life Cycle and Number of Generations under Field Weather Conditions.

Under the prevailing weather conditions of the Chaouia region of Morocco, BSGM has three generations, two complete and a third partial one.

First generation.

The first generation starts late October, about two weeks after the first significant rainfall. The first infested barley seedlings are observed in the field at the end of October. However the rate of adult emergence and seedling infestation is very slow. The peak of adult emergence in the field and collected stubble was observed during the second week of November. Sampling of infested plants showed that 100% of BSGM population was in the egg stage until mid-November when the first egg eclosion occurred. First instars were observed for about two weeks, until the end of November. Few larvae developed into second instars around November 25 (2.1%), and the percent of second instars in the population increased and reached 100% around the end of November. For about a week, all the population was in the second instar. The earliest third instars were observed by the end of first week of December. However, not all second instars completed development at the same time; many required a longer developmental time and were observed 20 days after the first third instar was observed. Gradually, the population of second instars decreased and that of third instars increased. This suggests that in addition to the five days difference in age that the population started with at the egg stage, second instars had different growth rates. The exposure to fluctuating day and night temperatures, the various larval densities, and the condition of the plants on which larvae were feeding may have been the major factors affecting the growth rate of these larvae. The developmental time of third instars were also stretched over a long period. Even though the highest percent of third instars was observed from second to third week of December, third instars were observed all through the generation time and after one hundred days since infestation (around mid February), about 6% of the population was still as third instars that failed to emerge. The first pupa was observed on mid December, and the first adult emerged at the end of December. Adult emergence peaked during the second week of January (Fig. 4). Likewise, the pupal stage and adult emergence were stretched out over a long period. Adults emerged for about a month after the peak emergence occurred. The remaining plants were examined by the end of June, and results

showed that about 6% of the population was still at the third instar.

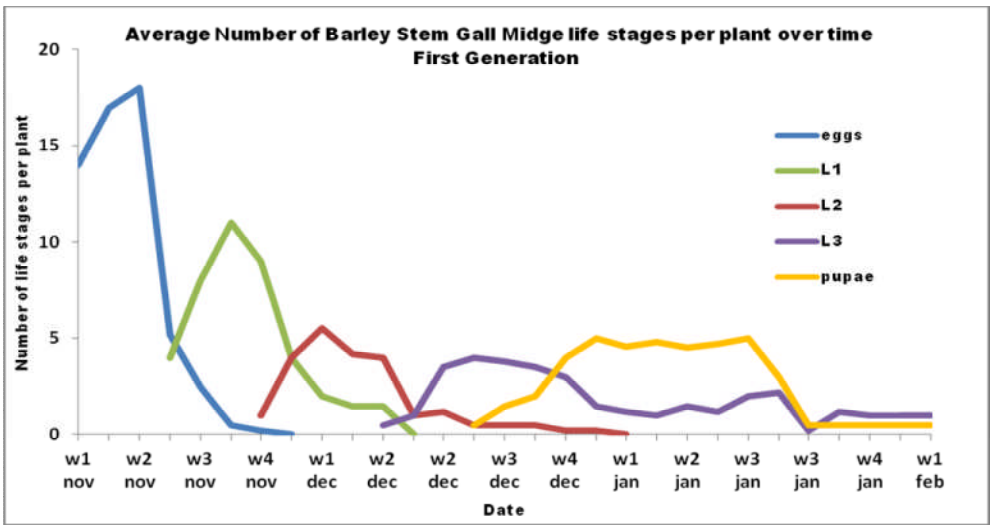

Figure 4: Developmental time for first generation of the barley stem gall midge in the Chaouia region, Morocco.

Second generation.

The second generation started early January. The peak of first generation adult emergence in the field was observed during the second week of January. Infested plants were collected from the field and adults that emerged from these plants were used to infest three new flats.

The earliest first instars of the second generation were observed starting the third week of January, about 11 days after infestation. This indicates that eggs required longer to complete development under the colder temperatures and shorter days of January than under the warmer temperatures of November. Most eggs eclosed during the fourth week and by the end of January, 100% of the population was in the first instar. First instars developed for about 10 days; and individuals issued from the earliest eclosions turned into second instars by about February 10. However, first instars are observed until about the end of third week of February. These individuals may have issued from later infestations (eggs laid by late emerged adults). At this time, all larvae in the experiment had turned into second instars. The earliest third instars were observed starting fourth week of February. But most second instars developed over a longer period than during the first generation; few were observed until mid-March, thus required about one month to complete development (Figure 5). Like discussed for the first generation, the developmental time of third instars was stretched over a long period, and about 80% of the population was in the third instar by mid march, the other 20% were already in the pupal stage. The first pupae were, however, observed during first week of March, and the first adults emerged at the end of second week. By the end of March, all pupae had turned into adults and adult emergence stopped completely. All larvae that remained in the plants were third instars that failed to pupate, and apparently went into

diapause. The examination of the puparia by the end of April revealed that aboutone third of the population were in the third instars. These constitute the proportion of individuals that did not emerge as adults.

