Egyptian Journal of
Biological Pest Control

RESEARCH Open Access

Determination of annual generations of *Phytomyza orobanchia* Kalt. (Diptera: Agromyzidae), using growing degree-days in Alexandria region, Egypt

M. A. S. Al-Eryan*, Amany M. H. Abu-Shall and Alaa H. Ibrahim

Abstract

In Egypt, parasitic weed species, *Orobanche crenata* Forsk is one of the major constraints to the production of faba bean, which is considered the most important food legume in the country. The broomrape fly, *Phytomyza orobanchia* Kaltenbach (Diptera, Agromyzidae) is particularly suitable for biological control since it is oligophagous feeding in the larval stage, only on *Orobanche* species. The objective of the present study is to estimate the number of annual generations of *P. orobanchia*, using growing degree-days (GDD) in Alexandria region, Egypt. Results revealed that development of *Phytomyza* immature stages (from egg deposition to adult emergence) required 103.5, 90.0, 79.5, and 93.5 DD through 31, 20, 18, and 16 days for the investigated four generations, respectively. Percentage of infested *Orobanche* capsules positively increased with increasing accumulated degree-days. A linear model for predicting infested capsules with *P. orobanchia* through knowing DD was estimated. The constructed model may be used also in predicting *Orobanche* capsule infestation with *P. orobanchia*. Results also indicated that estimating the degree-days will help in predicting the occurrence of the first egg laid for *P. orobanchia* in *O. crenata* flowers in the field for the first generation and subsequent generations. Knowledge of the *Phytomyza* activity will provide a prediction for adult emergence and subsequent egg laying and potential damage by larvae according to infestation percent of *Orobanche* capsules through the successive generations. This may help the biological control programs to justify the required numbers of *Phytomyza* and adequate time for releases to obtain the maximum destruction of *Orobanche* seeds.

Keywords: *Phytomyza orobanchia*, *Orobanche crenata*, Degree-days, Generations, Biological control, Egypt

Introduction

In Egypt, *Orobanche crenata* Forsk is one of the major constraints in faba bean productions, which is considered the most important food legume in the country. In the West Nile Delta region, the area infested with *O. crenata* was more than 25,000 feddan (feddan = 4200 m^2) (Zaitoun et al. 1991). It occurs in about 20% of the total area cropped with faba bean, of which about half suffers low to moderate infestation (less than 5 spikes per m^2) and the other half is infested with 5–20 spikes per m^2 or more. The loss in faba bean seed yield in El-Beheirah governorate, Egypt was 20,000 tons or about 10 million dollars (Hassanein et al. 1996).

* Correspondence: m_a_aleryan@yahoo.com
Applied Entomology and Zoology Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt

Many management strategies have been tried against *O. crenata* and other broomrape species, but few of them have proved reliable and these are just economical in high value agriculture. The strength of broomrape lies in its ability to form a considerable seed bank in soil. A management or eradication program must aim at reducing this seed bank, in order to minimize the production of new seeds and their dispersal to new sites.

Recent approaches are necessary to control parasitic weeds of the genus *Orobanche*. The broomrape fly, *Phytomyza orobanchia* Kaltenbach (Diptera, Agromyzidae), is particularly suitable for biological control since it is of oligophagous feeding, in the larval stage, only on *Orobanche* species. In total, of the 140 *Orobanche* spp. described, the occurrence of *P. orobanchia* was reported from 21 species. The use of *P. orobanchia* in biocontrol of *Orobanche* is based on inundative releases at the time of *Orobanche* emergence. The larvae of the fly mine in *Orobanche* shoots, capsules, and intervene at the sensitive reproductive stage of *Orobanche*. Hence, the reduction of *Orobanche* seed production prevents supplementary infestation and dissemination. The advantage of this control approach is its compatibility to all crop/*Orobanche* associations and that it can easily be combined with other control methods (Kroschel and Klein 2002).

