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Population dynamics of the weevil *Datonychus melanostictus* (Coleoptera: Curculionidae) on spearmint crop in Chaouia region, Morocco

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Abstract

The mint rhizome borer, *Datonychus melanostictus* (Marsham, 1802), is a new pest of spearmint crop in Chaouia region north central of Morocco. The main damage is caused by the larvae, which tunnel inside the rhizomes. In this study, we investigated the population fluctuation of *D. melanostictus* in spearmint fields during two seasons 2013 and 2014 using two sampling methods. The emergence of weevil begins in late March, followed by an increase of egg laying activity during May. A strong larval activity takes place at rhizomes below ground during late May to early June. Pupation takes place mainly inside the rhizomes. New generation adults reached three peaks between June and early August 2013 and two peaks during June and July in 2014. The highest larval density recorded before flowering resulted in significant reduction of the average fresh yields to 6.6 t/ha. Protection of mint fields against *D. melanostictus* is a daily challenge, population dynamics can be a tool to predict and to help farmers for applying protection methods in the best way

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Introduction

Morocco is the second most biologically diverse country in terms of species in the Mediterranean region, with 4500 species of vascular plants including 800 endemics, of which about 600 species are known for their medicinal use and/or aromatic properties (Montanari, 2014; Rankou *et al.,* 2013; Benabid, 2000). The genus *Mentha* of the family Lamiaceae, includes more than 25 known species. Commercially, the most important mint species are peppermint (*Mentha piperita* L.), spearmint (*M. spicata* L.), and corn mint (*M. arvensis*) (Kizil and Tonçer, 2006; Lawrence, 2007). In Morocco, spearmint is one of the aromatic plants that is largely consumed, daily preferred for flavoring tea, but is also known for its use in food, medicinal and aromatic purposes. It occupies an area of about 5510 ha and provides an average production of 91 658 t (FAOSTAT, 2017). The Settat province is ranked highest in the production of this crop in the country. However, this crop is liable to attack by several insect pests, diseases and weeds (El Fadl and Chtaina, 2010; El Fakhouri *et al.,* 2015; 2019). A broad diversification of pesticides is used to control all these pests; 27 active ingredients of insecticides and 11 fungicides belonging to different chemical families. Most of the commonly applied pesticides are not registered for mint; some have pre-cut duration exceeding 21 days, and others are now prohibited in some countries (El Fakhouri *et al.,* 2015; 2020a).

The most important pests of the mint crop in Chaouia region are the foliage feeding insects, a complex of Lepidopteran pests, the flea beetles, and aphids. For diseases rust and powdery mildew are the most common (El Fadl and Chtaina, 2010; El Fakhouri *et al.,* 2015). To this arsenal of enemies, joins a new insect pest identified as *Datonychus melanostictus* (Marsham, 1802) in mint growing areas of Chaouia region (El Fakhouri *et al.,* 2015; 2019). The weevil causes severe damage especially by larvae which feed on stems and rhizomes below ground. The most important insect pests in mint crop are subterranean feeding on the roots and crowns or boring into the underground rhizomes. In this category, Lacey & Kaya (2000) reported in western United States several insect pests, such as the mint root borer larvae of *Fumibotys fumalis* (Lepidoptera: Pyralidae), the strawberry root weevil *Otiorhynchus ovatus* and black vine weevil *Otiorhynchus sulcatus* (Coleoptera: Curculionidae), and the mint flea beetle *Longitarsus ferrugineus* (Coleoptera: Chrysomelidae).

D. melanostictus is native to Europe and North Africa (Dieckmann, 1972) with geographic distribution also in Western and Central Asia (Colonnelli, 2004). The weevil lives on Lamiaceae, *Lycopus europaeus* L., *Mentha longifolia* L. (wild mint), *Mentha suaveolens* Ehrh. (round-leafed mint), *Mentha aquatica* L. (water mint) (Delbol, 2008).

There is no insecticide registered against root and stem feeding insects on mint crop in Morocco, so the only strategy available to farmers for reducing the damage caused by *D. melanostictus* is an application of a broad-spectrum foliar and systemic insecticides increasing the residue problem on mint in the last few years (El Fakhouri *et al.,* 2020 b). Given the damage and the misuse of insecticides used against this weevil, it is necessary to know its bioecology and its population dynamics to develop the best strategy of management.

Materials and methods

Experimental sites

The experiments were conducted in two fields of spearmint crop near the town of Ouled Said located 21 km west of Settat city, 33°03’59’’N 7°53’05”W) and (33°04’05.0”N 7°53’13.6”W), with a history of weevil *D. melanostictus* occurrence throughout the growing season 2013 and 2014. The field studies of both years were conducted in 10 plots (10 m ×10 m for each plot) leaving 2 m between plots. Young shoots are planted with spacing of 30 cm between rows and 30 cm within rows. The middle of each plot (4 m²) was not sampled to take yield components. Cattle manure was used before planting with 30 t/ha and nitrogen fertilizer (urea 46%) was applied with average of 200 kg/ha in between mint harvests. Drip irrigation was applied two to three times per week and weeding was done when needed. No pest control products were applied for both fields.

