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IMPORTANT MORTALITY FACTORS AFFECTING ON THE CORN BORERS, *Sesamia cretica* Led. and *Ostrinia nubilalis* HUB. LARVAE During HIBERNATION PERIOD AT KAFR EL-SHEIKH REGION.

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ABSTRACT

The present investigation was carried out at Kafr El-Sheikh region during 2006/07 and 2007/08 seasons to evaluate the seasonal activity of the main mortality factors causing mortality among the hibernation larvae of *Sesamia cretica* Led. and *Ostrinia nubilalis* Hub.

The ectoparasitoid, *Bracon hebetor* Say was mainly observed associated with corn borers larvae. Percentages of parasitism against larvae of *S. cretica* varied from 2.56 – 6.82 % during 2006/07 and from 4.51–5.24 % during 2007/08, respectively. While percentage of parasitism of *O. nubilalis* larvae varied from 1.31-1.97 % and from 3.44-8.70 % during winter months. In spring (March–May) percentage of parasitism on *S. cretica* increased from 6.25 to 50.0 % and from 11.50 % to 33.30 %. It ranged from 2.50 to 45.36 % and from 6.70 to 42.71 % on *O. nubilalis* in 2006/07 and 2007/08, respectively.

The pathogenic bacteria *Serratia marcescens* Bizo caused disease in larvae. Percentage of diseased larvae of *S. cretica* ranged from 2.56 to 14.28 %, during a period extended from December to April in 2006/07, and between 2.94 to 13.18 % in 2007/08, respectively. On *O. nubilalis* it varied from 0.08 to 4.04 % and from 0.76 to 9.50, in the two seasons, respectively. The predators *Monomorium pharaonis* and *Labidura riparia* encountered in the course of the field work.

Biology of *B. hebetor* on hibernating *O. nubilalis* larvae was studied under constant condition of 20 + 2 ^{o}C and 70 + 5 % RH. Developmental durations were 2.6, 9.2, 2.3 and 9.7 days for egg, larva, prepupa and pupa, respectively. Longevity was 34.2 and 12.0 days, while total life-cycle amounted to 58.0 and 35.8 days for female and male, respectively. Fecundity averaged 210.2 and eggs hatchability was estimated as 93.8 %. Number of hosts destroyed per female was 39.8 through oviposition and 90.4 through paralysation. This finding is important from the biological point of view it may increase the value of the parasitoid. The peak period of egg-laying and destruction of hosts was that of 8 – 9 days of age. Pre-ovipostion and post-oviposition periods were 4.4 and 3.6 days. Sex-ratio was 2 ♂ : 1 ♀.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops in Egypt. The crop is heavily infested by the maize stem borers, *Sesamia cretica* Led., and *Ostrinia nubilalis* Hbn. Ahmed and Kira (1960), El-Sherif (1962), Ismail (1968), Mostafa (1981) and Metwally (2000). The pests overwinter as full grown larvae inside maize stalks until next spring.

Bracon hebetor Say (Hymenoptera: Braconidae) is a polyphagous gregarious larval ectoparasitoid mainly on stored product pests (Clark and Smith, 1967). *B. hebeter* an ectoparasitoid is known primarily as a parasitoid of pyralid moth larvae infesting stored grains, Heimpel *et al.,* 1997. A vast amount of literature has been accumulated on various aspects of this

parasitoid. The present work was undertaken to study winter mortality factors influencing the population size of the two pests during hibernation period. Also, to through some light on the value of *B. hebetor* as a biocontrol agent of *O. nubilalis*.

MATERIALS AND METHODS

1. Field experiments

The present study was carried out at Kafr El-Sheikh region during 2006/07 and 2007/08 maize seasons to evaluate the seasonal activity of the main mortality factors acting on *S. cretica* and *O. nubilalis* hibernating larvae

During November after harvesting of 2006 and 2007 maize stalks, heavily infested with maize borers were collected from the farm of Sakha Agric. Res. Station in groups of 100 stalks each, were packed together by means of a rope and heaped outdoors. A random sample of 100 stalks was taken and dissected at half-monthly intervals from December until mid May and the hibernating larvae and pupae of maize borers were counted. A larval ectoparasitoid was found and sent for identification. Larvae considered dead by the parasitoid were those having parasitoid eggs or larvae on them, or on which the parasitoids had completed feeding and pupating nearly the host's remains, or those paralized through stinging. Predators of corn borers larvae encountered in the course of the field study.

