Research Article

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Monitoring protocol using binomial counts of *Panonychus ulmi* wintering eggs (Acari: *Tetranychidae*)

Abstract

Binomial sampling plan based on the empirical model is developed to monitor wintering eggs of the European red mite *Panonychus ulmi*. Taylor power law used to establish the relationship mean-variance showed a higher aggregative distribution of wintering eggs. Mean number of eggs decreases during the winter period and the expected loss is estimated at 13eggs per obstacle. In years of biological control and before occurring of diapause, the predatory mite Typhlodromus (Typhlodromus) setubali reduces from 20 to 30% of total number of wintering eggs in late autumn. A post-flowering treatment with Oviphyt oil is applied to prevent the first attacks in early spring. Monitoring protocol using binomial count of wintering eggs is an efficient procedure to manage pest mite population in early growth season in accordance with the principles of integrated pest management.

Keywords: biological control, monitoring protocol, binomial count, wintering egg, *tetranychidae*, apple

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Said Ouassat, Latifa Allam, Abdelilah Ouahbi

Department of Biology, Faculty of sciences, University of Moulay Ismail, Morocco

Correspondence: Said Ouassat, Crop protection team, Health and Environment laboratory, Department of Biology, Faculty of sciences, University of Moulay Ismail, 11201, Zitoune, Meknes, Morocco, Tel +212 665 167 918, Email s.ouassat@edu.umi.ac.ma

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Introduction

Monitoring involves the assessment of the health of a crop, the presence of pests and gauging their population levels at regular intervals.[1] This is a critical component of integrated pest management as the identification of pest (and beneficial insects), and their relative densities is used to inform control decisions. Mites are among the most diverse and successful of all invertebrate groups. They are small in size and often go unnoticed, however mites are one of the most important pest groups attacking Moroccan apple orchards.[2] Some species have become more problematic over the last decade as farming practices have changed, and others are proving difficult to control due to tolerance and chemical resistance issues.

To assess the risks of abandoning insecticide treatments, entomologists use a method of estimating the density at single point in time. In this study, we give an efficient monitoring procedure to control wintering eggs of the European red mite *Panonychus ulmi* (Acari: *Tetranychidae)*. Monitoring procedure using empirical count of wintering eggs has been described in the studies.[3,4] The empirical model facilitates counting of wintering eggs based on economic thresholds without considering the theoretical distribution (normal, poisson law, negative binomial) of the eggs, this technique is adapted to evolution of crop management and leads an effective control of the population through the control of wintering eggs.

Material and methods

Study plot

The study area is located in the North Middle Atlas at an altitude of 1250m, Morocco (33°26'19.6 "N, 5°58'35.7"O) (Figure 1). The orchard contains a total of 53 plots cultivated of apple varieties of Golden Delicious, Granny smith, Red Shift, Jeromine, Skarlet and Gala Species of the family *Tetranychidae* mostly observed in study plot are *P. ulmi* (Koch); *T. urticae* (Koch) and often in late autumn *T.cinnabarinus* (Boisduval). Inundative release of the predatory mite T.(T.) setubali in sample plot showed an efficient control of pest mites and it be found compatible with pesticides used during season growth.

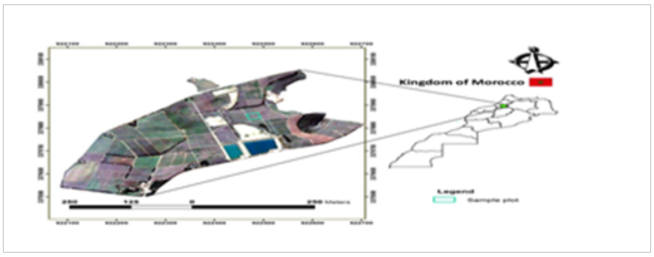

Figure 1 Geographic location of the study area.

Sampling data

Sampling procedure consists in collecting 50wooden portions of 20cm during December and January 2017/2018 taken on 10trees of 5rows, each portion of two-year old wood carry two obstacles. A set of 100 obstacles, density (eggs/obstacle) and data are recorded each sampling occasion. The choice of points and the sampling units must be carefully designed so that data does not influenced by the impact border on different developmental stage of *P.ulmi.*[5] Counting of wintering eggs is realised by using binocular loupe×15. Method proposed by Biological control organization is also applied, this technique consists in counting eggs according to the same class scale described above. Linear regression is tested by fitting the number mean of eggs per obstacle on the number total of eggs counted on 2m of wood, the total number of eggs correspond to number observed on sampling unit of 2m of length.

Distribution of wintering eggs

In order to characterize the distribution of eggs on wood, Taylor's law is used.[6] This law relates the variance (s2) to the mean density of eggs (m) according to the following relation: $s^2=[am]^b$, a and b are intercept and regression slope, respectively. Both parameters were expressed under a logarithmic scale $\log(s^2)=\log(a)+b\log(m)$.[7] Slope provides information about the distribution: when b=1, the species is distributed randomly, when b>1, the distribution is aggregative and regular when b<1.To establish such a relationship and calculate the b value, the mean density and variance of each row were calculated (and transformed into log) and a Student test was applied using software R.

Parameterization of monitoring protocol

An economic threshold of 10 eggs/obstacle is fitted first, for making decision to intervene before or after flowering or not intervening. Below this threshold, *P.ulmi* population remains within the acceptable range and doesn't require further interventions, except in late summer of growth season. The risk related to pest mite eggs is estimated conventionally according to a scaled density classes: 0 eggs 1-5 eggs; 6-20 eggs; 21-50 eggs; 51-100 eggs; 101-200 eggs; >200 eggs.[8] According to action thresholds proposed as standards of decision,[4] for the plots with a small population (less than 40% of sampling units carrying 0 to 20 eggs), winter treatment is not essential. When plot showing an average population (between 40 and 60% with an average number of eggs between 20 and 30 eggs per obstacle), the post-flowering treatment with oils must be scheduled. Finally, a level exceeds 60% request an intervention very early before flowering. The density intervals and the decisions relating to each population level are given in Table 1. The performance of empirical model in presence of natural enemies was studied and binomial count using class system was validated in field during winter period of 2018, the changes take into account the effect of some natural variations, which constitutes limiting factors such varietal system, climatic conditions and fungicidal effects.