In Egypt, biological control trials of the weed *O. crenata* were conducted by Kolaib (1991), Al-Eryan (1996), Al-Eryan and Zaitoun (1998), Zaitoun and Al-Eryan (1999), and Abu-Shall (2001). Although of the effectiveness of *Phytomyza* releases in reducing *Orobanche* seed production can reach (91.73%), the already infested soil in addition to new accumulated seeds will cause further infestations throughout the successive seasons. Biocontrol with *P. orobanchia* may be helpful in reducing further dissemination and infestation in light infested areas, and could be incorporated into an integrated control approach to reduce the seed bank in heavily infested soils (Rubiales et al. 2001). This effect can be substantially increased by massive propagation and releasing of the insect at the beginning of *Orobanche* flourishes as demonstrated by Klein et al. (2002).

In Egypt, Tawfik et al. (1976) studied the biology of *P. orobanchia* and found that the females insert their eggs in the *Orobanche* flower. The total number of deposited eggs was 32.93 eggs per female. The durations of development for the three larval instars lasted about 1.1, 1.93, and 2.22 days, respectively. The total larval period lasted about 5.3 days. The larvae feed on the immature seeds inside the capsule. Pupation takes place either in the capsules or in the stem. For pupation in the fruit, the mature larva bores into the enclosed seeds and tissues leaving a rounded thin membranous area on the pericarp through which the adult will emerge. At the end of season, the mature larva bores inside toward the stem to enter diapause till the next season. The estimated lifecycle from egg deposition to first egg laid in the following generation lasted 18.9 days (17–20 days).

Heat unit systems quantify the thermal environment of organisms and are commonly used in phenology models that relate organism growth and development to local weather/climate conditions. Several methods of calculating heat units have been developed and are presently used worldwide in phenology models. The accumulation of degree-days has become a useful tool in monitoring the emergence and development of insect populations (Jyoti et al. 2003).

The objective of the present study was estimating the number of annual generations of *P. orobanchia*, using growing degree-days at Alexandria region.

Materials and methods

Estimation of generation numbers of *P. orobanchia*

In faba bean fields, *Orobanche* spikes emerge aboveground on different growth stages from January until end of April 2015. To estimate the first generation of *P. orobanchia***,** 40–50 flowering *Orobanche* spikes were randomly selected at the beginning of *Orobanche* emergence. Collected spikes were picked up from faba bean field in Nubaria region located at Sahara adjacent south Alexandria region, Egypt. Collected plants were transferred to the laboratory, placed in plastic containers, and then left to complete maturation under natural conditions. Three flowers or capsules were daily detached from each *Orobanche* spike and examined under stereomicroscope to record immature stages of *P. orobanchia*. Immature stages were classified into sequential developmental stages, i.e., egg, 1st, 2nd, and 3rd larval instars and pupae (Fig. 1) in conjunction with the three growth stages of *Orobanche*: GS 6, 65: fleshy spike (growth stage full flowering: 50% of flowers open, first petals may be fallen), GS 6, 69: semi-mature spike (growth stage, end of flowering: fruit set visible), and GS, 7, 79: mature spike (growth stage: nearly all fruits have reached final size normal) according to description of Hess et al. (1997) and Al-Eryan et al. (2003). Generation period was expressed as from egg to adult emergence. Percentage of *Orobanche* capsules infested with *P. orobanchia* was estimated daily.

After adult emergence of the first generation, new group (40–50) of flowering *Orobanche* plants were randomly collected from the faba bean field cultivated in Abees region, Alexandria to follow up development of the second generation. The same method was applied to determine the third and fourth generations of *P. orobanchia*. During the period of study, daily maximum and minimum temperatures were obtained from data of Egyptian Meteorological Authority station (E.M.A.S).

Determination of growing degree-days

Degree-days (DD) are used to measure the number of accumulated heat units above temperature threshold that are required for insect development. The first date at which *P. orobanchia* female laying the first egg in the *Orobanche* flower was considered as the beginning of calculation of the degree days accumulation (biofix date). The lower threshold temperature for development of *P. orobanchia*was12.5 °C, daily average (base temperature), calculated by Abu-Shall (2007) and used in the present study.