2. Laboratory experiments:

Laboratory culture of *Bracon hebetor* was initiated in January, 2008 from five parasitized *O. nubilalis* larvae collected from maize stalks. On emergence, adult parasitoids were confirmed in pairs in Petri-dishes (9 cm in diameter) each containing 10 host larvae which were daily renewed and hosts with parasitoid eggs laid on them were incubated. This procedure was followed all throughout study period, thus assuring continuous supply of parasitoids, parasitoids culture was maintined at constant conditions of 20 + 2 ^{o}C and 70+5 % RH and experiments were conducted at the same condition. Four batches of host larvae (10 each in a Petri-dish) on which parasitoid eggs were laid the preceeding night were selected, each batch bearing about 80 eggs. The batches were daily examined under binocular microscope to record the stage of development for each parasitoids individual until all were transformed into adults. Mean duration for each stage (egg, larva, prepupa and pupa) was then calculated.

Thirty newly emerged parasitoids (10 females and 20 males) were randomly chosen. Each female accompanied with two males were placed in a Petri-dish containing 10 healthy hosts which were daily renewed until death of the parasitoid.

A sample of this parasitoids (160 cocoons) were collected from the field on January and February 2008 to determine whether the parasitoid has any hyperparasitoid. Fifty individuals of those cocoons were dissected under binocular microscope, while the others were kept individually in vials and daily observed for emergence of hyperparasitoid which appeared and sent for identification.

Hosts on which eggs had been deposited, those destroyed through paralysation, eggs laid by each female and eggs hatchability were daily recorded. Pre-oviposition and post-oviposition periods for each female and longevity of individuals of both were recorded. A lot of 200 individuals were selected randomly from the culture to determine sex ratio.

2.1. The potential of the parasitoid *B. hebetor* as a vector for pathogenic bacteria *S. marcescens*:

Five further diseased larvae were found parasitized by *B. hebetor*. These larvae had a total of 60 parasitic larvae, out of which 32.2 % completed their development. The parasitoids females produced on these diseased hosts were allowed to oviposit on healthy hosts in order to test whether they will transmit the pathogen. Diseased larvae of the borers were recognized by their red color and softened body. A sample of these diseased larvae were sent to the faculty of Agric. at Kafr El-Sheikh for identifying.

RESULTS AND DISCUSSION

1. Field studies:

1. 1. Mortality factors acting on *S. cretica* hibernating larvae:

The maize stalks had been examined in the laboratory and numbers of hibernated larvae and pupae were recorded, classified as follow; a) parasitizied (by *Bracon hebetor*). b) diseased with the pathogenic bacteria, *Serratia marcescens* and c) healthy larvae.

The obtained results Tables (1 & 2) indicated that, the population of hibernated larvae was markedly lower in 2007/08 season than that of the previous season 2006/07. Larval numbers in December and January was relatively higher than throughout the rest of the season, decreased from 29 to 2/larvae/100 stalks and completely disappeared in the second half of May. The resulted pupae began to appear from first week of March, therefore, larval population decreased gradually, on the other hand the pupal number began to increase.

The parasitoid found on *S. cretica* and *O. nubilalis* hibernated larvae was identified as *Bracon hebetor* Say (Hymenoptera: Braconidae) which is a larval gregarious ectoparasitoid.

Percentage of parasitism by *B. hebetor* among hibernated larvae of *S. cretica* given in Tables (1 & 2), during 2006/07 increased from 2.56 in mid December to 50 % in mid May. While, during the next season 2007/08, the percentage ranged from 4.51 % to 42.85 %. Results inferred that, this rate of parasitism appeared to be related to the size of existing larval population.

A solitary, internal hyperparasitoid was found on *B. hebetor* which identified as *Dibrachys cavus* Walker (Hymenoptera: Pteromalidae) percentages of hyperparasitism reached 19.2 %.

As for the pathogen, preliminary diagnosis indicated the presence of a bacterial disease it was identified as *Serratia marcescens* Bizio (*Tribe serrateae*, family Enterobacteriaceae). It is a gram negative rodshaped bacterium characterized by the production of a red pigment and non-sporeforming bacterium.