Direct sampling

Quadrat sampling of 0.5×0.5 m was used in random way. Sampling was done by taking 10 quadrats by digging the soil samples and by tearing all mint plants from the root to the stem of each sample. The evaluation of the presence of the weevil was carried out by shaking the soil surrounding the roots of mint plants also by beating the mint foliage causing the weevils to drop to the soil and inspecting the soil surface. The mint plants with soil were bagged, labelled, and returned to the laboratory to dissect carefully with scissors under a binocular from the root up to the stem. Different notations were performed weekly by examining 10 quadrats sample. The number of adults, larvae and pupae per m² counted from each quadrat sample (leaf, stem, rhizome and soil samples). The number of laying holes per stem and exit holes per rhizome were counted. The identification of the weevil in the laboratory was mainly based on external morphological characters using the identification keys (Hoffmann, 1958; Dieckmann, 1972; Tempere and Pericart, 1989).

Traps for monitoring adults

Ten circular (20 cm diameter and 7 cm deep) yellow traps (YWT) were chosen for their effective attraction for *Ceutorhynchus* species (Smart *et al.,* 1997; Laska *et al.,* 1986). The yellow traps used in the study contained 75% water and 25% propylene glycol as a preservative, which was changed weekly. The traps were placed in soil in the middle of each plot and attached to stakes that allowed for the traps to be moved upward to the top of the plant canopy as the plants grew, YWT were set 20, 40, 80, 120 cm. These traps were monitored weekly to make notations on the dynamics of the adult weevils. All adults collected were placed into vials with 70% ethanol for preservation.

The monitoring of all insect stages per m² and per trap were followed under the climatic conditions described in Tables 1 and 2 throughout four harvesting times for both 2013 and 2014. The first mint cut (2 February to 15 April), the second (16 April to 1 June), the third (2 June to 20 July), fourth and the last harvest (21 July to 27 September).

Data on yield

The fresh mint plants were harvested by taking 1 m² × 4 on each plot (fresh mint: stem + leaf), considering two harvest periods (pre-flowering and full flowering stage). The first-year harvest was done on 01 June 2013 at pre-flowering stage and on 20 July 2013 at full flowering stage. The second-year harvests were done on 3 June 2014 and 26 July 2014 for pre-flowering and full flowering, respectively with aim to evaluate the impact of the weevil infestation on plant height and fresh herbage yield.

Statistical analysis

Data on the different stages (adult, larva and pupa) of the weevil, laying holes per stem and exit holes per rhizome, plant height and fresh herbage yield were analyzed using harvest time as the

Table 1. Weather conditions in the Settat region during the 2013 cropping season.

Cropping season 2013	January	February	March	April	May	June	July	August	September
Mean temperature °C	16	17	19	21	20	26	30	32	30
Max temperature °C	20	22	22	27	27	33	37	39	33
Min temperature °C	12	13	15	15	14	19	23	26	27
Total rainfall (mm)	29.9	17.8	92	50.6	0	0	0	0	6.3
Photoperiod (LD)	10:14	11:13	12:12	13:11	14:10	14:10	14:10	13:11	12:12¥

main factor. All data were analyzed by individual one-way ANOVAs using Genstat-18.1 software for statistical analysis. The differences among the mean values were tested using the Fisher test ($P<0.05$). The simple correlation coefficient *r* between number of adult weevils collected per m² and per YWT were calculated.

Results

Monitoring adult weevil

Adult weevils of *D. melanostictus* have round black bodies tinted with grey and brown with average size of 2.95 mm (2.4-3.5 mm length), covered with short squamules, rounded, whitish, or greyish, with a long rostrum curved and brown antennae. The legs are black brown, marked by a strong toothed femur with toothed tarsus as distinctive characteristic of the species. This small weevil began to appear in quadrat and YWTs on 22 March 2013 (Figure 1) under photoperiod 12L: 12D and mean temperature 19°C (Table 1). In the second year, the first adult was captured on 29 March 2014 (Figure 2) under photoperiod 12L: 12D and mean temperature of 15°C (Table 2).

The number of adult weevils increased gradually with increasing temperature and photoperiod, to achieve three peaks of infestation in 2013. The first recorded as maximal value on 28 June with 16 adults/m² reached at average temperature of 29°C and photoperiod 14L:10D. The second adult peak recorded on 19 July with 10 adults/m². For the third peak 7 adults/m² was recorded in early August. The number of adult weevils was high in the field in June and July 2014 (Figure 2). Two peaks were recorded, the first on 14 June with 13 adults/m² at mean temperature of 32°C and photoperiod 14L:10D. The second peak occurred on 12 July with 10 adults/m².

