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INFLUENCE OF TEMPERATURE ON BREAKING DIAPAUSE, DEVELOPMENT AND EMERGENCE OF *MEGACHILE MINUTISSIMA* (HYMENOPTERA, MEGACHILIDAE)

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Influence of Temperature on Breaking Diapause, Development and Emergence of *Megachile minutissima* (Hymenoptera, Megachilidae). Kamel, S. M., Osman, M. A. M., Mahmoud, M. F., Haggag, El-S. I., Aziz, A. R., Shebl, M. A. — The leafcutting bee, *Megachile minutissima* Radoszkowski, 1876, is a valuable pollinator of alfalfa grown for seed production in Egypt. In order to control adult emergence of *M. minutissima* to be synchronized with the expected changes in flowering times of alfalfa crop, breaking the prepupal diapause to initiate the subsequent developmental stages of this insect pollinator is necessary. Laboratory experiments were conducted to estimate the influence of incubation temperature at 30 ± 0.4 °C after different intervals of cold storage at 10 ± 0. 4 °C on loose bee cells containing diapaused prepupae, which were obtained during the previous season from the successful nesting straws of the artificial nests. Results indicated that the shortest incubation time required for breaking prepupal diapause was about 12 and 19 days to reach the pupal and adult stages, respectively. The percentage of emerged adults was about 60 % at 152 days cold store, and gradually decreased with the increase of cold storage periods in 2009. Meanwhile, the percentage of emerged adults ranged between 40–60 % at all cold storage periods in 2010. Moreover, the results indicated that the optimal period of cold storage at 10 ± 0.4 °C for diapaused prepupae in their loose cells should be between 146 to 153 days, where the maximum rate of adult emergence was occurred. The maximum rate of newly emerged males and females of *M. minutissima* was recorded at 10 % and 26 % in April 26, 2009 and 30 % and 20 % in May 4, 2010, respectively. However, the minimum rate of adult emergence was occurred in August, September and October months.

Key words: Adult emergence, cold storage, diapause, loose cell management, incubation, artificial nesting, *Megachile minutissima*.

Introduction

Alfalfa, *Medicago sativa* L., is a high quality forage and green manure crop that is well adapted to the situation and resists drought and heat. The importance of alfalfa has been increased after the expansion in cultivation of the reclaimed desert in Egypt with approximately of 80–120,000 ha, and acreage is noticeably rising each year especially in the newly reclaimed lands (Shebl et al., 2008 a; 2009). However, alfalfa seed productivity is considered relatively low (averaged about 150 kg/ha) in comparison with world's records (El-Nahrawy & Rammah, 1995). One of the major problems that face most of the newly reclaimed areas is the relatively low production of crops due to the lack of insect pollinators. For maximum production of high-quality seed yield, the alfalfa flowers must be tripped and cross-pollinated by specialized group of bees (Rashad & Ewis, 1985; Abrol, 1993). Tripping occurs when the action of a flower-visiting bee release pressure on interlocking keel petals, abruptly snap upward from within the keel (Frank, 2003).

Solitary bees such as leafcutting bees (Megachilidae, Hymenoptera) are the most effective pollinators of alfalfa and can increase seed yield as much as 20 folds with good management (Richards & Kevan, 2002; Shebl et al., 2009). The importance of using leafcutting bees to pollinate alfalfa was recognized in the late of 1950's, and shortly thereafter developed methods to manage and propagate such bee pollinators (Stephen, 1962; Hobbs, 1967; Richards, 1984; Pesenko & Radchenko, 1993; Shebl et al., 2008 b). The alfalfa leafcutter bee, *Megachile minutissima* Radoszkowski is a common solitary cavity-nesting bee widely distributed across Egypt, with a gregarious habit, each female builds her own nest by cutting, transporting, and placing suitable leaf pieces of alfalfa or other plants in a tunnel creating a series of brood thimble-shaped cells, collecting provisions of pollen and nectar, and laying eggs in the cells. Completed nests are sealed with a cut-leaf plug. By the end of the summer, most bee larvae enter diapause as prepupae and wait to resume development in the following summer (Alqarni et al., 2014; Shebl et al., 2013).