Calculating growing degree days (GDD) was applied according to Pruess (1983) and Fry (1983) as follows:

$$\text{Daily GDD} = [(\text{ max temperature} + \text{minimum temperature})/2] \\ -\text{base temperature}$$

If the maximum temperature for the day never rises above the base temperature, then no development occurs

Fig. 1 Synchronization between phonological stages of *O. crenata* and immature stages of *P. orobanchia*

and zero degree-days accumulated (negative degree-day values are not calculated since the development of organisms does not continue when it is cold).

Prediction of *P. orobanchia* annual generations was carried out by determining the relationship between the thermal heat units, expressed as DD and development of *P. orobanchia* stages, expressed as percentage of infested capsules during the period from early January to late April 2015.

Statistical analysis

For statistical analysis, the correlation between degree-days and percentage of capsule's infestation with *P. orobanchia* were calculated, using least squares regression (Sokal and Rohlf 1973).

Results and discussion

Accumulated degree-days required for development of four generations of *P. orobanchia* in *O. crenata* spikes are presented in Table 1 and Fig. 2. *Orobanche* spikes were examined at the three growth stages: GS 6, 65 (flowering *Orobanche*), GS 6, 69 (semi-mature *Orobanche*), and GS 7, 79 (mature *Orobanche*) through all generations of *P. orobanchia*. Results showed that eggs of *P. orobanchia* started to be laid on Jan. 11 at 0.5 accumulated degree-days. This was considered as the date of starting the development of *P. orobanchia* and calculation of accumulated degrees-days. Development of *Phytomyza* immature stages (from egg to adult emergence) required 103.5 accumulated degree-days through 31 days (from Jan. 11 to Feb. 10). Infestation percent of *Orobanche* capsules with *P. orobanchia* reached the maximum (25.4%) by the end of this generation. This generation appeared in faba bean fields cultivated early in Sahara regions adjacent to Alexandria region.

Accumulated degree-days required for development for the second generation of *P. orobanchia* in *O. crenata* spikes were indicated in Table 1 and Fig. 2. Results showed that the eggs of *P. orobanchia* were laid on Feb. 20 at 139.5 DD. Development of *Phytomyza* immature stages (from egg to adult emergence) required 90 accumulated day-degrees through 20 days (from Feb. 20 to Mar. 11). Infestation percent of *Orobanche* capsules with *P. orobanchia* reached the maximum (50%) by the end of this generation.

In the third generation, the eggs of *P. orobanchia* were laid on Mar. 12 at 226.5 DD. Development of *Phytomyza* immature stages (from egg to adult emergence) required 79 accumulated degrees-days through 18 days (from Mar. 12 to Mar. 29). Percentage of infestation to *Orobanche* capsules with *P. orobanchia* reached the maximum (64.7%) by the end of this generation (Table 1 and Fig. 2).

Degree-days accumulations required for development of the fourth generation are presented in Table 1 and Fig. 2. Results showed that the eggs of *P. orobanchia* were laid on Mar. 30 at 308 DD. Development of *Phytomyza* immature stages (from egg deposition to adult emergence) required 93.5 accumulated degrees-days through 18 days

Table 1 Develoment of *Phytomyza. orobanchia* generations synchronized with three growth stages of *Orobanche crenata* spikes in Alexandria region

Generations	Date 2015	*Orobanche* growth stage	*Phytomyza* growth stages	% infested capsules	Accumulated Degree-Days
I	Jan., 11	Flowering	Eggs	2.77	0.5
	Jan., 18		Larvae	5.55	11
	Jan., 29	Semi-mature	Pupae	12.25	52.5
	Feb., 10	Mature	Pupae	25.4	116.5
II	Feb., 20	Flowering	Eggs	1	139.5
	Feb., 23		Larvae	15.5	156
	Mar., 4	Semi-mature	Pupae	16.5	187.5
	Mar., 11	Mature	Pupae	50	224
III	Mar., 12	Flowering	Eggs	5.5	226.5
	Mar., 13		Larvae	7.5	229
	Mar., 16	Semi-mature	Pupae	36.2	240
	Mar., 29	Mature	Pupae	64.4	304
IV	Mar., 30	Flowering	Eggs	33.3	308
	Mar., 31		larvae	61.6	313.5
	Apr., 3	Semi-mature	Pupae	66.6	333
	Apr., 14	Mature	Pupae	100	463.5