The yellow water traps appeared to be less attractive to the adults *D. melanostictus*, reaching the maximum value of 6 and 7 adult weevils/week on 21 June in both years 2013 and 2014, respectively (Figures 1 and 2). The data of the current study showed that the total number of adult weevils was much higher in the quadrat than in the yellow water traps. The total number of weevils registered using the direct sampling reached 108 and 89 adult weevils/m², instead of 60 and 52 adult weevils caught using YWTs in both years 2013 and 2014, respectively.

The abundance of *D. melanostictus* adult weevils was significantly positively correlated ($p<0.001$) for quadrat and YWTs ($r=0.7$, $r=0.78$) in 2013 and 2014, respectively.

The larvae and pupae populations

The number of laying holes in the stems increased significantly during May in 2013 and 2014. After hatching, the larvae bore into and feed on rhizomes of mint plants from Mid-March. The larvae

Table 2. Weather conditions in the Settat region during the 2014 cropping season.

Cropping season 2014	January	February	March	April	May	June	July	August	September
Mean temperature °C	16	16	20	24	25	26	27	30	30
Max temperature °C	20	20	25	30	33	33	34	36	33
Min temperature °C	12	12	16	17	17	19	20	23	27
Total rainfall (mm)	76.1	21.9	14.7	38.4	5	0	0	0	23
Photoperiod (LD)	10:14	11:13	12:12	13:11	14:10	14:10	14:10	13:11	12:12

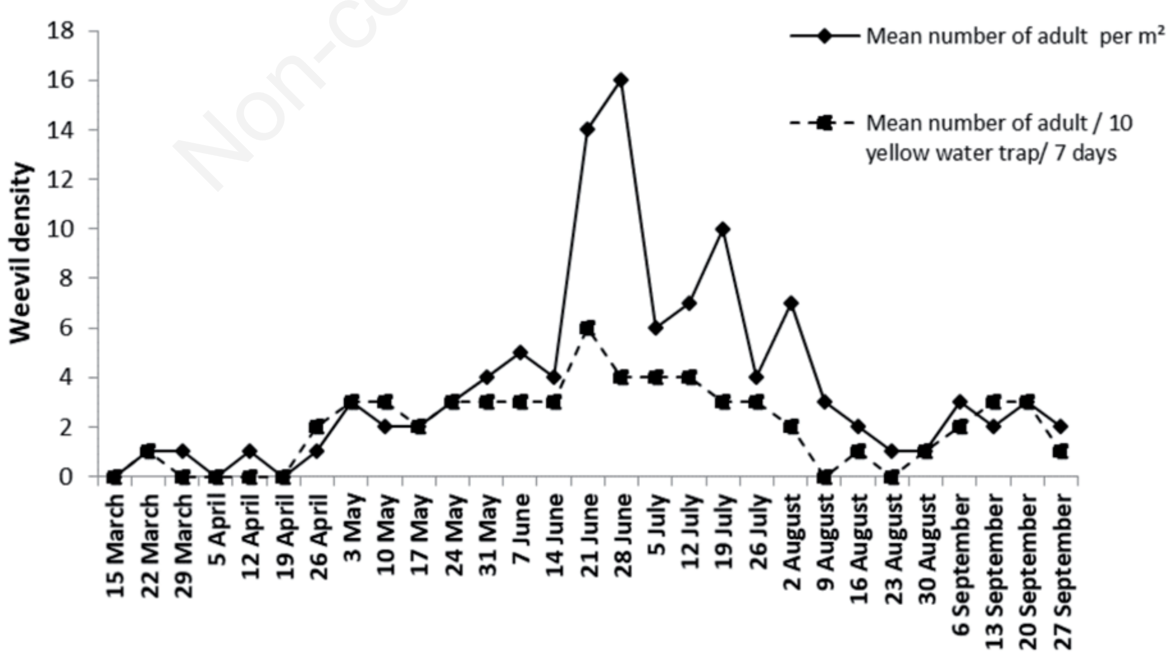

Figure 1. Activity monitoring of the mean number of adult weevils from 10 samples during 2013.

are legless with a creamy white body and a brown head capsule. They reach between 3.4 mm to 5.6 mm length. The number of larvae gradually increases during April and peaked in May. The larval peaks in the rhizomes were reached with 13 and 14 larvae/m² on 31 May 2013 and 24 May 2014. Although few larvae can be found inside rhizomes until the end of August (Figures 3 and 4).

The larvae stage started into stems and continued their development mainly in the rhizomes of mint plants. In fact, they began to feed on the tissues around the egg deposition sites from April to June, and then, they penetrate to the centers of the stems. Afterwards, the larvae dig into the rhizomes and complete their development inside during the summer cut (June and July).