The evolution of management practices for the leafcutting bee has depended on improvements in artificial nest materials such as polystyrene, paper, and wood-laminate (Richards, 1978; Parker et al., 1983; Kamel et al., 2007; Shebl et al., 2018). Great numbers of leafcutter bees (50,000–75,000 / ha) are needed to pollinate alfalfa crop. For this reason, the loose-cell system of leafcutter bee management was developed (Hobbs, 1973; Richards, 1987). This system places the optimum number of bees on the crop at the appropriate time to obtain a high seed set and an adequate return of viable bees for the following year (Richards, 1984).

The loose-cell system enables easy removal of bee cells from the artificial nesting material made of pine wood, polystyrene or rolled fluted paper nest materials during the annual management cycle for storage over the winter and help to control the natural enemies of the bees and diseases (Richards & Kevan, 2002). The development and emergence of bees can be regulated more easily by using controlled incubation facilities as compared to relying on field conditions and controlling the blooming of the crop (Bohart, 1972; Baird & Bitner, 1991). In this system, the cocoons containing diapaused prepupal stage of leafcutting bees are stripped or punched out of the nest materials at the end of the activity season. The loose cocoons are kept in boxes, cans or bags in cold storage in order to direct release in the alfalfa fields after incubation or for commercial trade nationally or internationally (Richards & Kevan, 2002). Incubation of the cells must perform approximately 21 days before peak bloom is expected to synchronize the emergence of the adult bees with 10 % bloom in the field that begins in late May to early June (Fairey et al., 1984).

Diapause is an important physiological mechanism regulating the timing of development and reproduction by which leafcutting bees overwintering. The prepupae of *M. minutissima* must enter in diapause for long time reached to 8.9 months for females and 9.8 months for males before their transformation into pupae. Temperature plays a major role in regulating the breaking of prepupal diapauses (Kireyeva, 1984; Rashed & Ewis, 1985; Kireyeva & Butuzova, 1986; Kireyeva & Bodnarchuk, 1990). However, the establishment of such pollinator faced several obstacles such as insect parasitoids and diseases, as well as asynchronous adult emergence with the alfalfa blooming. Therefore, the aim of this study is to use the temperature treatment in loose system management as an attempt to break the prepupal diapause of this insect pollinator and control of adult emergence to be synchronized with the expected changes in flowering seasons of alfalfa crop under Egyptian condition.

Material and methods

Establishment and nesting of alfalfa leafcutting bee *M. minutissima*

Several attempts to establish *M. minutissima* were conducted from the beginning of 2000 till now at the bee research center, Suez Canal University in Ismailia, Egypt. Nesting shelters with artificial foam nests were prepared as a tool to attract bees and boost their local populations as published in several papers (Kamel et al., 2007; Shebl et al., 2008 a, b).

Obtaining the loose leafcutting bee cells

The loose cells or free cells containing prepupae of *M. minutissima* were obtained from the prior successful nesting straws of the artificial nests, placed at the Experimental Farm, Faculty of Agriculture, Suez Canal University. These straws of rolled paper nest materials are available for the loose-cell system. At the end of the flowering season, the nesting straws were removed from their holes in the foam boards, and cells containing the bee cocoons were easily removed, taken apart and stripped from excess leaf material and nest-destroying

or predacious insects (Richards, 1984). In order to obtain an appropriate numbers of prepupae of homogenous age, an examination of a large numbers of straws up to 1440 straw were performed. The completed straws were taken and dissected to separate cells containing the living prepupae. The collected prepupae were placed in 10 jars with 100 prepupae in each and a total of 1000 prepupae were used to conduct this experiment.

Incubation of the loose cells

Jars containing the diapaused prepupae were covered with a piece of muslin and refrigerated at 10 ± 0.4 °C in November, 2008. These prepupae were observed and examined weekly to ensure that the prepupae are still alive and not change into pupae, as well as to record the parasitic wasps appeared during storage. The diapausing prepupae were remained under cold storage for different periods ranged between 5 to 10 months. First sample of 50 prepupae was taken after 152 days from cold storage and each prepupa was placed inside a small glass tube. Tubes containing prepupae were covered with parafilm and transferred to other incubator at 30 ± 0.4 °C. Similar samples were taken from the cold storage at 13 different intervals: 164, 180, 193, 200, 207, 214, 221, 235, 238, 241, 286, 296 and 300 days and transferred to incubate in (Raven) incubator at 30 ± 0.4 °C. The same work was repeated in November 15th, 2009. While only 6 different intervals (146, 153, 170, 174, 181 and 188 days) were studied and methodology was the same as in 2008/2009.