(from Mar. 30 to April 14). Infestation percent of *Orobanche* capsules with *P. orobanchia* reached the maximum (100%) by the end of this generation, most pupae entered summer diapause up to the new flourishing season.

A linear regression equation provided a good mathematical description of the effect of degree-days on the infestation of *Orobanche* capsules with *P. orobanchia* (Fig. 3). Percentage of infested *Orobanche* capsules positively increased by increasing accumulated degree--days. Where the regression coefficient ($R^2 = 0.964$) was significant.

Under field conditions, adults of *P. orobanchia* are common from the beginning of *Orobanche* flourishing. The mated females search for suitable buds and flowers to lay their eggs. In this context, Klyueva and Pamukchi (1978) in Moldova noticed that adults of *P. orobanchia* appeared in the field from the beginning of *Orobanche* emergence, and hatching took place at a mean air temperature of 20 °C and 530 accumulated degree-days. In Ukraine, Kott (1969) observed adults at mean temperatures of 22–23 °C from the beginning of emergence of *Orobanche*. Also, in the former USSR, Tsybul'skaya et al. (1978) found that the

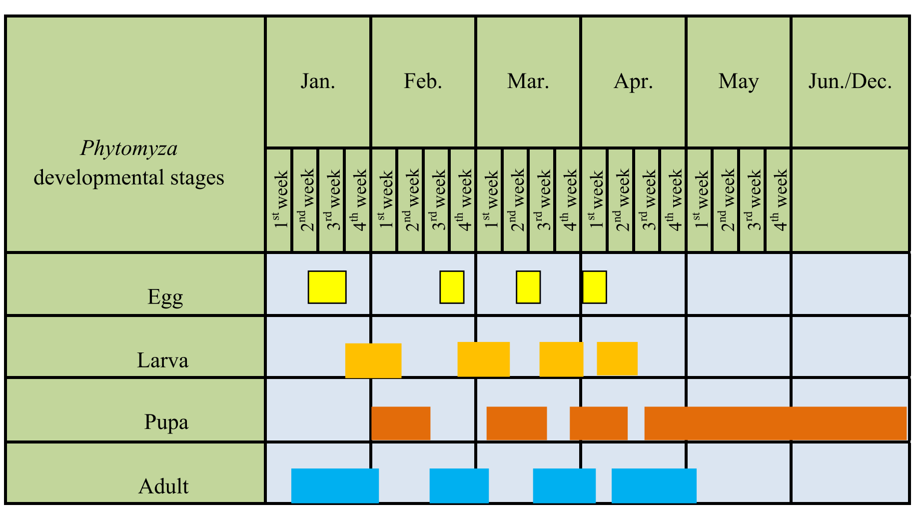

Fig. 2 Phenological stages of *Phytomyza orobanchia* on *Orobanche crenata* parasitizing on faba bean crop showing four generations in Alexandria region