The statistical analysis showed no difference in terms of the number of larvae per stems between the harvest times of April-June which reached 1.57±0.52 and 1.43±0.61 larvae/m²and summer cut June-July 0.29±0.18 and 0.13±0.12 larvae/m² obtained in 2013 and 2014, respectively. The data presented (Table 3) indicated that the mean number of larvae per rhizome at April-June ranged between 6.86±1.33 and 8.43±1.28 in 2013 and 2014, respectively. The mean number of larvae per rhizome during the

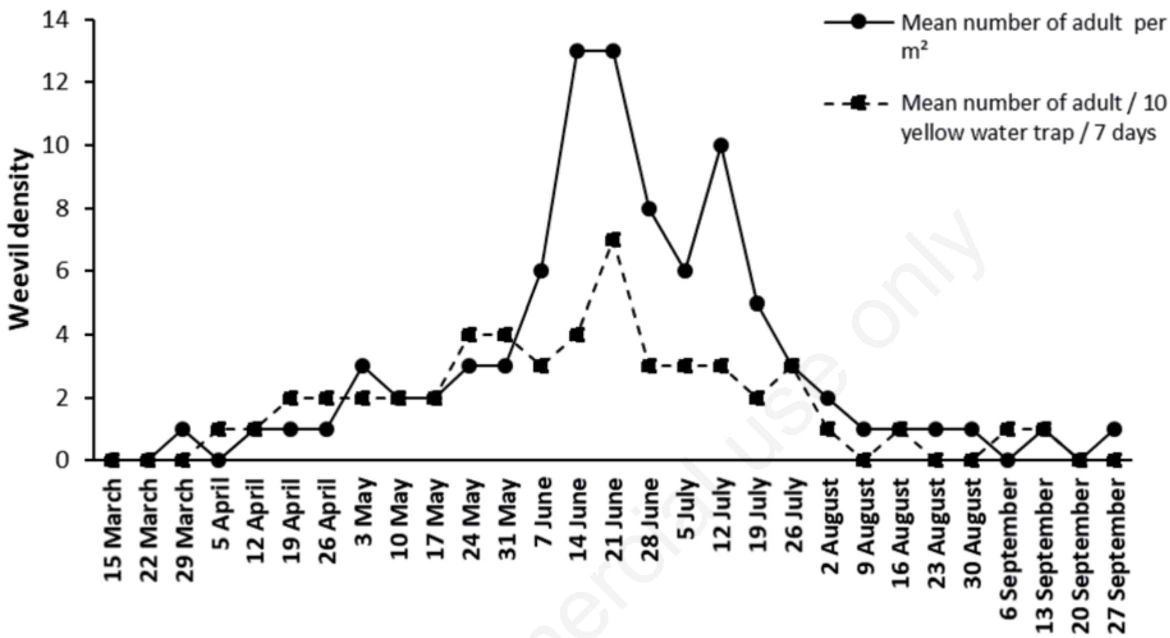

Figure 2. Activity monitoring of the mean number of adult weevils from 10 samples during 2014.