Samples in incubator were observed daily to record the time needed to break the diapausing state of incubated prepupae and the beginning time of its transformation into pupae and complete their development to the adult stage. The time of adult emergence was recorded in each interval, and the percent of parasitism with *Coelioxys* sp. was also recorded. The emerged adults of *M. minutissima* were sexed then released later in the alfalfa fields during the blooming period.

Results

Effect of different intervals of cold storage at 10±0.4°C on the postdiapause development of *M. minutissima* in incubator at 30 ± 0.4 °C

Table 1 showed the percentage of immature survival of incubated *Megachile minutissima* prepupae, which prior stored at 10 ± 0.4 °C for different intervals. The obtained results indicated that the survivorship of pupal stage was highest at the periods of 152, 146, 153 and 164 days cold store with percent of 74, 70, 70 and 64 %, respectively. The highest rate of prepupal adult survival was recorded at 136, 146, 152 and 153 days cold store as 20, 20, 38 and 30 for females and 30, 40, 22 and 30 for males, respectively. The prepupal adult survival decreased with the increase of cold storage periods. Our observations showed that the first occurrence of pupal stage was recorded after 7 days of incubation and represented by 1, 2, 2, 1 and 1 pupa for 164, 181, 193, 207, 214 days cold storage in season of 2009, while represented by 4 pupae after 9 days of incubation for the 188 days cold storage. The transformation into pupal stage delayed with the increase of cold storage periods during diapaused prepupal stage. Also, the first appearance of adults was recorded after 19 days of incubation and represented by 3 females and 5 males for 152 days of cold storage in 2009 season. Similar data of adult occurrence were observed for 164, 181, 193, 200 and 207 days of cold storage in 2009 season and for 170 and 188 days of storage in 2010 season. Adult emergence delayed with the increase of cold storage periods over than 200 days. Results also revealed that the diapaused prepupae could transform in incubator at 30 ± 0.4 °C into pupae reached to the adult emergence after 17–19 and 19–26 days in 2009 and 2010, respectively. The incubation time required to occur the subsequently developmental stages of *M. minutissima* was 12 days for pupal stage, and 19 days for adult stage of both sexes. Moreover, data indicated that the largest number of emerged adults of the parasitic bee *Coelioxys* sp. was recorded after 17–26 days of incubation for the different tested periods of cold storage in 2009 season with a parasitism percentage of about 3.57 %. The sex ratio of emerged bees was 1.7 ♀ : 1 ♂. Data further indicated that mortality rate of prepupal stage was higher than those of pupal and adult stages (table 1).

Effect of cold storage periods on the incubation time (days) needed for transformation into pupae and the rate of adult emergence of *M. minutissima*

As shown in table (2), the shortest incubation time needed for breaking prepupal diapause and occurrence the subsequently developmental stages with maximum percentage

Table 1. Percentage of immature survival of *Megachile minutissima* prepupae stored at 10 ± 0.4 °C for different intervals prior to incubation at 30 ± 0.4 °C

Stored intervals (days)	n	Prepupal mortality		Transformed pupae		Pupal mortality		Time needed for prepupal-pupal transformation (days)		Adult emergence				Time needed for prepupal-adults transformation (days)		Parasitism*	
										♀		♂					
		No.	%	No.	%	No.	%	Min.	Max.	No.	%	No.	%	Min.	Max.	No.	%
2008/2009																	
152	50	13	26	37	74	2	4	12	183	19	38	11	22	19	26	5	10
164	50	18	36	32	64	1	2	7	23	16	32	10	20	19	26	5	10
181	50	26	52	24	48	0	0	7	118	13	26	8	16	17	122	3	6
193	50	27	54	23	46	5	10	7	108	11	22	3	6	19	108	4	8
200	50	31	62	19	38	3	6	10	101	4	8	9	18	17	108	3	6
207	50	27	54	23	46	6	12	7	101	12	24	4	8	17	111	1	2
214	50	37	74	13	26	5	10	7	115	2	4	4	8	76	94	2	4
221	50	27	54	23	46	11	22	66	108	4	8	5	10	80	118	3	6
235	50	38	76	12	24	2	4	52	73	6	12	4	8	66	104	0	0
238	50	42	84	8	16	4	8	59	91	2	4	2	4	63	80	0	0
241	50	48	96	2	4	1	2	52	66	0	0	1	2	59	59	0	0
286	50	46	92	4	8	0	0	52	58	0	0	2	4	58	70	2	4
296	50	46	92	4	8	0	0	42	53	1	2	2	4	53	67	1	2
300	50	48	96	2	4	0	0	38	44	1	2	1	2	56	56	0	0
2009/2010																	
136	10	5	50	5	50	0	0	16	17	2	20	3	30	33	33	0	0
146	10	3	30	7	70	0	0	18	24	2	20	4	40	24	42	1	10
153	10	3	30	7	70	0	0	17	24	3	30	3	30	21	28	1	10
170	10	5	50	5	50	0	0	11	18	4	40	1	10	18	27	0	0
174	10	7	70	3	30	0	0	14	23	1	10	2	20	23	33	0	0
181	10	5	50	5	50	0	0	16	56	2	20	3	30	26	70	0	0
188	10	6	60	4	40	0	0	9	9	2	20	2	20	19	19	0	0

