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Phenological Stages of the Parasitic Weeds *Orobanche crenata* Forsk. as a Guide for Collecting a Parasitoid-free *Phytomyza orobanchia* Kalt.

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ABSTRACT

The hymenopteran, *Tetrastichus phytomyzae* Kost. (Eulophidae) is the only encountered parasitoid of the bio-control agent, *Phytomyza orobanchia* Kalt. (Diptera: Agromyzidae) in Egypt till now. This study aims to develop a simple technique for collecting the unparasitized *Phytomyza* pupae from the parasitic weed, *Orobanche crenata* Forsk. at end of the season, in order to be used as a mass culture for field release. During April 2000, nine Faba bean fields infested with *O.crenata* in West Nile Delta were selected. *Orobanche* spikes were divided into three phenological stages, fleshy,semi-fleshy and dry. The percentages of emerged *Phytomyza* adults, unemerged and parasitized pupae with *Tetrastichus* were calculated for each *Orobanche* stage. Results revealed that the highest percentage of emerged *Phytomyza* adults were found in fleshy *Orobanche* spikes collected in early and mid April. While *Phytomyza* pupae collected from the same *Orobanche* stage were completely unparasitized through April. The highest percentages of unemerged pupae from the three investigated phenological stages were observed during late of April which entered a long period of diapause. Data confirmed that, it is possible to collect the highest numbers of unparasitized *Phytomyza* pupae from fleshy *Orobanche* spikes (principal growth stage 6-65, full flowering: 50% flowers open, first petals may be fallen) during April. To use in mass-culture for any bio-control programe of the weed, *O.crenata.*

Key Words: *Phytomyza orobanchia, Orobanche crenata, Tetrastichus phytomyzae*, Biological control, Egypt.

INTRODUCTION

Biological control of the fully parasitic weed *Orobanche* species by the agromized, *Phytomyza orobanchia* Kalt. has been successfully applied in the former USSR and Eastern Europe. The natural capacity of *P.orobanchia* to reduce the *Orobanche* population is limited by several factors such as low temperature, cultural practices, insecticide application and parasitoids (Kroschel & Klein, 1999). The most predominant parasitoid species are the braconids *Opius occulus* Tel., *Crataepiella carlinarum* Szel., *Diglyphous isaea* Walk. And the eulophid *Tetrastichus phytomyzae* Kost., as well as the pteromalid *Sphegigaster orobanchiae*, as reported by Mihajlovic (1986) and Horvath & Wittmann (1988) in Europe . In this concern, Klyueva *et al.* (1978) and Bondarenko (1986) added that parasitism rates by the above mentioned species may reach up to 90% in some countries, which means a considerable population reduction of the *Phytomyza* flies leading to a possible outbreak in the broomrape population.

A reduction of the *Orobanche* seed production can only be achieved if the natural population of *P.orobanchia* is strengthened. This can be secured either by the creation of better conditions for *P.orobanchia* to reproduce and increase in number or by mass-rearing and targeted releases at the beginning of the *Orobanche* flourish (Trenchev, 1981). Rearing on artificial diets has not been successful so far (Cubero & Moreno, 1979), hence other methods which have been developed in the 1960s and 1970s in the former Soviet Union are the most suitable for bio-control of this parasitic weed. In Egypt, a biological control trial of the weed *O.crenata* was conducted by Kolaib,1991 at El-Tahrir area by spreeding the *P.orobanchia* pupae.

The species *Tetrastichus phytomyzae* is the only encountered parasitoid of *P.orobanchia* in Egypt till now (Tawfik *et al.*, 1976, Al-Eryan *et al.*, 2001). In order to reach their hosts, parasitoid females have to pierce into the broomrape stems locating *Phytomyza* maggots or pupae (Kostyukov, 1977, then deposite more than one egg into the puparium. After hatching, larvae feed gregariously in one host until pupation (Al-Eryan *et al.* 2001). The present study aims to develop a simple technique for collecting unparasitized *Phytomyza* pupae from parasitic *Orobanche* plants for the purpose of bio-control of that weed.

MATERIALS AND METHODS

At the end of the faba bean growing season, *i.e.*, during April, *Phytomyza* maggots mine toward the under`-ground parts of *Orobanche* to undergo pupation and start aestivation until the next season (Fig. 1).

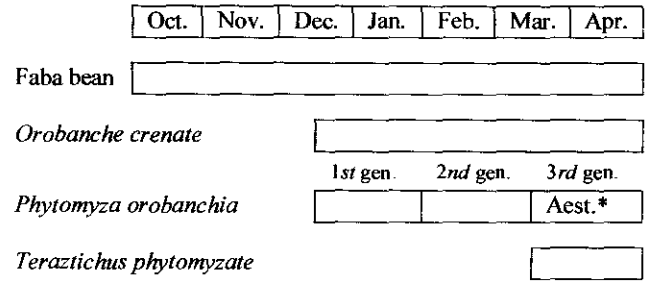

Fig. (1) seasonal distribution of faba bean, *Vicia faba* and its bio- associates. (* Aest. = Aestivation).