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S. marcescens played a role as natural mortality factor of hibernated larvae of *S. cretica* under field conditions.

Results in Table (1) indicated clearly that, in 2006/07 percentage of diseased larvae ranged from 2.56 to 14.28 % during a period extended from December until April. In the second season (2007/08) the percentage of diseased larvae ranged from 2.94 to 13.18 during January until the end of April (Table 2).

The parasitoid *B. hebetor* seems to be responsible for disseminating this pathogen as it has been shown here that females transmited it to new host presumably through the ovipositor. Temerak (1982) reared the parasitoid *Bracon brevicornis* Wesm. (Hym., Braconidae) on infected larvae collected from tunnels of the hibernating pink borer, *S. cretica*) in stalked sorghum stalks revealed that through contamination of ovipositor, the non sporeforming bacterium *S. marcescens* has more pathogenic effect on the parasitoid and its progeny than the sporeforming bacterium *Bacillus thuringiensis* (Berliner).

1. 2. Mortality factors acting on *O. nubilalis* hibernating larvae:

The half-monthly samples of 100 dry maize stalks had been taken from the field during the two seasons (2006/07 and 2007/08). As shown in Tables 1 & 2, the recorded larvae of *O. nubilalis* which decreased gradually during hibernating period as time progressed whereas, the pupae began to appear from the beginning of March and gradually increased.

The parasitoid *B. hebetor* was the only parasitoid recorded attacking the hibernating larvae. This parasitoid did not attack the larvae of this borer in growing seasons (Metwally, 2000).

As seen from Tables (1 & 2), the parasitoid began its activity from December to May. Percentage of parasitism increased gradually from 1.31 to 45.36 % and from 3.44 to 42.71 % in the two seasons, respectively. Also, Abd El-Rahman *et al.* (1983), recorded *B. hebetor* as an ectoparasiotid of *O. nubilalis*.

In respect to larval mortality due to pathogenic bacteria *S. marcescens*, percentage of diseased larvae ranged from 0.08 to 4.04 % in 2006/07 and from 0.76 to 9.50 % in 2007/08, respectively.

The first symptoms of *S. marcescens* appeared as round red colored spots on the host's body, which grew larger as time progressed and finally covered the whole body of the host. The parasitoid oviposited on infected hosts but the majority of these hosts (73.4 %) died before the parasitoid larvae completed feeding. Parasitoid larvae on other infected hosts (26.6 %) completed feeding and spun the cocoons. Of the latter, 29.3 % failed to pupate and 10.9 % failed to emerge as adults. Steinhaus (1959) reported that *O. nubilalis* larvae were susceptible to *S. marcescens* by injection. Transmission of bacterial pathogens by the ovipositor of hymenopterous parasitoids has been reported previously.

The same pathogen considered here, *i.e.*, *S. marcescens* was transmitted to pupae of *Galleria mellonella* L. by *Itoplectis conquisitor* (Say) (Bucher, 1963).

In this respect, hibernated larvae of corn borers were subjected to the factors of environment (biotic and a biotic) which are responsible for reducing

their numbers. Therefore, the numbers of these insects during overwintering were continuously decreased as time progressed and only a small portion survived. Similar results were obtained by Tawfik and Awadallah (1970) and Tawfik and El-Sherif (1974) they stated that, by the end of May cold weather of winter and natural enemies caused a great reduction in its numbers. On the other hand the present results revealed that *S. marcescens* killed a portion of those hibernated borers and a portion of its parasitoid *B. hebetor* which acted as a mechanical carrier of the pathogen. Thus *S. marcescens* is also a natural enemy for the parasitoid *B. hebetor* . Hereby, the latter have two natural enemies; a hyperparasitoid, *D. cavus* and a pathogen *S. marcescens*.

Two predators encountered in the course of the field work were; the ant, *Monomorium pharaonis* L. (Hymenoptera: Formicidae) and the earwig, *Labidura riparia* Pall. (Dermaptera: Labiduridae).