n = initial number of prepupae per each storage period, * — rate of parasitism was calculated based on number of storage prepupae.

of adult emergence of *M. minutissima* were recorded in 136, 146, 152, 153, 164 and 170 days cold storage. The highest rate of adult emergence of *M. minutissima* was reached to 60 % at 146, 152 and 153 days cold storage. Apparently, there was an apparent decrease in rates of adult emergence with the increase of cold storage periods. For instance the percentage of emerged adults was 60 % at 146 days storage, 40 % at 188 days storage, and decreased sharply to 28 % and 4 % at 193 and 300 days, respectively.

Data in figure 1 showed that the highest percentage of newly emerged females of *M. minutissima* was recorded at 152 and 171 days of cold storage, and decreased slightly with the increase of cold storage periods. However, the highest percentage of newly emerged male was recorded at 146, 153 and 188 days of cold storage. Also, the obtained results

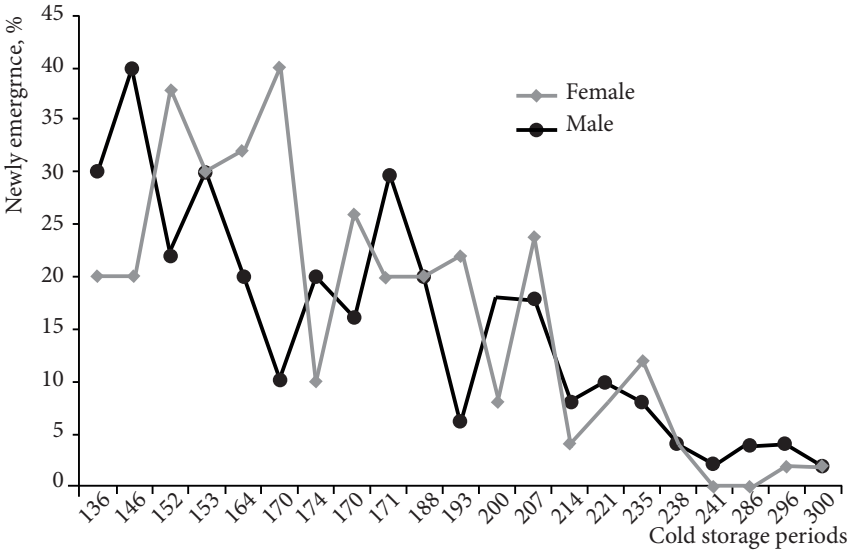

Fig. 1. Percentage of newly emergence of *M. minutissima* males and females under incubation conditions at 30 ± 0.4 °C after different cold storage periods.

proved that the sex ratio of *M. minutissima* was 1.41 ♀ : 1 ♂ at 146 to 188 days storage.

Data in table 3 represented the maximum level of newly emerged adults of *M. minutissima* during the period from April 2nd, 2009 till June 6th, 2010. Data revealed that the maximum rate of adults' emergence was recorded in April 26th, 2009 and May 4th, 2010 at 10 % and 26 % for males and 30 % and 20 % for females, respectively.

The maximum rate of newly emerged males and females of *M. minutissima* was 10 % and 26 % in April 26th, 2009 and 30 % and 20 % in May 4th, 2010, respectively. While the maximum rate of emerged females was recorded by 30% in May 22nd, 2010. On the other hand, the minimum rate of adult emergence for both males and females were occurred in August, September and October in both seasons (table 3).

