The Effect of Various Temperature Treatments on the Development of *Megachile minutissima* in Egypt

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Abstract: Laboratory experiments were conducted as an attempt to break the prepupal diapause of this insect pollinator, in order to control adult emergence to be synchronized with the expected time of flowering seasons of alfalfa crop. Regarding the effect of incubation temperature on post-diapause developmental stages of *M. minutissima*, results indicated that the shortest period of incubation at 30±0.4°C needed for breaking prepupal diapause to initiate the subsequently developmental stages of *M. minutissima* was about 12 days for pupal stage, and 19 days for adult stage of both males and females, while the longest period lasted about 26 days for each of pupae, males and females. The percentage of emerged adults of *M. minutissima* was 60% for the 152 days cold store, and gradually decreased with the increase of the cold storage period in 2009. Meanwhile, the percentage of emerged adults ranged between 40-60% for all cold storage periods in 2010. The sex ratio of emerged bees was 1.7 ♀: 1 ♂. The overlap percentage of parasitism by a Cuckoo bees, *Coelioxys* sp. (Megachilidae: Hymenoptera) was 3.57%. Regarding the effect of cold storage period at 10±0.4°C on the rate of *M. minutissima* adult emergence, results indicated that cold storage period of diapaused prepupae should be between 136 to 188 days at which maximum rate of adult emergence was occurred. Moreover, the percentage of adult emergence decreased with the increase of cold stored periods. Also, results revealed that the maximum rate of newly emerged males and females of *M. minutissima* was recorded at 10% and 26% in April 26th, 2009 and 30% and 20% in May 4th, 2010, respectively. Meanwhile, the maximum rate of emerged females was recorded at 30% in May 22nd, 2010. However, the minimum rate of adult emergence was occurred in August, September and October months.

Keywords: Adult emergence, cold storage, diapuase, 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 hectares, and acreage is noticeably rising each year especially in the newly reclaimed lands (Shebl *et al.*, 2008). However, alfalfa seed productivity is considered relatively low (averaged about 150 kg/ha) in comparison with world's records (El-Nahrawy and 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. The alfalfa flowers must be tripped and cross-pollinated by specialized group of bees for maximum production of high-quality seed yield (Rashad and Ewis,1985,and Abrol, 1993). Tripping occurs when the action of the bee releases pressure on interlocking keel petals, allowing the flowers fused reproductive column to 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 yields as much as 20 folds with good management (Richards and Kevan, 2002). 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 populations (Stephen, 1962; Hobbs, 1967; Richards, 1984; Shebl *et al.*, 2008). The alfalfa leafcutter bee, *Megachile minutissima* Radoszkowski is a solitary cavity-nesting bee with a gregarious habit and attracted to previously used nests. At the nests, each female builds her own nest in natural or artificial cavity by cutting, transporting, and placing suitable leaf pieces of alfalfa or other plants in the accurate diameter of (6 mm) 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. *M. minutissima* populations are partially bivoltine. Other individuals, fraction depends of same genetic and environmental factors (so-called "second generation bees") skip prepupal diapause and develop to adulthood to produce a new generation before the end of the summer (Theresa *et al.*, 2010). For commercial management, the diapausing prepupal cells are stored at cold winter temperatures and are artificially incubated early in the following summer to synchronize adult emergence with alfalfa bloom (Richards, 1984).

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). Great numbers of leafcutter bees (50,000 - 75,000 per hectare) 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 without destroying the nesting materials (Richards and Kevan, 2002). This system was developed in order to control the potential build-up of populations of natural insect enemies of the bees. Efficient use of cold storage and incubation facilities leads to synchronisation of bee emergence with the beginning of flower bloom, so that adult bees can be released into the field in a timely manner. 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. Also, the loose-cell system allows better control of many leafcutting bee enemies, reduces the cold storage space required, and reduces the spread of disease specially chalkbrood disease (Bohart, 1972; Baird and 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 growing 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 and 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). If weather is cooler than normal and blossoming is expected to be delayed, the bees can be held mid-incubation during up to 15-19 days at 15-20°C, without adverse effects (Rank and Goerzen, 1982).