2. Biological studies on *B. hebetor* on hibernating larvae of *O. nubilalis*:

The adult female *B. hebetor* stings host larvae by the ovipositor and thereby renders them permanently paralyzed., this paralysis facilitates feeding and oviposition by the wasp. It then feeds on the fluids exuding from the punctures made by the ovipositor, prior to and between egg-laying. Round black spots appeared on the cuticle of host larvae as a result of parasitoid stinging.

Upon hatching, the parasitic larvae pierces host's body with its mouthparts and feeds continuously except for three intervals during which it moults. Thus, there are four larval instars. On completion of feeding, the larvae leaves the host's remaining and move away a distance of 0.5 – 2.9 cm to spin a silk white cocoon. It then goes through a period of rest. Thus, larval stage can be divided into three periods owing to different behaviours: feeding on the host, spinning the cocoon and at rest.

Following the rest period, the larva transforms into prepupa and then pupa. Development of larva at rest, prepupa and pupa inside the cocoon can be clearly observed through glass surface of the Petri-dish.

Table (3) shows duration periods of all stages of *B. hebetor* as well as total life cycle for both sexes. Developmental durations were 2.6, 9.2, 2.3 and 9.7 days for egg, larva, prepupa and pupa, respectively. Larval duration was divided into three periods 3.9 days feeding on the host, 1.6 days spinning the cocoon and 3.9 days at rest. Longevity was 34.2 and 12.0 days, while total life-cycle amounted to 58 and 35.8 days for female and male, respectively.

Values for durations of developmental stages of *B. hebetor* obtained in this study at 20 ^{o}C are comparable and nearly similar to others reported in a previous work (Patel and Gangrade, 1965) in India but with the use of different host, *i.e.*, full grown larva of the caster capsule borer *Dichocrcis punctiferalis*.

Table (4) presents data on different biological characters of *B. hebetor* . Number of hosts destroyed per female was 39.8 through oviposition and 90.4 through paralyzation, thus making a total destruction of 130.2 hosts per female. Hosts destroyed through paralyzation were about more twice as much as those destroyed through oviposition. Fecundity averaged 210.2.

Eggs hatchability was estimated as 93.8 % in a radom sample of 262 eggs. Eggs failing to hatch (6.2 %) shrinked soon after oviposition without showing any signs of development. Pre-oviposition and post-oviposition periods were 4.4 and 3.6 days, respectively. Sex-ratio was 2 ♂ : 1 ♀. These finding are in agreement with pervious studies conducted by Soliman (1940) and El-Sufty *et al.* (1983).

Table (3): Duration of development, longevity and total life cycle for both sexes of *Bracon hebetor* Say reared on larvae of *Ostrinia nubilalis*, at a temperature of 20 + 2 °C and 70 + 5 % RH.

	Duration in days + S.D	Range
Eggs	2.6+0.38	1.9 – 3.3
Larvae:		
Feeding on the host	3.7+0.60	3.0 – 5.0
Spinning the cocoon	1.6+0.38	1.0 – 2.0
At rest	3.9+0.92	2.6 – 6.0
Total	9.2	
Prepupae	2.3+0.51	1.7 – 4.2
Pupa	9.7+2.10	6.1 – 15.4
Adult:		
Female	34.2+9.25	22.0 – 52.0
Male	12.0+4.39	6.0 – 22.0
Total life cycle		
Female	58.0	
male	35.8	

Table (4): Some biological determinations for *B. hebetor* raised on larvae of *O. nubilalis*, at a temperature of 20 +2 °C and 70 + 5 % RH.

Determination	Mean + S.D	Range
Number of hosts destroyed/♀:		
Through oviposition	39.8+14.87	20 – 60
Through paralyzation	90.4+36.19	49 – 149
Total	130.2+51.96	68 – 191
Number of eggs laid/♀	210.2+57.43	142 – 310
Eggs hatchability (%)	93.8	
Pre-oviposition period (days)	4.4+1.17	3 – 7
Post-ovipostion period (days)	3.6+1.08	2 – 3
Sex ratio (♂:♀)	2:1	

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أهم عوامل الموت التى تؤثر على يرقات دودة القصب الكبيرة ودودة الذرة الأوربية أثناء فترة البيات الشتوى بمنطقة كفر الشيخ