Discussion

Efficient use of cold storage and incubation facilities leads to synchronise of bee emergence with the beginning of flower bloom, so that adult bees can be released into the field in a timely manner (Kireyeva, 1987; Richards & Kevan, 2002). The obtained results revealed that the cold stored diapaused prepupae could transform in incubator at 30 ± 0.4 °C into pupae reached to the peak of adult emergence after 17–19 and 19–24 days in 2009 and 2010 which is more closely obtained in some published papers (Pitts Singer & James, 2005). The peak of adult emergence was occurred after 19–23 days at 30 °C. Data indicated that the developmental periods for males and females in incubation time at 30 °C was 17 and 19 days (Peterson et al., 1991). The developmental periods for males and females of diapausing bees were 18.1 days at 22 °C and 10 days at 29 °C (Petrowski, 1991). The time of pre-emergence adults increased with increasing of storage time in cold incubator. Our results are different with some other published data of the high survival of prepupae, emergence of cocoons and the emergence time after incubation (Richards et al., 1987; Richards & Whitfield, 1988; Bosch & Kemp, 2004, a, b).

The development of *Megachile rotundata* was affecting by the changed temperature in the autumn-winter period (Kireyeva, 1990; Radchenko et al., 1993). In our earlier study, cold storage periods at 10 ± 0.4 °C significantly affected the time needed

Table 2. Cold storage periods of diapaused prepupae of *M. minutissima* at 10 ± 0.4 °C and the incubation time at 30 ± 0.4 °C needed for breaking their diapause and transformation into pupae and pre-emergence adults

Cold storage (10 ± 0.4 °C) periods (days)	Incubation period at 30 ± 0.4 °C		
	Time needed (days) for transformation into:		Emergence (%) *M. minutissima*
	pupae	Pre-emergence adults	
136	16	33	50
146	24	24–42	60
152	12–26	19–26	60
153	17	21-28	60
164	7–23	19–26	52
170	11	18–27	50
174	14	23–33	30
180	7–26	17–26	42
181	56	26	50
188	9	19	40
193	7–21	19–24	28
200	10–17	17–108	26
207	7–16	17–111	32
214	7–69	76–94	12
221	66–76	76–118	18
235	52–66	66–73	20
238	59–91	63–81	8
241	52	59	2
286	52	58–70	4
296	42	53–67	6
300	38	56	4
Cold storage periods (days)	Time needed (days) for transformation into:		Emergence (%) *M. minutissima*
	pupae	Pre-emergence adults	
136	16	33	50
146	24	24–42	60
152	12–26	19–26	60
153	17	21–28	60
164	7–23	19–26	52
170	11	18–27	50
174	14	23–33	30
180	7–26	17–26	42
181	56	26	50
188	9	19	40
193	7–21	19–24	28
200	10–17	17–108	26
207	7–16	17–111	32
214	7–69	76–94	12
221	66–76	76–118	18
235	52–66	66–73	20
238	59–91	63–81	8
241	52	59	2
286	52	58–70	4
296	42	53–67	6
300	38	56	4

for breaking the prepupal diapause and the rate of adult emergence of *M. minutissima* under incubation conditions of 30 ± 0.4 °C. The statistical analysis of three phases of incubation temperatures (phase I from 1 to 14 days, phase II from 15 to 19 days and phase III from 20–78 days) showed a significant effect on the rate of emergence (Rank & Goerzen, 1982).

Table 3. Incubation periods (days) at 30 ± 0.4 °C needed for maximum emergence of *M. minutissima* adults and the rate of newly emerged males and females