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 (Rashed and Ewis,1985). Therefore, the aim of this study is to use the loose system management as an attempt to break the prepupal diapause of this pollinator and control of adult emergence to be synchronized with the expected changes in flowering seasons of alfalfa crop under Egyptian condition.

MATERIALS AND METHODS

Establishment and Nesting of Alfalfa Leafcutting Bee *M. minutissima*

Several attempts to establish *M. minutissima* were conducted during the period from 2006-2010 year, in new alfalfa cultivated areas in Ismailia, Egypt. Nesting shelters with artificial foam nests were prepared as a tool to attract bees and boost their local populations (Fig. 1). The artificial polystyrene foam nests, which contain particular numbers of rolled paper straws in their wooden shelters, were transferred to the natural nests sites in different villages of Tel El Kebir, about 50 km west of Ismailia (30°33' 30"N, 31°56' 13"E) on the Delta of River Nile, during the period from April to the end of July each year. By the end of flowering season of alfalfa, the nesting shelters of successful artificial nests were transferred to the experimental field in July and August for over wintering period (Kamel *et al.*, 2007). The artificial nests were protected from any damage or attack by ants or any other pests. This procedure was repeated yearly during the period between 2006 – 2010 to propagate and establish this insect pollinator in the new alfalfa field at the Experimental Farm, Faculty of Agriculture, Suez Canal University. 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. So that the loose cell management was conducted in the season of 2009-2010 to maximum preserve of the immature stages of *M. minutissima* in store in order to break the prepupal diapause to control the time of adult emergence synchronizing with the expected dates in flowering seasons of alfalfa crop.

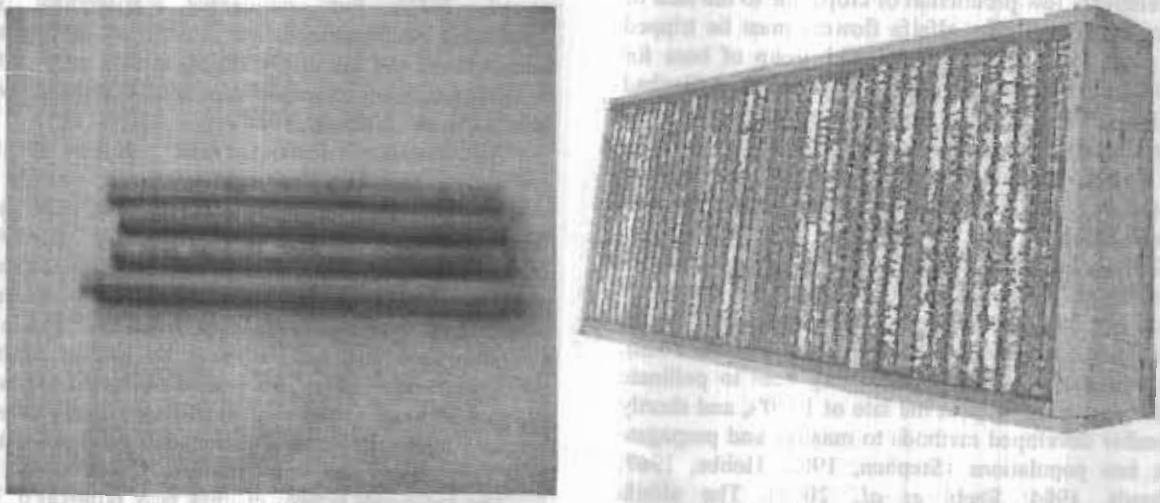

Figure 1. Nesting shelters with artificial foam nests and paper straws as a tool for renesting the insects.

Figure 2. Jars containing loose cells of *M. minutissima* in cold storage

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. 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 punched out, taken apart and stripped from excess leaf material. These straws of rolled paper nest materials are available for the loose-cell system, thus these cells were easily removed because the leaf pieces are held together by the silken cocoons spun by the prepupae. After removal from nest materials, cells were sifted in a screened tumbler to remove excess leaf pieces 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 (Fig. 2).