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تعتبر ثاقبات الذرة، دودة القصب الكبيرة ودودة الذرة الأوربية من الآفات الرئيسية على محصول الذرة الشامية حيث تقضى الآفات بياتاً شتوياً فى طور اليرقة الكاملة النمو فى أحطاب الذرة الجافة بعد الحصاد وقد استهدف البحث دراسة بعض عوامل الموت المؤثرة على هاتين الآفتين أثناء فترة البيات الشتوى حيث أجريت الدراسة بمحطة البحوث الزراعية بسخا – كفر الشيخ خلال موسمى 2007/2006 و 2008/2007 حيث أختير حقل الذرة بعد جمع المحصول وتم تخزين الأحطاب تحت الظروف الحقلية – وأخذت العينات بمعدل 100 عود/15 يوم ثم شرحت النباتات وتم تسجيل أعداد اليرقات والعذارى لهذه الآفات وتم حصر وتعريف الأعداء الحيوية التى وجدت وسجلت نسب التطفل على اليرقات البائتة بطفيل *Bracon hebetor* وكذلك اليرقات المرتبطة بالمسبب البكتيرى *Serratia marcescens*

وتم التوصل إلى النتائج الآتية:

1- تناقص تعداد اليرقات الحية فى دودة القصب الكبيرة تدريجياً خلال فترة البيات فى موسمى 2007/2006 ، 2008/2007 على اليرقات على الترتيب فى منطقة كفر الشيخ حيث بدأ البيات لهذه اليرقات فى أواخر سبتمبر وأستمر طيلة أشهر الشتاء وتراوحت نسبة التطفل بالطفيل *B. hebetor* على دودة القصب الكبيرة 2.56 إلى 6.82 فى موسم 2007/2006 ومن 4.51 إلى 5.24 % فى موسم 2008/2007 أما على دودة الذرة الأوربية فكانت 1.31 : 1.97 فى موسم 2007/2006 ومن 3.44 إلى 8.70 % فى موسم 2008/2007 على التوالى – أما فى اشهر الربيع مارس إلى مايو فقد ازدادت نسب التطفل على دودة القصب الكبيرة من 6.25 إلى 50.1 % ومن 11.50 إلى 33.33 % خلال الموسمين وتراوحت ما بين 2.5 إلى 45.36 % ومن 16.70 إلى 42.71 % على دودة الذرة الأوربية خلال موسمى الدراسة على الترتيب.

2- تسبب الممرض البكتيرى *Serratia marcescens* فى إصابة يرقات كلا الآفتين وبلغت نسب الإصابة ليرقات دودة القصب الكبيرة من 2.56 إلى 14.28 % خلال الفترة من ديسمبر إلى أبريل ومن 2.94 إلى 13.18 % خلال الفترة نفسها فى موسمى 2007/2006 ، 2008/2007على الترتيب. أما بالنسبة لدودة الذرة الأوربية فبلغت نسب الإصابة من 0.08 إلى 4.04 % ومن 0.76 إلى 9.5 % فى الموسمين السابقين على الترتيب .

3- وقد وجد أن الطفيل *B. hebetor* هو الناقل الميكانيكى للمسبب المرضى *S. marcescens* عن طريق آلة وضع البيض كما سجل طفيل *Dibrachys cavus* على الطفيل السابق حيث كانت نسبة التطفل الثانوى 19.2 % وأن جزءاً كبيراً من اليرقات البائتة للآفتين وكذلك طفيل *Bracon hebetor* يموت بسبب هذا الممرض البكتيرى.

4- درست بيولوجية الطفيل *B. hebetor* على يرقات دودة الذرة الأوربية البائتة تحت درجة حرارة 20 °م ورطوبة 70 % واستغرق طور كل من البيضة واليرقة وما قبل العذراء والعذراء 2.6 ، 9.2 ، 2.3 و 9.7 يوماً على الترتيب واستغرقت الأنثى البالغة والذكر 34.2 ، 12.0 يوماً على الترتيب. وبلغ تعداد اليرقات التى ماتت بسبب وضع بيض الطفيل 39.8 والتى أصيبت بالشلل التام أكثر من ضعف هذا العدد 90.4 يرقة/♀ - وبلغ عدد البيض الذى وضعته الأنثى الواحدة خلال فترة حياتها التى استمرت 34.22 يوماً ، 210.0 بيض/♀ وبلغت نسبة الفقس 93.8 ، وكانت النسبة الجنسية 2 : 1 لكل من الذكر والأنثى على الترتيب.