In April 2000, three regions of West Nile Delta, namely Abbis, Kafr El-Dawar and Abu El-Matamir were selected for the experiments. Three *faba bean* fields highly infested with *O.crenata* were chosen in each region. *Orobanche* spikes were divided into three phenological stages. According to Hess *et al.* (1997) these stages were described as fleshy (principal growth stage 6-65, full flowering: 50% of flowers open, first petals may

be fallen); semi-fleshy (principal growth stage 6-69, end of flowering: fruit set visible); and dry (principal growth stage 7-79, nearly all fruits have reached final size normal). Fifteen spikes from each growth stage were collected randomly from each field. Samples were taken three times in April and in labeled paper bags to the laboratory. Each growth stage was kept in a labeled cage and covered with muslin cloth until *Phytomyza* pupation. When all spikes ripened, the *Orobanche* stems were dissected to detach and count the pupae. The collected pupae from each *Orobanche* growth stage were separately preserved in a covered glass vial. At the time of adults emergences (May,2000 for *Tetrastichus* and January,2001 for *Phytomyza*) parasitoid or *Phytomyza* adults were collected daily and recorded weekly. The percentages of emerged *Phytomyza* adults and parasitized pupae by *Tetrastichus* were calculated as follows:

$$(1)\ \%\ \textit{Phytomyza}\ \text{adult emergence} = \frac{\text{No. emerged adults}}{\text{Total No. of pupae}}$$

$$(2)\ \%\ \text{Parasitism} = \frac{\text{No. of emerged parasitoids/6* X 100}}{\text{Total } \textit{Phytomyza} \text{ pupae}}$$

* = Mean No. of parasitoids per pupa (Al-Eryan *et al.*, 2001)

The results were statistically analyzed by ANOVA.

RESULTS

The seasonal abundance of *P. orobanchia* and its parasitoid, *T. phytomyzae* in three locations of West Nile Delta are presented in Fig. (2). The results indicate that the adults of *P. orobanchia* made their first appearance in January and reached the first peak late in January. The second peak occurred late in March. At that time, emergence of *Phytomyza* adults synchronized with *Orobanche* flourishing in the field. The emerged parasitoid adults had two peaks per year. The first peak was recorded in June 2000 and the second was during April 2001.

Percentages of emerged adults from the three growth stages of *Orobanche* spikes in the three experimental locations are presented in Table (1). Statistical analysis revealed that the percentage of emerged *Phytomyza* adults from fleshy *Orobanche* was significantly higher (66.89%) than those collected from both semi-fleshy (39.16%) and dry *Orobanche* (46.67%). Irrespective of growth stages, late April emergence was significantly lower than both mid and early April recording 31.56, 56.17 and 64.98%, respectively. Meanwhile, no significant differences were observed between early and mid April. In general, the highest numbers of healthy *Phytomyza* pupae were found in fleshy *Orobanche* collected in early and mid April from the experimental locations (Table 1).

Table (2) shows the percentages of *Phytomyza* pupae parasitized by *T. phytomyzae* collected from the three growth stages of *Orobanche* spikes. The results proved that the *Phytomyza* pupae collected from fleshy *Orobanche* spikes were completely unparsitized in the three collecting dates. The higher percentages of parasitized *Phytomyza* pupae were recorded in semi-fleshy (25.84%) and dry spikes (15.34%). Analysis of variance indicated significant differences between the three investigated growth stages of *Orobanche* spikes in hosting parasitized *Phytomyza* pupae. Irrespective of growth stages, the lowest percentage of parasitism occurred early in April (2.62%) followed by mid April (12.93%) and late April (25.63%). The statistical analysis showed significant differences between the three investigated collecting dates (Table 2).

Table (1): Percentages of adults emerged from *phytomyza* pupae separated from three growth stages of *Orobanche* spikes during April 2000 in West Nile Delta.

Collecting date	% *Phytomyza* emergence			Mean
	Fleshy	Semi-fleshy	Dry	
April,11	76.18	57.48	61.29	64.98a
April,18	70.04	50.52	47.96	56.17a
April,25	54.44	9.49	30.75	31.56b
Mean	66.89a	39.16b	46.67b	50.56

L.S.D. $_{0.05}$ (collecting date) = 11.85
L.S.D $_{0.05}$ (Growth stages) = 11.85

Table (2): Percentages of parasitized pupae of *P. orobanchia* with *Tetrastichus phytomyzae* separated from three growth stages of *Orobanche* spikes during April 2000 in West Nile Delta.