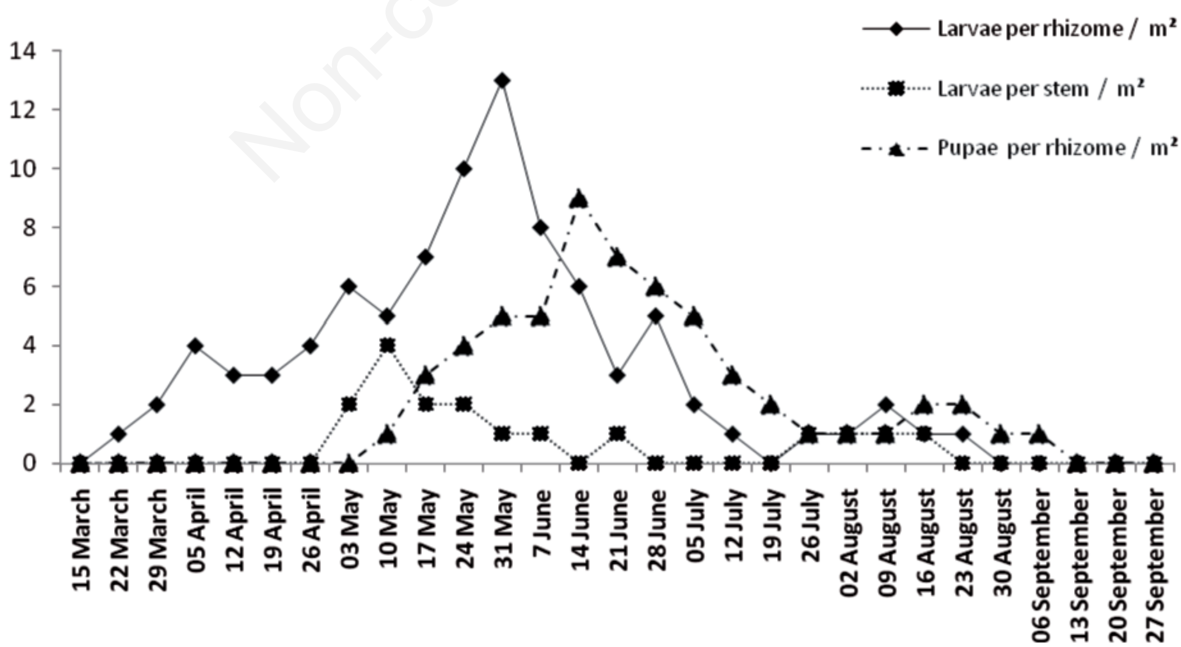

Figure 3. Mean number of larvae and pupae in stems and rhizomes/ m² from 10 samples during 2013.

summer cut decreased significantly to reach 3.57±1.08 and 3.25±0.74 in 2013 and 2014, respectively.

The earliest pupae were "C" shaped, found at the ends of their feeding tunnels inside the rhizomes on 10 May 2013 and 26 April 2014. The pupae with projecting legs, rostrum and elytra were found in the rhizomes surrounded by their exudate left after feeding larvae. The pupae peaks were reached at rhizomes level during Mid-June 2013 with 9 pupae/m² and 10 pupae/m² recorded from 7 to 14 June 2014 (Figures 3 and 4).

Emergence of new generation adult weevils

After pupal development which lasts about two weeks, the adults chew small circular exit holes (2 mm) and begin to emerge from the rhizome and rarely from stem to start a new infestation in the fields. The number of exit holes increased gradually from Mid-May to reach the maximum value during June and July in both years. The average of exit holes was much lower at pre-flowering (April to early June) reaching the average 3.14±1.29 and 7.86±1.83 in both years 2013 and 2014, respectively (Figures 5 and 6).

The data presented (Table 4) showed a lower number of emerged adults during the pre-flowering stage. The mean number of emerged adults per m² at this period recorded 2.14±0.50 and 2.1±0.34 in both years 2013 and 2014, respectively. Whereas the mean number of adult exit holes in the rhizomes level were most numerous during the full-bloom stage (June and July) reaching the average of 16.29±1.83/m² and 18.63±1.38 in 2013 and 2014,

Table 3. Mean number of different stages of *D. melanostictus* per 10 yellow water traps and per m² sampling.

Sample period	Mean adults/ m² (S.E)	Mean adults/ yellow water traps (S.E)	Larvae per rhizome/m² (S.E)	Larvae per stem/m² (S.E)	Pupae per rhizome/m² (S.E)	Mean exit holes in rhizome/m² (S.E)	Mean laying holes per stem/m² (S.E)
16 April - 1 June 2013	2.14(0.50) a	2.29(0.42) a	6.86(1.33) ab	1.57(0.52) a	1.86(0.80) a	3.14(1.29) a	6 (1.27) a
2 June - 20 July 2013	8.86(1.75) b	3.86(0.40) b	3.57(1.08) a	0.29(0.18) a	5.29(0.89) b	16.29(1.83) c	3.42 (0.48) b
18 April - 3 June 2014	2.1(0.34) a	2.57(0.39) a	8.43(1.28) b	1.43(0.61) a	3.57(0.48) ab	7.86(1.83) b	6 (1.71) a

Sample means were compared with Fisher's Protected LSD (P=0.05). Means with the same letter within the same column are not significantly different (Petersen 1985).

Table 4. Plant height and fresh herbage yield of spearmint at different harvest times.

Harvest time	Plant height (cm) 2013	Plant height (cm) 2014	Plant height (cm) Mean	Fresh herbage yield (t/ha) 2013	Fresh herbage yield (t/ha) 2014	Fresh herbage yield (t/ha) Mean
Pre-flowering (01.6.2013) (3.6.2014)	25.5 a	27.5 a	26.5	7.2 a	6 a	6.6
Full flowering (20.7.2013) (26.7.2014)	30 b	34 b	32	9 b	8.5 b	8.75
Mean	27.75	30.75		7.5	7.85	

Means with the same letter within the same column are not significantly different (Petersen 1985).