5- سجل مفترسان هما حشرة النمل *Monomorium pharaonis* ، وإبرة العجوز *Labidura reiparia* على يرقات كل من دودة الذرة الأوربية ودودة القصب الكبيرة البائتة فى أحطاب الذرة.

قام بتحكيم البحث

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Table (1): Percentage of healthy, parasitized and diseased larvae of *S. cretica* and *O. nubilalis* during 2006/07 season at Kafr El-Sheikh region.

Sampling date	*S. cretica*							*O. nubilalis*							Mean	
	Larvae						Pupae	Larvae						Pupae		
	Total	H.*	P.	D.	% PL	% DL		Total	H.	P.	D.	% PL	% DL		Temp °C	RH %
Dec. 1/06	0	0	0	0	0	0	0	613	600	8	5	1.31	0.08	0	19.4	57.7
15	39	37	1	1	2.56	2.56	0	675	662	9	4	1.33	0.06	0	13.4	66.8
Jan. 1/07	44	40	2	2	4.5	44.54	0	712	701	5	6	0.70	0.80	0	14.5	66.0
15	49	46	2	1	4.08	2.04	0	699	690	5	4	0.70	0.57	0	14.1	65.3
Feb. 1/07	32	30	1	1	3.13	3.13	0	650	642	3	5	0.46	0.76	0	14.0	62.9
15	29	25	2	2	6.89	6.89	0	639	621	12	6	1.97	0.93	0	13.8	62.2
Mar. 1/07	16	15	1	0	6.25	0	3	624	602	15	7	2.50	1.12	4	15.9	57.0
15	14	12	1	1	7.14	7.14	5	578	554	22	2	3.80	0.34	8	16.7	65.0
Apr. 1/07	9	6	2	1	22.2	11.11	6	456	440	10	6	2.10	1.30	42	19.2	60.3
15	7	4	2	1	28.57	14.28	8	431	401	22	8	5.11	1.90	81	19.5	59.9
May 1/07	5	3	2	0	40.0	0	2	298	202	84	12	28.2	4.04	66	19.9	64.0
15	2	1	1	0	50.0	0	0	132	72	60	0	45.36	0	25	22.3	59.8

H= Healthy larvae
P= Parastized larvae
D= Diseased larvae
Sample 100 stalks

Table (2): Percentage of healthy, parasitized and diseased larvae of *S. cretica* and *O. nubilalis* during 2007/08 season at Kafr El-Sheikh region.

Sampling date	*S. cretica*							*O. nubilalis*							Mean	
	Larvae						Pupae	Larvae						Pupae		
	Total	H.	P.	D.	% Pl	% DL		Total	H.	P.	D.	% Pl	% DL		Temp °C	RH %
Dec. 1/07	20	20	0	0	0	0	0	523	501	18	4	3.44	0.76	0	19.7	59.5
15	22	21	1	0	4.51	0	0	519	500	14	5	2.78	0.96	0	17.3	60.0
Jan. 1/08	34	30	3	1	8.88	2.94	0	451	422	22	7	4.90	1.55	0	14.2	62.3
15	28	22	4	2	14.30	7.15	0	429	400	24	5	5.60	1.17	0	14.0	62.1
Feb. 1/08	23	28	3	2	13.01	6.9	0	406	380	22	4	5.41	0.98	0	12.4	59.9
15	19	16	3	1	15.7	5.24	0	345	310	30	5	8.70	1.13	0	12.5	59.8
Mar. 1/08	17	14	2	1	11.12	5.70	4	313	290	19	4	6.70	1.24	6	12.5	64.2
15	15	12	2	1	13.33	6.66	6	275	248	22	5	8.81	1.70	24	13.2	60.1
Apr. 1/08	15	10	4	1	26.66	6.66	8	193	102	82	9	42.7	4.70	49	15.5	56.5
15	7	3	3	1	42.85	13.18	9	140	71	59	10	42.1	7.10	56	15.9	55.6
May 1/08	6	3	2	1	33.33	6.66	3	21	11	8	2	38.1	9.50	43	19.2	57.5
15	0	0	0	0	0	0	0	13	7	5	1	42.7	7.80	19	22.9	51.8