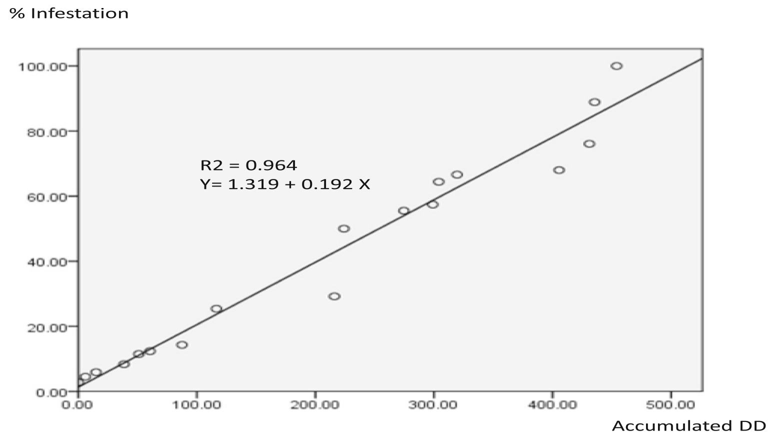

Fig. 3 Regression line of accumulated degree-days and percentages of infested *Orobanche* capsules with *P. orobanchia*

flies of *P. orobanchia* overwinter in the pupal stage and the adults emerge when the temperature reaches 20 °C. In the same subject, *P. orobanchia* was affected by high and low temperature degrees in Uzbekistan, where Kurbanov (1970) reported that up to (35%) of the pupae were destroyed by temperature under zero during hard winter. While low temperatures during winter can cause a high mortality of *Phytomyza* pupae (Lekic 1970). In the south of Ukraine, there are 2–3 generations in a year and each requiring 20–36 days for development. Most of the larvae moved to the underground parts of the *Orobanche* stems for pupation (Tsybul'skaya et al. 1978).

Also, unsuitable temperature forced the *Phytomyza* larvae to enter diapause in the pupal stage. Klyueva and Pamukchi (1980) reported that the percentage of the diapause for the three generations took place as: 2–22, 33–87, and almost 100% for first, second, and third generations, respectively. But most of the diapaused pupae emerged in the subsequent year and only 13% remained in a prolonged diapause for longer than 1 year. Sometimes, the diapause lasts 4 years in order to cover the absence of host-plant. Diapause is induced by a day-length of less than 14 h, but the optimum photoperiod for the midge's development was 16 h. The percentage of diapaused pupae increased with declining temperatures to 16 and 8 °C. In Syria, Linke et al. (1991) studied the relationship between the appearance of *P. orobanchia* and *Orobanche* spp. The first appearance of the fly coincided with the first emergence of the *Orobanche* shoots. They suggested that its arrival was most probably related to temperature. Al-Eryan (1996) recorded three generations of *P. orobanchia* per year under field conditions. The first and second generations required about 3 weeks for development, while the third one required 2 weeks. The present study indicated that additional generation appeared early in January and February 2015 in faba bean fields cultivated in Sahara regions at daily mean temperature 15.1 °C (11.5–21.0 °C).

Since the temperature is considered as an important environmental factor that affects the rate of development of the insect and controlled the success of the insect to survive in a given temperature, it was particularly as practical point of view interesting for economic insects to obtain a useful and good forecast and prediction system for insect population (Wagner et al. 1984). The degree-days (DD) forecasting method has been successfully used in the past for forecasting several economic pests. In the formulation of forecasting models, developmental data from a range of temperatures help to determine the insect developmental threshold (Herms 1998).

Similar studies were conducted on related species of agromized flies. Petitt et al. (1991) and Petitt and Wietlisbach (1994) reported that (99.9%) of *Liriomyza sativae* emerged from leaves after 95 DD (threshold temperature, 10 °C). Millia (2005) stated that the Holly leaf miner (*Phytomyza ilicis*) larvae and adults needed 246 DD, while native holly leaf miner (*Phytomyza iliciola*) larvae and adults needed 192 and 298 DD.

Conclusion

Results of the present study certified four generations for *P. orobanchia* on *O. crenata* in faba bean field. Knowledge of *Phytomyza* activity will provide a prediction for adult emergence and subsequent egg laying and potential damage by larvae as infestation percent of *Orobanche* capsules through the successive generations. This may help the biological control programs to justify the required numbers of *Phytomyza* and adequate time for releases to obtain the maximum destruction of *Orobanche* seeds. The constructed model may be used in predicting *Orobanche* capsule infestation with *P. orobanchia*, but the validity of the model should be carried out using new field observations through several seasons.