Season	Date of transferred samples to incubator	Maximum of adult emergence		No. (%) ♀	No. (%) ♂
		Date	Periods in days		
2009	2.4.2009	26/4/2009	24	13 (26)	5 (10)
	14.4.2009	5/5/2009	21		3 (6)
		7/5/2009	23	8 (16)	
	30.4.2009	21/5/2009	21	5 (10)	4 (8)
	14.5.2009	2/6/2009	18	5 (10)	2 (4)
	21.5.2009	7/6/2009	16		4 (8)
		10/6/2009	19	1 (2)	
	28.5.2009	14/6/2009	17		2 (4)
		6/9/2009	101	6 (12)	
	4.6.2009	30/8/2009	87		2(4)
		6/9/2009	94	2 (4)	
	11.6.2009	26/8/2009	77		3 (6)
		30/8/2009	81	1 (2)	
	25.6.2009	31/8/2009	67		2 (4)
		17/9/2009	84	2 (4)	
	28.6.2009	28/8/2009	61		1 (2)
		5/9/2009	69	1 (2)	
	2.7.2009	20/8/2009	49		1 (2)
	16.8.2009	13/10/2009	58		1 (2)
	26.8.2009	18/10/2009	53		1 (2)
		1/11/2009	67	1 (2)	1 (2)
	30.8.2009	25/10/2009	56	1 (2)	1 (2)
2010	1.4.2010	4/5/2010	33	2 (20)	3 (30)
	10.4.2010	4/5/2010	23	1(10)	3 (30)
	17.4.2010	8/5/2010	21		2 (20)
		15/5/2010	28	2 (20)	
	4.5.2010	22/5/2010	18	3 (30)	
		31/5/2010	27		1 (10)
	8.5.2010	31/5/2010	23	1 (10)	
		10/6/2010	33		2 (20)
	15.5.2010	10/6/2010	26	2 (20)	
		24/7/2010	70		2 (20)
	22.5.2010	10/6/2010	19	2 (20)	2 (20)

From the obtained results, it was clear that diapaused prepupae should be cold stored (10 °C) for 136 to 188 days, and then incubated at (30 °C) until adult emergence, three weeks prior to alfalfa bloom (Peterson et al., 1994). Also, the pupation of managed populations is delayed by holding prepupae in cells at 2–3 °C for 3 weeks before the blooming of Lucerne. Cells are then incubated at 25 °C, the first bees emerged 3 weeks later, and emergence was completed within a further 10 days (Donovan, 1980).

Based on the data obtained during five years of study (from 2006 to 2010), it was noticed that when the straws were left out side during the winter, mortality for various reasons was high. For best results, cells should be stored in a dry, cool place, and then incubated for the following spring to adjust adult emergence when needed (Hobbs, 1969). Therefore, it was clear that the prepupae should be incubated at 30 °C, the prepupae of

leaf cutting bee exposed to 35 °C and 37 °C emerged later than bees incubated at 30 °C (Richards & Whitfield, 1988).

Conclusion

It could be conclude that, temperature plays an important role in regulating the breaking process of prepupal diapause in alfalfa leafcutting bee, *M. minutissima*. As the results indicate, the incubation time needed for transformation of diapaused prepupae into pupae reached to adult stage is greatly influenced by the different cold storage periods at 10 ± 0.4 °C before incubation at 30 ± 0.4 °C. The rate of pupal and adult survival is considerably decreased with the increase of cold storage period over than 153 days. The optimal period of cold storage at 10 ± 0.4 °C for diapaused prepupae in their loose cells should be between 146 to 153 days, where the maximum rate of adult emergence was occurred. Our results suggest that, the loose cells of *M. minutissima* containing prepupal stage must be stored for proper cold periods at 10 °C, and then transferred to incubator at 30 °C for three weeks prior to alfalfa bloom for breaking the prepupal diapause and encourage the adult emergence as required.

This work is considered as a first step to establish leaf cutting bees industry in Egypt.

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References

Abrol, D. P. 1993. Energy intake and expenditure in alfalfa pollinating bee *Megachile femorata* (Hymenoptera : Megachilidae). *Journal of Tropical Ecology*, **34**, 173–180.

Alqarni, A. S., Hannan, M. A., Gonzalez, V. H., Engel, M. S. 2014. Nesting biology of the leafcutting bee *Megachile minutissima* (Hymenoptera: Megachilidae) in Central Saudi Arabia. *Annals of Entomological Society of America*, **107** (3), 635–640.

Baird, C. R., Bitner, R. M. 1991. *Loose cell management of alfalfa leafcutting bees in Idaho*. Current Info. Ser. No. 588, University of Idaho Cooperative Extension, Moscow, ID.

Bohart, G. E. 1972. Management of wild bees for the pollination of crops. *Annual Review of Entomology*, **17**, 287–312.

Bosch, J., Kemp, W. P. 2004 a. Effect of wintering duration and temperature on survival and emergence time in males of the orchard pollinator *Osmia lignaria* (Hymenoptera: Megachilidae). *Environmental Entomology,* **32**, 711–716.