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 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 hot (Raven) incubator. 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.

Samples in incubation were observed daily to record the time needed to break the diapausing state and the beginning of its transformation into pupae and complete their development to the adults. 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 emerged adults were released later in the alfalfa fields during the blooming period.

RESULTS AND DISCUSSION

Effect of Incubation Temperature on Development of Immature and Mature Stages of *M. minutissima*

Table (1) showed the percentage of immature survival of *Megachile minutissima* prepupae stored at 10±0.4°C for different intervals. The obtained results indicated 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 formation of pupae delayed with the increase of cold storage periods of diapaused prepupae. Also, the first appearance of adults was recorded after 19 days of incubation and represented by 3 females ar 5 males for 152 days of cold storage in 2009 seaso Similar data of adult occurrence were observed for 1(181, 193, 200 and 207 days of cold storage in 20 season and for 170 and 188 days of storage in 2(season. Adult emergence delayed with the increase the cold storage periods over 200 days of cold storag Results also revealed that pupae stage could transfor into adult stage. These results were consistent wi those obtained by Pitts Singer and James (2005). Als Peterson *et al.* (1991) found that the peak of adu emergence occurred after 19-23 days at 30°C.

Data indicated that the most of adult emergence of the parasitic wasp *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 3.57%. Moreover, the obtained data indicated that the shortest time of incubation needed for breaking prepupal diapause and the 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, while the longest time was 26 days for each of pupae, males and females. The percentage of adult transformation was 60% for the 152 days cold storage treatment; then it decreased gradually with the increase of the cold storage period in 2009. The percentage of emerged adults ranged between 40-60% for all cold storage periods in 2010. 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 Rate of *M. minutissima* Adult Emergence

As shown in Table (2), the highest rate of adult emergence of *M. minutissima* stored at 10°C was observed for diapaused prepupae that were stored for 136-188 days. 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 Fig. (3) showed that the highest percentage of newly emerged females of *M. minutissima* was recorded after 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 after 146, 153 and 188 days of cold storage. Also, the obtained results proved that the sex ratio of *M. minutissima* was 1.41 ♀: 1 ♂ at 146 to 188 days storage.

Data indicated that the minimum incubation time at 30°C needed to break diapause and start to transform the prepupae into pupae, adult males and females was 7, 17 and 19 days, respectively, while the maximum incubation time was 26 days for all stages. These results were in agreement with those obtained by Petrowski (1991), who demonstrated that the developmental periods for males and females of diapausing bees were 18.1 days at 22°C and 10 days at 29°C. He found that the time of pre-emergence adults increased with increasing of storage time in cold incubator. Our results are disagreed with those obtained by Richards *et al.* (1987) and Richards and Whitfield (1988) who recorded high survival of prepupae during incubation when cocoons stored at 5°C for 7 months. Also, they recorded

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 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