Collecting date	% Parasitized pupae			Mean
	Fleshy	Semi-fleshy	Dry	
April,11	0.00	7.86	0.00	2.62 c
April,18	0.00	19.57	19.22	12.93 b
April,25	0.00	50.07	26.81	25.63 a
Mean	0.00 c	25.84 a	15.34 b	13.73

L.S.D. $_{0.05}$ (collecting date) = 9.82
L.S.D. $_{0.05}$ (Growth stages) = 9.82

After adult emergence of both *P. orobanchia* and the parasitoid, *T. phytomyzae*, remained *Phytomyza* pupae did not emerge and entered a prolonged diapause. The percentages of unemerged pupae separated from the three investigated growth stages of *Orobanche* spikes in the three locations are shown in Table (3). The highest percentages of non-emerged pupae (45.56%) were observed during late of April. At the same time and irrespective of collecting dates, no significant difference was observed between the investigated growth stages of *Orobanche* spikes in hosting non-emerged pupae.

Table (3): Percentages of unemerged pupae of *P. orobanchia* separated from three growth stages of *Orobanche* spikes during April 2000 in West Nile Delta.

Collecting date	% Unemerged pupae			Mean
	Fleshy	Semi-fleshy	Dry	
April,11	23.82	34.66	38.71	32.39 b
April,18	29.96	29.91	32.82	30.89 b
April,25	45.56	40.43	42.44	42.81 a
Mean	33.11	35.001	37.99	35.36

L.S.D. $_{0.05}$ (collecting date) = 7.35

DISCUSSION

The parasitoid, *T. phytomyzae* was recorded in high numbers in the pupae of the broomrape fly, *Phytomyza*