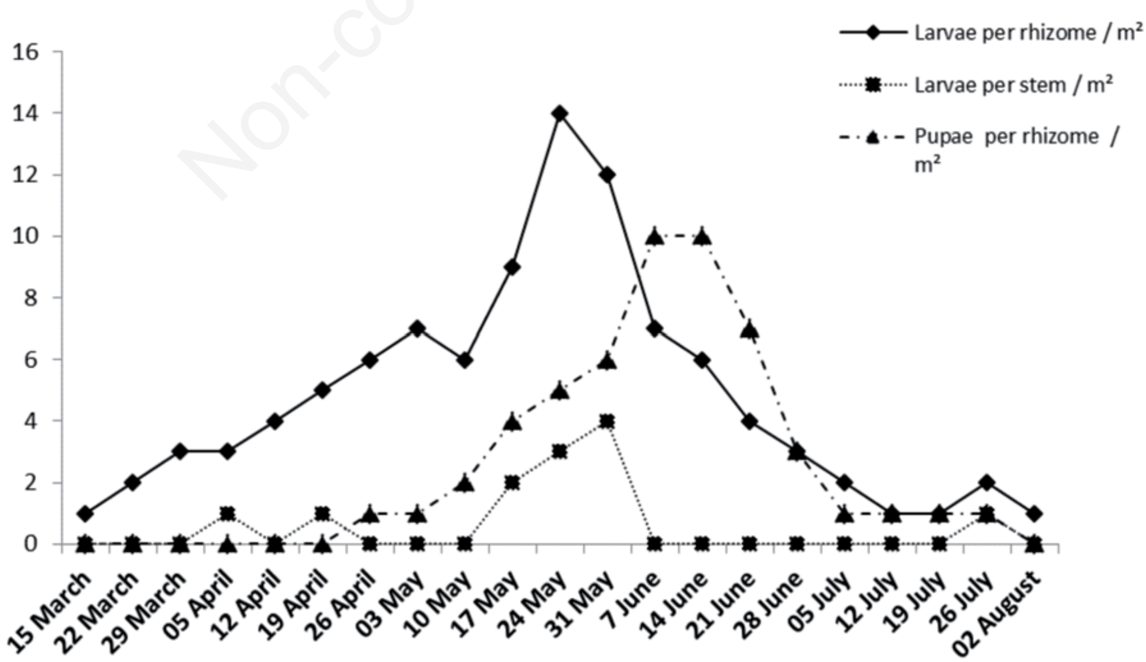

Figure 4. Mean number of Larvae and pupae in stems and rhizomes/ m² from 10 samples during 2014.

respectively. During this stage, the mean number of emerged adult weevils/m² increased significantly to reach 8.86±1.75 and 8±1.3 /m² in both years 2013 and 2014, respectively.

Positive and significant correlations (p=0.005, p=0.0005) were established between

number of exit holes per rhizomes and new emergence of adult weevils (r=0.71; r=0.78) in both years 2013 and 2014, respectively. However, several overlapping of the different insect stages was recorded from April through August of both years study.

The impacts of infestation

The highest number of larvae of *D. melanostictus* per rhizome was reached between April to June (pre-flowering). This larval infestation affected the mint fresh yields which reached 7.2 and 6 t/ha in both years 2013 and 2014, respectively (Table 4). The average height of plants was noticeably reduced to 25.5 cm and 27.5 cm in 2013 and 2014, respectively.

During full-bloom stage period from early June to late July, the number of larvae in the rhizomes dropped to 3.57±1.08 and

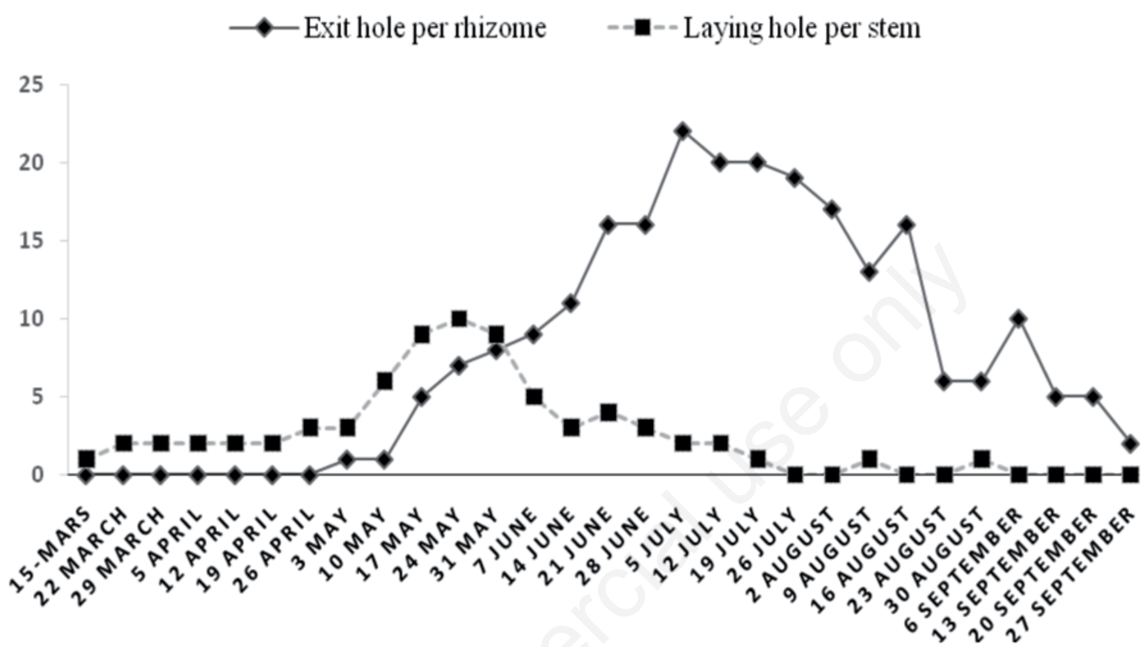

Figure 5. Mean exit holes and laying holes in stems and rhizomes/m² from 10 samples during 2013.