Bosch, J., Kemp, W. P. 2004 b. Effect of pre-wintering and wintering temperature regimes on weight loss, survival, and emergence time in the mason bee *Osmia cornuta* (Hymenoptera: Megachilidae). *Apidologie,* **35**, 469–479.

Donovan, B. J. 1980. Interactions between native and introduced bees in New Zealand. *New Zealand Journal of Ecology*, **3**, 104–116.

El-Nahrawy, M. A., Rammah, A. 1995. Current status and prospects of alfalfa seed production and use of seed in Egypt. *Proc. 3rd International Herbage Seed Conference.* Halle, Germany, 18–23 June, 336–340.

Fairey, D. T., Lieverse, J. A. C., Siemens, B. 1984. Management of the Alfalfa Leafcutting Bee in Northwestern Canada. Contribution No. NRG 84-21. *Agriculture Canada*, 1–31. Beaverlodge, Alberta.

Frank, G. 2003. *Alfalfa seed and leafcutter bee: productions and marketing manual*. Irrigated Alfalfa Seed Producers Association, Brooks, Alberta, Canada, 1–160.

Hobbs, G. A. 1967. *Domestication of alfalfa leaf-cutter bees*. Canada Department Agriculture Publ. 1313.

Hobbs, G. A. 1969. Further information on alfalfa leafcutter beekeeping. *Agriculture Canada,* Lethbridge, 1–9.

Hobbs, G. A. 1973. *Alfalfa leafcutter bees for pollinating alfalfa in Western Canada*. Canada Department of. Agriulture, Publ. 1495.

Kamel, S. M., Abu Hashesh, T. A., Osman, M. A., Shebl, M. A. 2007. A new polystyrene foam for nesting

leafcutting bees (*Megachile* spp., Megachilidae, Hymenoptera). *Agriculture Research Journal*, Suez Canal University, **7**, 97–101.

Kireyeva, I. M. 1984. On the preimaginal development of *Megachile rotundata* (F.) at different temperatures; p. 218. *In*: Vasil' yev, V. P., ed. *The 9th Congress of the All-Union Entomological Society (Kiev, October 1984). Abstracts. Pt.1,* 1–300, Kiev [In Russian].

Kireyeva, I. M. & Butuzova, L. N. 1986. On some characters of the immature development of *Megachile rotundata* (F.) at different temperatures, 15–19. *In*: Cherkasova, A. I., ed. *Apiculture. A Republican Inter-Departmental Topic Collection of Scientific Papers. No. 17,* 1–56. Urozhay, Kiev [In Russian].

Kireyeva, I. M. 1987. Optimal conditions of incubation by mass-rearing of the leaf-cutter bee, 83–84. *In*: Vasil'yev, V. P., ed. *The 3rd Congress of the Ukrainian Entomological Society (Kanev, September 1987). Abstracts.* Ukrainian Entomological Society, Kiev 1–254.

Kireyeva, I. M., Bodnarchuk, L. I. 1990. The effect of different temperatures on the development and survival of *Megachile rotundata* in the autumn-winter period; pp. 63–65. *In*: Tobias, V. I., L'vovskiy, A. L., eds. *Advances of Entomology in the USSR: Hymenoptera and Lepidoptera. The 10th Congress of the All-Union Entomological Society ([Leningrad], 11–15 September 1989*). *Proceeding s*pp. Zoological Institute of the Academy of Sciences of the USSR, Leningrad, 1–231 [In Russian].

Parker, F. D., Teranishi, R., Olson, A. C. 1983. Influence of attractants on nest establishment by the alfalfa leafcutting bee Hymenoptera: Megachilidae) in styrofoam and rolled paper. *Journal of the Kansas Entomological Society*, **56**, 477–482.

Pesenko, Yu. A. & Radchenko, V. G. 1993. The use of bees (Hymenoptera, Apoidea) for alfalfa pollination: the main directions and modes, with methods of evaluation of populations of wild bees and pollinator efficiency. *Entomological Review*, **72** (2), 101–119.

Peterson, S. S., Baird, C. R., Bitner, R. M. 1991. Variation in weight and postdiapause development among groups of alfalfa leafcutting bees, *Megachile rotundata* (F.), reared in different locations. *Bee Science*, **1**, 230–236.