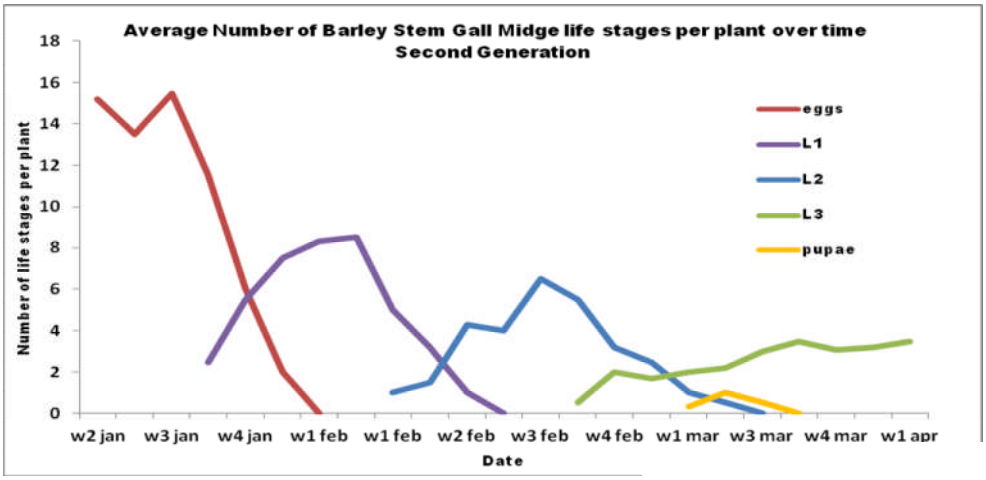

Figure 5. Developmental time for second generation of the barley stem gall midge in Settat region, Morocco.

Third generation.

Since the second generation adults were abundant during fourth week of March, plants designated to study the third generation were infested during the end of third week. The eggs of this generation eclosed in 7 to 8 days like for those of the first generation. First instars were observed from late march until mid April, when almost 100% of the population turned into second instars. The earliest second instars were, however, observed at the end of first week of April. The earliest third instars were observed starting the third decade of April, and near the end of April, more than 85% of the population was in the third instar. Unlike for the first two generations, the growth rate of second instars was less stretched out in time. All the population turned into thirds instars by the end of April. Also all third instar failed to pupate; none developed into pupae (Figure 6). This indicates that third instars of this generation all go into summer diapause. Plants of this generation were examined through the end of June, and results revealed that all individuals were third instar. This indicates that all larvae of the third generation would diapause as third instars, and not resume development until the next fall, when the environmental conditions are favorable for the host growth. However, the rate of parasitism started building up from early May and reached a mean of 64.2% of the population by the end of June. Only 35.8% of the population was not parasitized (third instars). The examination of the puparia collected from the field just before emergence of adults of the oversummering generation revealed that 8.7% of the puparia existing on all stems had either third instars or pupae, the remaining puparia were

empty, and had either BSGM adult emergence splitting, or parasite emergence holes.

In the field, it was noticed that the third generation of barley stem gall midge was very light; only very rare larvae were found on the youngest barley tillers. This could mainly be explained by the fact that most barley plants were in the full maturity phase by the end of March, when adults of the second generation were emerging. In Morocco, barley cultivars grown in the coastal plains have spring growth habits, and mature completely by late April. Oversummering larvae of barley stem gall midge terminate diapause and resume development only in the following fall after the first significant rainfall, when barley seedling of new barley crops or volunteer barley, are emerging in the field.

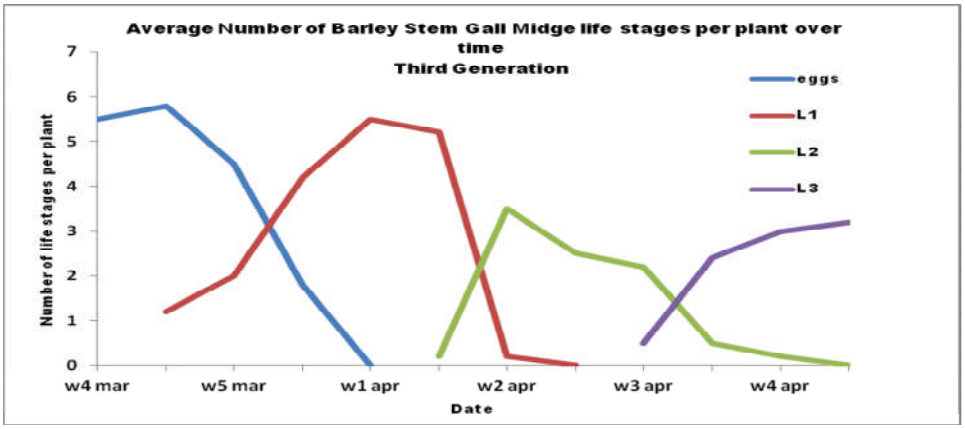

Figure 6. Developmental time for third generation of barley stem gall midge in Settat region, Morocco.

In summary, these results clearly show that the life cycle of the BSGM is very well synchronized with its host, barley. Gagné (1989) has reported that in deed, the life cycle of all Cecidomyiids is very well synchronized with that of their hosts. In the field, the peak of adult emergence of the oversummering BSGM generation was observed when barley seedlings were emerging in the fall, after the first significant rainfall. This behavior shows that BSGM is closely linked to its host, barley, and is active in the field only when this host is available (Gagné & *al.*, 1991). Similar behavior was reported for H. fly, demonstrating host specificity of this pest (Harris & Rose 1989). The timing and duration of the BSGM developmental generations were also similar to those described for H. fly (Coutin 1974, Lhaloui 1986, Lhaloui & *al.*, 2005), as both wheat and barley grown in Morocco have similar physiology and habits; both are spring crops with winter habits, seeded in the fall. BSGM and H. fly are sympatric species of these two crops in Morocco (Gagné & *al.*, 1991).

CONCLUSION

This study revealed several developmental similarities between the BSGM and H. fly. Like for H. fly, the

BSGM has 2 feeding instars and a third non-feeding one, reproduces mostly by unisexual progenies, and has a sex ratio of about 1:1. The BSGM also develops over 2 complete generations and a third partial one because most barley matures by late April and is no longer a suitable host. However, few discrepancies were recorded; some life stages take longer to grow and complete development than for H. fly

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