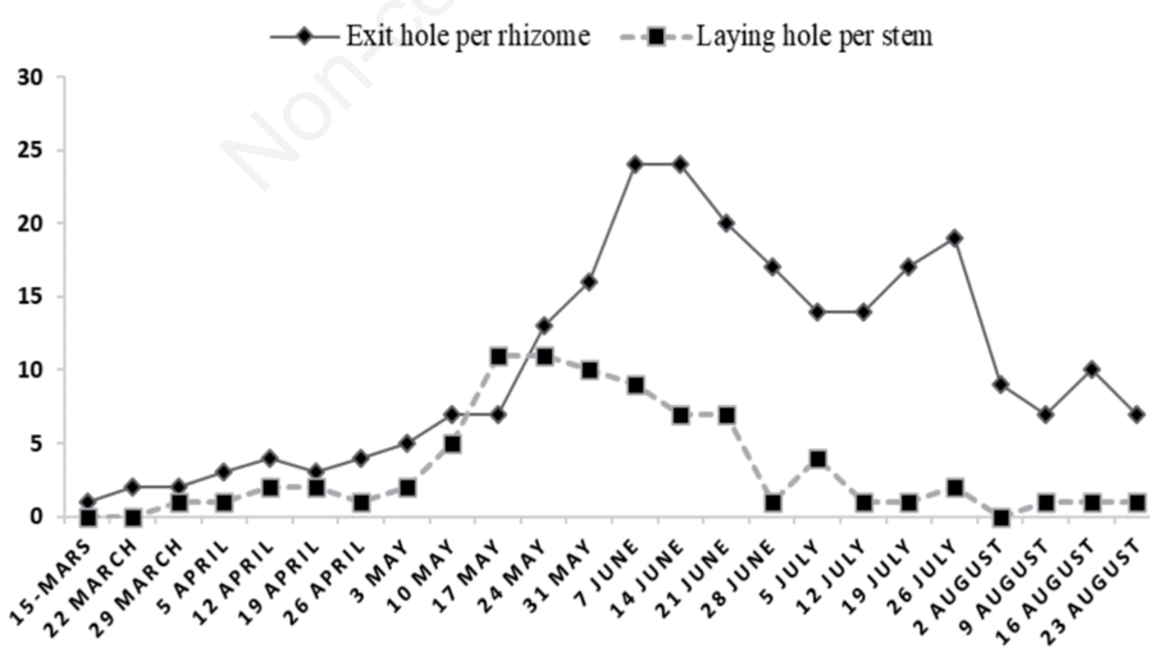

Figure 6. Mean exit holes and lying holes in stems and rhizomes/m² from 10 samples during 2014.

3.25±0.74/m² in 2013 and 2014, respectively. However, the mean number of exit holes and the emergence of new adult weevils from rhizomes increased significantly. The damage of the new generation of adult weevils *D. melanostictus* is limited by feeding on leaf tips of mint plants which does not significantly affect the fresh yields (8.75 t/ha) in both years (Table 4).

Discussion

According to the results, *D. melanostictus* is found on mint plants in Chaouia region of north-central of Morocco between March and September. In Poland Burakowski *et al.* (1997) indicated that the weevil is observed two months later between May to October in different species of mints. The emergence of adults occurred in early spring in March at temperature >15°C, by feeding over spearmint leaves. They are very visible on mint fields, and usually hide on the lower leaves and on the ground level with rising day temperatures. The infestation of adult weevils increases gradually with increasing temperature and photoperiod. A similar observation was reported by Dosdall (2009) on a similar species, *Ceutorhynchus obstrictus*, one of the major pests in the production of canola (*Brassica napus* L.) in North America and Europe. The dispersion of *C. obstrictus* from overwintering sites in spring start when mean air temperature reaches 9-12°C and peaks when ground temperatures reach 15°C at depths of 5 cm (Ulmer and Dosdall, 2006). For the other similar species *C. napi*, Maceljski (1974) indicated their emergence between February and March on oilseed rape with a mass flight occurring at temperature between 12 to 15°C.