Peterson, S. S., Baird, C. R., Bitner, R. M. 1994. Heat retention during incubation in nests of the alfalfa leaf cutting bee (Hymenoptera: Megachilidae). *Journal of Economic Entomology*, **87**, 345–349.

Petrowski. 1991. *Forage seed production manual*, USA, 13–15.

Pitts-Singer, T. L., James, R. R. 2005. Emergence success and sex ratio of commercial alfalfa leafcutting bees from the United States and Canada. *Journal of Economic Entomology*, **98**, 1785–1790.

Radchenko, V. G., Pesenko, Yu. A. & Malysheva, N. Yu. 1993. Some pathways of improving the fecundity of the alfalfa leaf-cutter bee (Hymenoptera, Apoidea) under rearing conditions. *Vestnik Zoologii*, **6,** 75–82.

Rank, G. H., Goerzen, D. W. 1982. Effect of incubation temperatures on emergence of *Megachile rotundata* (Hymenoptera: Megachilidae). *Journal of Economic Entomology*, **75**, 467–471.

Rashad, S. E., Ewies, M. 1985. *Chalicodoma mucorea*, a promising Egyptian species for alfalfa pollination, *Proc. 3rd International Conference of Apiulture Tropical climates, Nairobi, 1984*, 134–137.

Richards, K. W. 1978. Comparisons of nesting materials used for the alfalfa leafcutter bee, *Megachile pacifica* (Hymenoptera: Megachilidae*). Canadian Entomologist*, **110**, 841–846.

Richards, K. W. 1984. *Alfalfa leafcutter bee management in western Canada*. Agriculture Canada, Ottawa, Canada. Publication 1495 /E, 1–53.

Richards, K. W. 1987. Alfalfa leafcutter bee management in Canada. *Bee World*, **68**, 168–78.

Richards, K. W., Whitfield, G. H., Schaalje, G. B. 1987. Effects of temperature and duration of winter storage on survival and period of emergence for the alfalfa leafcutter bee (Hymenoptera: Megachilidae). *Journal of the Kansas Entomological Society*, **60**, 70–76.

Richards, K. W., Whitfield, G. H. 1988. Emergence and survival of leafcutter bees, *Megachile rotundata*, held at constant incubation temperatures (Hymenoptera: Megachilidae). *Journal of Apicultural Research*, **27**, 197–204.

Richards, K. W., Kevan, P. G. 2002. Aspects of bee biodiversity, crop pollination, and conservation in Canada. *In*: Kevan, P., Imperatriz Fonseca, VL, eds. Pollinating bees — the conservation link between agriculture and nature. Ministry of Environment, Brasília, 77–94.

Shebl, M. A**.,** Kamel, S. M., Abu Hashesh, T. A., Osman, M. A. 2008 a. The impact of leafcutting bees (*Megachile minutissima*, Megachilidae, Hymenoptera) (Radoszkowski, 1876) artificial nest sites on seed production of alfalfa, Ismailia, Egypt. *Agricultura*, **5**, 33–35.

Shebl, M. A., Kamel, S. M., Abu Hashesh, T. A., Osman, M. A. 2008 b. Seasonal abundance of leafcutting bees (*Megachile minutissima*, Megachilidae, Hymenoptera). *World Journal of Agricultural Sciences*, **4** (2), 280–287.

Shebl, M. A., Kamel, S. M., Abu Hashesh, T. A., Osman, M. A. 2009. The impact of using leafcutting bees (Megachilidae: Hymenoptera) with different fertilization treatments on alfalfa seed production. *Revista de la ciencia del suelo y nutrición vegetal*, **9** (2), 134–141.

Shebl, M. A., Kamel, S. M., Mahfouz, H. 2013. Bee fauna (Apoidea: Hymenoptera) of the Suez Canal Region, Egypt. *Journal of Apicultural Science*, **57**(1), 33–44.

Shebl, M. A., Hassan, H. A., Kamel, S. M., Osman, M. A., Engel, M. S. 2018. Biology of *Osmia latreillei* Spinola under artificial nesting conditions in Egypt**.** Asia *Pacific Entomology*, **21** (3), 754–759. https://doi.org/10.1016/j.aspen.2018.05.008

Stephen, W. P. 1962. Propagation of the leaf-cutter bee for alfalfa seed production. *Agricultural Experiment Station, Oregon State University, Station Bulletin*, 586.

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