Abbreviations
DD: Degree-days; GDD: Growing degree-days

Acknowledgements
The authors express deep thanks to Prof. Dr. Fatthalla Mohammad Zaitoun, Professor of flowering Parasitic Plants, Department of Phytopathology, Faculty of Agriculture, Alexandria University, for helping in revising the manuscript.

Funding
There is no funding source to be declared for this study.

Availability of data and materials
Data will not be shared.

Authors' contributions
All authors read and approve the manuscript or all authors approve to publication.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 16 March 2018 Accepted: 16 October 2018
Published online: 04 December 2018

References
Abu-Shall AMH (2001) Applied study on utilization of *Orobanche* fly and other biotic agents in the biological and integrated management of broom-rape and other noxious weeds, M.Sc. Thesis. Faculty of Agriculture, Alexandria University, p 125

Abu-Shall, Amany M.H. (2007). Investigations on certain factors affecting the efficacy broomrape fly associated with the biological and integrated management of faba bean broomrape. Ph.D. thesis, Faculty of Agriculture, Alexandria University,134pp

Al-Eryan, M.A.S. (1996). Role of certain bioassociates in the biological and integrated pest management *Phytomyza orobanchia* Kalt. and Glyphosate as biochemical control of agents of *Orobanche crenata* Forsk. Ph.D. Thesis, Faculty of Agriculture, Alexandria University, 143 pp.

Al-Eryan MAS, Altahtawy MMM, El-Sherief HK, Abu-Shall AMH (2003) Phenological stages of the parasitic weeds *Orobanche crenata* Forsk. as a guide for collecting a parasitoid-free *Phytomyza orobanchia* Kalt. Egypt J Biol Pest Control 13(1&2):105-109

Al-Eryan MAS, Zaitoun FMF (1998) Efficiency of the Dipteran, *Phytomyza orobanchia* and rot fungi as biocontrol agents of broomrape, *Orobanche crenata* in Egypt. Submitted to VIth European Congress of Entomology, Ceske Budejovice, August 23–29, 1998, Czeck Republic, Book of Abstracts, pp 493–494

Fry, K.E. (1983). Heat-unit calculations in cotton crop and insect models.USDA-ARS advances in agricultural technology, Western Series No. 23, Oakland CA, 23 pp. (c.f. Thomas, 1997)

Hassanein, E.E.; Ellan, M.E.; Abo, K.G. and El-Enin, R.A. (1996). *Orobanche crenata* Forsk. survey and control in Faba bean (*Vicia faba* L.*)* and Peas (*Pisum sativa* L.). Sixth International Parasitic Weed Symposium, Cordoba Spain, 16–18

Herms, A.H. (1998). Ornamental plants. Annual reports and research reviews of entomology. Ohio Agriculture Research Development Centre, Special Circular, 165–169

Hess, M.; Barralis, G.; Bleiholder, H.; Buhr, L.; Th-Eggers; Hack, H. and Stauss, R. (1997). Use of the extended BBCH scale-general for the descriptions of the growth stages of mono- and dicotyledonous weed species. Weed Res 37: 433–441

Jyoti JL, Shelton AM, Barnard J (2003) Evaluation of degree day and Julian- day logistics models in predicting cabbage maggot (Diptera: Anthomyiidae) emergence and flight in upstate New York. J Entomol Sci 38(4):535–532

Klein, O.; Kroschel, J and Elzein, A. (2002). Status quo of *Phytomyza orobanchia* research. Parasitic plant management in sustainable agriculture WG2+WG4 +MC meeting Obermarchtal, Germany 25–27

Klyueva MP, Pamukchi GV (1978) Broomrape midge the natural enemy of broomrape in Moldavia. Izvestiya Akademii Nauk Moldaveskoi SSR, Seriya Biologiches Kikhi Khimicheskikh Nauk 4:21–25