After mating, the female *D. melanostictus* chews a single hole in the lower level of stem near the soil and deposits eggs from early March. The same character is noticed for mint stem borer *Pseudobaris nigrina* (Coleoptera: Curculionidae) in mint growing areas of Idaho and eastern Oregon in U.S.A. (Baird *et al.,* 1990). Similarly, Burakowski *et al.* (1997) reported in Poland that the females of *D. melanosticus* lay eggs on stems of different species of mints and *Lycopus europaeus.*

This study indicates that the development of larvae is realized mainly in the rhizomes of mint plants, after initiating their feeding on stems. The larval activity in mint rhizomes starts from March until the late August. The highest monthly intensity of infestation with the total number of larval stages was recorded between April to June. A general weakness of infested plants occurs with visible symptoms such as yellowing, stunting and wilting. In addition, a reduction in mint plants density and the dominance a wide range of weeds. Young plants are often killed at the soil, but new shoots can emerge from rhizomes. Vigorously growing mint generally has no symptoms.

The weevil *D. melanostictus* realizes his pupation mainly inside the rhizomes, which is not the case for other related species of the genus Ceutorhynchus that is performed in soil level (Dieckmann, 1972). This current study showed that the application of drip irrigation had a significant positive impact in larval development. Similarly, Burakowski *et al.* (1997) indicated that *D. melanostictus* prefers to live on wetlands, bogs, water edges, wet and marshy meadows and ditches. The severity of infestation of the weevils can be affected also by cultural practices, such the excessive nitrogen fertilization that can stress more infested plants by the larvae. Aljmli (2007) showed that higher doses of nitrogen fertilizer applied to oilseed rape increased significantly the density of adult weevils within the *Ceutorhynchus* genus; such as the species *C. pallidactylus* and *C. obstrictus*. This positive effect of nitrogen fertilizer application was not only seen on the adult stage, but also on larval density of *C. napi* and *C. pallidactylus* and *C. obstrictus*. The weevils *D. melanostictus* seem to cause the greatest damage among stressed plants and old mint fields (El Fakhouri *et al.,* 2015).

The increase of mean number of larvae per rhizome during April to June (preflowering stage) had a significant negative impact on the length of mint plants which reached in average of 26.5 cm in both years. Usually, the average of mint stem height that are ready to harvest in Chaouia region is between 50 to 80 cm (Tanji, 2008). In addition, the larval rhizome feeding significantly decreased the fresh weight to 6.6 t/ha during the pre-flowering stage in both years. Whereas the average fresh mint yield in Morocco is around 10 t/ha per cut (El Fadl and Chtaina, 2010). According to this study, the yield losses is inflicted by the larval infestation within the stem and mainly into the rhizomes of mint plants. In contrast, the average fresh mint yield during full flowering stage (June to late July) reached 8.75 t/ha in both years. During this period the emergence of the new generation of adult weevils with the reduction of mean number of larvae had less impact on the average fresh yield. Foliage feeding caused by adults is usually insignificant since the damage are limited to the leaf tips. The damage can be indirectly caused by the adult exit holes at the rhizomes, which facilitates infection of a wide range of soil fungi in the rhizomes. According to Pike and Glazer (1982) and Pike *et al*. 1988, the damage caused by Mint root borer, *Fumibotys fumalis* (Guenée) (Lepidoptera: Pyralidae) in peppermint can be increase winter kill or stand loss during the following spring, because of damaged rhizomes having a greater susceptibility to infection by soil-borne pathogens. Cárcamo *et al.* (2001) reported a similar damage caused by the weevil *C. obstrictus* under moist conditions, with reduction of seed yield and quality affected by fungal pathogens through larval exit holes or feeding punctures on oilseed rape.

The results showed that the population density of the new generation of adult weevils *D. melanostictus* increased significantly during full flowering stage of mint plants. Three infestation peaks were recorded between late June to early August 2013, ranging from 7 to 16 adults per m². Two peaks were recorded in June and July 2014 with average ranging between 10 and 14 adults per m². These results are consistent with those of Dosdall *et al.* (2006), showing that the numbers of the closely related species adults *C. obstrictus* captures per trap greatly increased during mid to late flowering period of canola plant. This shows that population dynamics of *C. obstrictus* depends on the growth stage of the plant.

The adult weevils *D. melanostictus* overwinters inside hollowed out mint plants, in contrast to the most studied related species of genus *Ceutorhynchus*. The adult weevils *C. obstrictus* spend the winter in diapause under leaf litter in treed areas and soil beneath, shelterbelts and field margins (Dmoch, 1965; Ingrid, 2010). The winter diapause for the adult *C. pallidactylus* is occurred at plant debris at the edges of woods and shrubs (Notle, 1957).

Conclusions

In summary, although the weevils *D. melanostictus* are a relatively recent invasive species on mint crop in Chaouia region and its distribution continues to expand, across uninfested mint fields. The yield losses caused by weevil are associated with the direct larval feeding and tunneling in the underground rhizomes of plants. The only strategy available for the farmers of mint in Chaouia region to reduce the damage caused by the pest is the spray of a broad-spectrum of insecticide without any result. Protection of Mint crop against *D. melanostictus* is a daily challenge. The farmer must apply combining methods including population dynamics of the weevil for the best control.

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