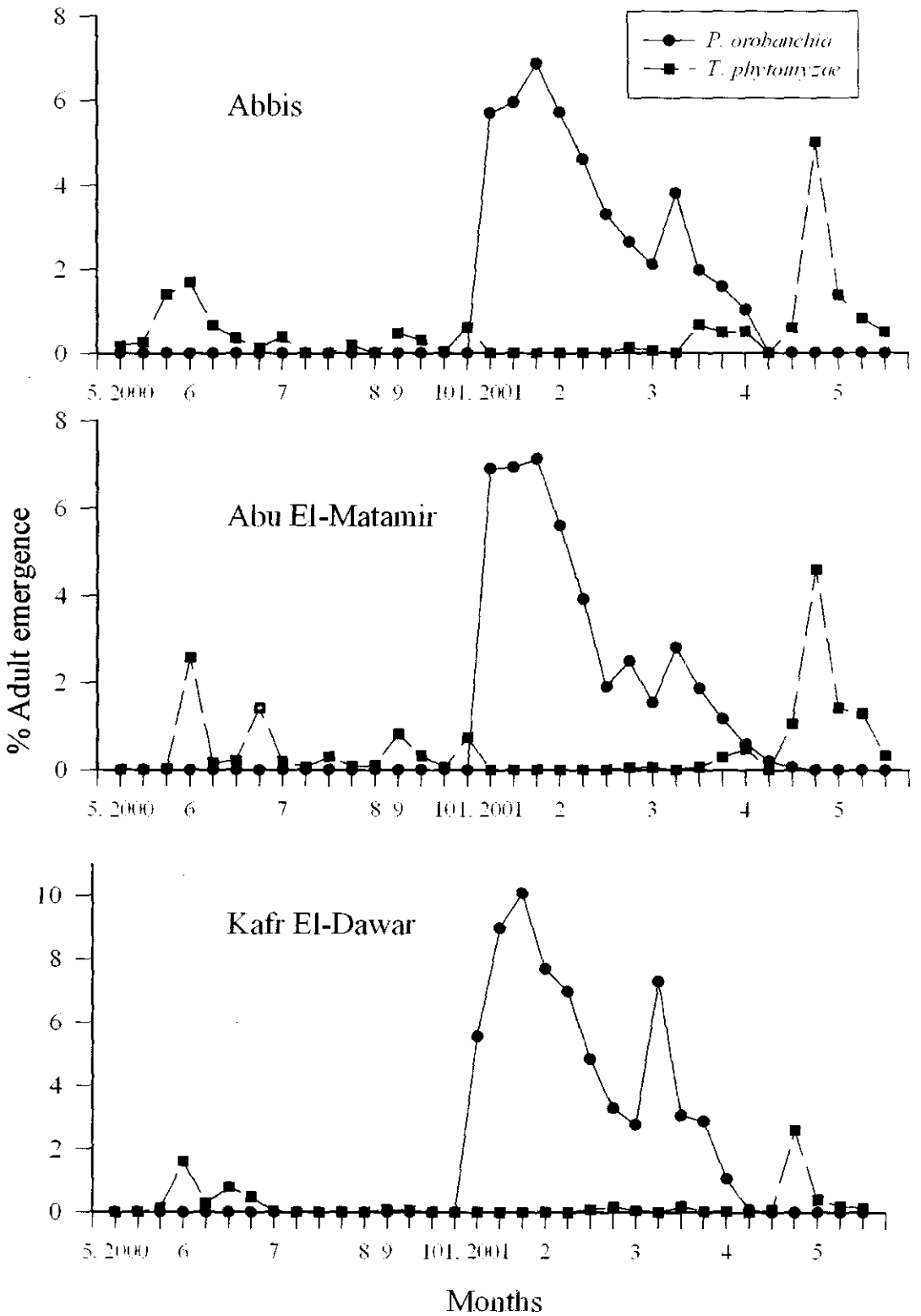

Fig. (2): Percentage of *Phytomyza orobanchia* and its parasitoid, *Tetrastichus phytomyzae* adults emerged from collected *Phytomyzae* pupae at three locations of West Nile Delta

orobanchia. This parasitoid was carefully studied and described by Al-Eryan *et al.* (2001). The results in the present study agree with those recorded by Al-Eryan *et al.* (2001) who recorded a parasitization rate by *T. phytomyzae* ranging between 16.0%-67.77%. Similar results were reported from different countries in the former USSR and East Europe. In the former USSR, Klyueva *et al.* (1978) found that high numbers of *P. orobanchia* pupae were heavily parasitized by hymenopterous parasitoids and other pupae remained in diapause. In Yugoslavia, the recorded parasitization rate by hymenopterous parasitoids was 32% (Mihajlovic, 1986). Parasitism rates up to 90% were reported from some countries (Klyueva *et al.* 1978 and Bondarenko, 1986). These results disagree with those reported by Tawfik *et al.* (1976) in Egypt, who recorded low percentage of parasitism, not exceeding 3% with *Tetrastichus* sp. in Giza region, may be due to inproper collecting date.

Synchronization between the parasitoid, *T. phytomyzae* and its host, *Phytomyza* pupae enter aestivation by end of the growing season, *i.e.*, in April. The absence of parasitism in fleshy *Orobanche* spikes (Table 2) may be due to the fact that this *Orobanche* growth stage was mainly infested with eggs and first and second instar larvae of *P.orobanchia* (Al-Eryan, 1996), while the suitable host stages, *i.e.*, *Phytomyza* pupae, for the parasitoid are not yet formed. Accordingly, the fleshy stage is unattractive for the parasitoid. In case of the semi-fleshy and dry *Orobanche* spikes, the *Phytomyza* pupation synchronizes with the formation of those two growth stages in the field (Al-Eryan, 1996). Thus, they are attractive for the parasitoid and consequently some of the *Phytomyza* pupae separated from them were found parasitized, *i.e.*, the suitable host stage for parasitism.

The occurrence of high parasitism rate creates a control problem since the remaining numbers of *P. orobanchia* may fail to control *Orobanche*. This was pointed out by Klyueva *et al.* (1978) and Pamukchi (1979), who stated that Calcididae-parasitism rates on *P. orobanchia* pupae collected from *O. cumana* were higher (34%-94%) than those from *O.ramosa* (5%-54%). They suggested collecting the required *Phytomyza* pupae for *Orobanche* control from the *Orobanche* species having lower rates of parasitism. Another approach was suggested by Klyueva and Pamukchi (1982) when they developed a release methodology by which they could separate the emerged *Phytomyza* adults and capture the parasitoid adults.

By using this method, they could decrease the population of *Tetrastichus* leaving enough numbers of the *Phytomyza* flies to feed on the *Orobanche* seeds. In the Ukraine, Tsybul'skaya *et al.* (1978) used a quick and accurate determination of the number of healthy, empty and parasitized pupae using X-ray diagnosis. Another approach to avoid parasitization was suggested by Klyueva and Pamukchi (1980). They stated that the material to be used for biological control in the next season should be taken from the first generation of *P. orobanchia*. This generation is characterized by less *Tetrastichus* parasitism than late generation. The pupae of the first generation were collected from *O. cumana* and artificially induced to diapause until the new season.

In conclusion, collecting *Phytomayze* pupae from fleshy *Orobanche* spikes may assure obtaining unparasitized pupae. Accordingly, the natural capacity of *P. orobanchia* to reduce the *Orobanche* population will be improved. Although this technique seems to be simple and economical, the identification of phenological stages of *Orobanche* should be certain and accurate.

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