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

Stored intervals	n	Prepupal mortality		Transformed pupae		Pupal mortality		Time needed for prepupal-pupal transformation (days)		Prepupal - adult survival				Time needed for prepupal-adults' transformation (days)		Parasitism*	
										♀		♂					
		No.	%	No.	%	No.	%	Min.	Max.	No.	%	No.	%	Min.	Max.	No.	%
2007/2008																	
152	50	13	26	37	74	2	5.4	12	183	19	54.3	11	31.4	19	26	5	14.3
164	50	18	36	32	64	1	3.1	7	23	16	51.6	10	32.3	19	26	5	16.1
181	50	26	52	24	48	0	0.0	7	118	13	54.2	8	33.3	17	122	3	12.5
193	50	27	54	23	46	5	21.7	7	108	11	61.1	3	16.7	19	108	4	22.2
200	50	31	62	19	38	3	15.8	10	101	4	25.0	9	56.3	17	108	3	18.8
207	50	27	54	23	46	6	26.1	7	101	12	70.6	4	23.5	17	111	1	5.9
214	50	37	74	13	26	5	38.5	7	115	2	25.0	4	50.0	76	94	2	25.0
221	50	27	54	23	46	11	47.8	66	108	4	33.3	5	41.7	80	118	3	25.0
235	50	38	76	12	24	2	16.7	52	73	6	60.0	4	40.0	66	104	0	0.0
238	50	42	84	8	16	4	50.0	59	91	2	50.0	2	50.0	63	80	0	0.0
241	50	48	96	2	4	1	50.0	52	66	0	0.0	1	100	59	59	0	0.0
286	50	46	92	4	8	0	0.0	52	58	0	0.0	2	50.0	58	70	2	50.0
296	50	46	92	4	8	0	0.0	42	53	1	25.0	2	50.0	53	67	1	25.0
300	50	48	96	2	4	0	0.0	38	44	1	50.0	1	50.0	56	56	0	0.0
2008/2009																	
136	10	5	50	5	50	0	0	16	17	2	40.0	3	60.0	33	33	0	0.0
146	10	3	30	7	70	0	0	18	24	2	28.6	4	57.1	24	42	1	14.3
153	10	3	30	7	70	0	0	17	24	3	42.9	3	42.9	21	28	1	14.3
170	10	5	50	5	50	0	0	11	18	4	80.0	1	20.0	18	27	0	0.0
174	10	7	70	3	30	0	0	14	23	1	33.3	2	66.7	23	33	0	0.0
181	10	5	50	5	50	0	0	16	56	2	40.0	3	60.0	26	70	0	0.0
188	10	6	60	4	40	0	0	9	9	2	50.0	2	50.0	19	19	0	0.0

n = initial number of prepupae per each storage period.

* rate of parasitism was calculated based on number of storage prepupae.

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 & % of females	No & % of males
		Date	Periods per day		
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%)

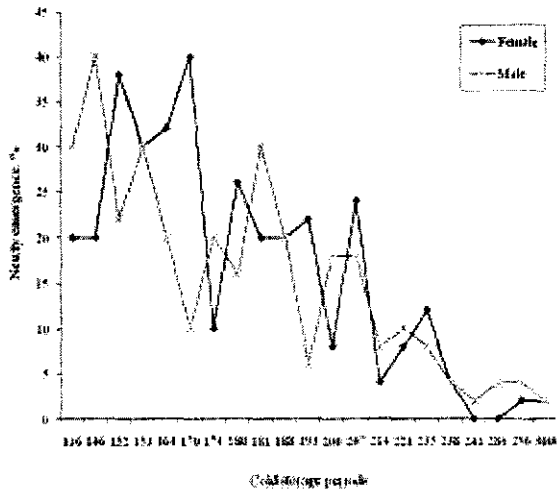

Figure 3. Percentage of newly emergence of *M. minutissima* males and females under incubation conditions at 30±0.4°C after different cold storage periods.

61% emergence of cocoons that incubated at 30 °C with no cold storage and up to 90% emergence or greater after 4 to 5 months of cold storage at temperatures of 20-32°C. Also, the obtained results in the current study are disagreed with those of Bosch and Kemp (2004, a, b), who indicated that the emergence time after incubation decreased with increasing both of wintering duration and wintering temperature.

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).

In our earlier study, cold storage periods at 10±0.4°C significantly affected the time needed for breaking the prepupal diapause and the rate of adult emergence of *M. minutissima* under incubation conditions of 30±0.4°C. These results are in agreement with those obtained by Rank and Goerzen (1982), who indicated that the statistical analysis of three phases of incubation temperatures [Phase I from 1 to 14 days, phase II from days 15 to 19 and phase III from 20-78 days] showed a significant effect on the rate of emergence.

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, which agreed with Peterson *et al.* (1994). They demonstrated that approximately 21 days prior to alfalfa bloom, the bees should be incubated at 30°C until adults emergence. Also, Donovan (1980) found that 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.

Based on the data obtained during five years of study (from 2006 to 2010), it could be conclude 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. These observations were in agreement with Hobbs (1969). Therefore, it was clear that the prepupae should be incubated at 30°C. These findings are in agreement with those reported by Richards and Whitfield (1988), who showed that the prepupae of leaf cutting bee exposed to 35°C and 37°C emerged later than bees incubated at 30°C.

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