Klyueva MP, Pamukchi GV (1980) The diapause of the phytophage *Phytomyza* and its causes. Sel'skokhozyaist vennaya Biologiya 15(3):396–400

Kolaib MO (1991) Biological control of the broomrape weed, *Orobanche crenata* Forsk., in Egypt Menoufia. J Agric Res 6(1):867–876

Kott, S.A. (1969). Biolgical control of broomrape. In: [Weeds and their control], Kolos, Moskva, USSR, 169–171. (c.f. Kroschel, J. and Klein, O. 1999)

Kroschel, J. and Klein, O. (2002). Biological control of *Orobanche* spp. in the Near East and North Africa by Inundative releases of the herbivore *Phytomyza orobanchia*. In integrated management of *Orobanche* in food legumes in the Near East and North Africa, 55–66

Kurbanov T (1970) The use of *Phytomyza* on the fields of Solchos. Biological control of the Egyptian broomrape in vegetables and melons. Sel, skoe Chozjajstvo Usbekistana 9:50

Lekic MB (1970) The role of the dipteran *Phytomyza orobanchia* Kalt. (Agromyzidae) in reducing parasitic phanerogam populations of the *Orobanche* genus in Vojvodina. Contemporary Agric 18(7/8):59–68

Linke K.H.; Weigand, S. and Saxena, M.C. (1991). Biological control of *Orobanche*. ICARDA, Legume program: Annual Report 231–233

Millia K (2005) Spatial/temporal model for survivability of pea leaf miner (*Liriomyza huidobrensis*) in warm climates: a case study in South Florida, USA. Eur J Sci Res 7(5):65–73

Petitt FL, Allen JC, Carl S (1991) Degree-day model for vegetable Leafminer (Diptera: Agromyzidae) phenology. Barfield Environ Entomol 20(4):1134–1140

Petitt FL, Wietlisbach DO (1994) Laboratory rearing and life history of *Liriomyzasativae* (Diptera: Agromyzidae) on Lima bean. Environ Entomol 23:1416–1421

Pruess KP (1983) Day-degree methods for pest management. Environ Entomol 12:613–619

Rubiales, D.; Moreno, M.T.; Kharrat, M.; Zermane, N. and Khalil, S. (2001). Broomrape (*Orobanche crenata*) control in sustainable agriculture. In: AEP (Ed.), Legumed Symposium Grain Legumes in the Mediterranean Agriculture. Rabat, pp. 25–27

Sokal RR, Rohlf FI (1973) Introduction to biostatistics. Freeman, San Francisco

Tawfik MFS, Awadallah KT, Shalaby FF (1976) Biology of *Phytomyza orobanchia* kalt. (Diptera: Aagromyzidae). Bull Soc Ent Egypte 60:53–64

Tsybul'skaya GN, Degtyarev BG, Fedoryak NA, Skoklyuk AN (1978) Determination of viability of *Phytomyza* puparia. Zascita Rastenij 5:29–30

Wagner TL, Wu HI, Sharpe PJ, Schoolfield RM, Coulson RN (1984) Modeling insect development rate: a literature review and application of a biophysical model. Ann Ent Soc Am 77:208–225

Zaitoun FMF, Al-Eryan MAS (1999) Loss assessment and forecasting work on plant diseases: 2-prediction of *Orobanche crenata* seed yield and its reduction due to *Phytomyza orobanchia* and rot fungi. In: Kroschel J, Abderabihi M, Betz H (eds) Advances in parasitic weed control at on-farm level. Vol. II. Joint Action to Control *Orobanche* in the WANA Region. Maragraf Verlag, Weikersheim, pp 185–195

Zaitoun, F.M.F.; Al-Menoufi, O.A. and Weber, H.C. (1991). Loss assessment and forecasting work on plant disease: 1- A new method for assessment of loss in *Viciafaba* through infection with *Orobanche crenata*.In: Wegmann, K. and L. J. Musselman (eds.), Progress in *Orobanche* Research. Eberhard-Karls-Universitat, Tubingen, FRG, 167–184