Table 1 The values used in the prediction of pest mites attacks

Eggs/ obstacle	Population level	Control
0-20	carrier obstacles more than ten eggs < 40%	Not necessary to treat
20-30	40-60 %	Treatment after flower
> 30	> 60%	Treatment before flower

Results

Distribution of wintering eggs

A significant correlation is observed between log (eggs/obstacle) and log(s2) (r2=0.81) (Figure 2) and the slope value (1.58±0.12) shows an aggregative distribution of wintering eggs (P< 0.05). In the general case, the aggregative distribution of eggs offers the possibility to develop a sequential analysis according to the negative binomial law. A common K (Kc) for the negative binomial distribution must be estimated by using the relationship K_c=d^2/((s^2-d)) , with (d) and s2 are mean density and variance respectively.[9] The empirical model based on class system and used to calculate the average number of eggs per obstacle consists to multiply the number of obstacles by the multiplication factor. This factor is commonly assigned to the median density of each class.[4] The findings obtained for a set of 20 obstacle corresponding to 10 sample units during December 2017 ranged from 27 to 46 eggs/ obstacle (Table 2). This result is practically accepted comparing to values observed in other conventional plots.

Monitoring protocol is conducted on same sampled rows to evaluate the expected loss occurring during January 2018. For each sampled row, a proportional correspondence is observed between the mean number of eggs per obstacle and the number loss recorded in December and January respectively. Mean number of eggs per obstacle is higher in row1 (27 eggs/obstacle), while the lowest is observed in row5 (14 eggs/obstacle) (Table 3).

Count of wintering eggs of *Panonychus ulmi* was conducted during 5 seasons (from 2012 to 2018) and the results are given on (Table 4). Wintering eggs are counted at 72h after mineral oil spraying in the experimental plot and simultaneously in temoin plot, in where no chemical or biological treatment did not scheduled during survey periods. Typhlodromus (T.) setubali showed its regulatory capacity of the *P.ulmi* population in the experimental plot.[10] However, release of this species significantly decreases the number of adult females in late autumn. Biological control by (T.) (T.) setubali effectively contributes to reducing from 20 to 30% of the total number of eggs which, are destroyed before the predator females leave in diapause. Result obtained by using the International Organization of Biological Control method shows a conformity with those of count binomial. Total number of eggs on 2m of wood is counted on the same trees at 15th January 2018. A very significant linear relationship between mean numbers of eggs per obstacle and the total number of eggs on 2m of wood is observed (Eggs/obstacle = 0.05*(Eggs/2m of wood)-0.16;r2=0.99;P<0.05) (Figure 3).

Table 2 Findings of monitoring conducted during December

Class		0	1	2	3	4	5	6	Total	Average number of eggs
Number of Eggs		0 eggs	1-5 eggs	6-20 eggs	21-50 eggs	51-100 eggs	101-200 eggs	> 200 eggs		
Multiplication factor		0	2	10	30	70	150	300		
Row 1	nb.obstacles	0	4	6	4	2	4	0	20	
	nb. Eggs	0	8	60	120	140	600	0	928	46
Row 2	nb.obstacles	1	2	6	6	4	1	0	20	
	nb. Eggs	0	4	60	180	280	150	0	674	34
Row 3	nb.obstacles	2	2	4	7	5	0	0	20	
	nb. Eggs	0	4	40	210	350	0	0	604	30
Row 4	nb.obstacles	1	5	6	4	3	3	0	22	
	nb. Eggs	0	10	60	120	210	450	0	850	39
Row 5	nb.obstacles	0	6	5	6	2	1	0	20	
	nb. Eggs	0	12	50	180	140	150	0	532	27
							Mean (Eggs / obstacle)			35
							Standard deviation			8

Table 3 Findings of monitoring conducted during January

Class		0	1	2	3	4	5	6	Total	Average number of eggs
Number of Eggs		0 eggs	1-5 eggs	6-20 eggs	21-50 eggs	51-100 eggs	101-200 eggs	> 200 eggs		
Multiplication factor		0	2	10	30	70	150	300		
Row 1	nb.obstacles	0	3	7	6	4	0	0	20	
	nb. Eggs	0	6	70	180	280	0	0	536	27
Row 2	nb.obstacles	3	2	7	5	3	0	0	20	
	nb. Eggs	0	4	70	150	210	0	0	434	22
Row 3	nb.obstacles	2	5	5	5	2	1	0	20	
	nb. Eggs	0	10	50	150	140	150	0	500	25
Row 4	nb.obstacles	3	4	4	5	4	0	0	20	
	nb. Eggs	0	8	40	150	280	0	0	478	24
Row 5	nb.obstacles	4	5	5	5	1	0	0	20	
	nb. Eggs	0	10	50	150	70	0	0	280	14
							Mean (Eggs/ obstacles)			22,28
							Standard deviation			5

Table 4 Number of *P. ulmi* wintering eggs per 100 obstacles during 5 seasons in experimental and temoin plots. Sampling was carried out 72h after intervention

Number total of wintering eggs / 100 obstacles		
spray date	**oils**	**No oils**
24 January 2012/2013	194	1842
04 February 2013/2014	235	2275
12 February 2014/2015	188	1930
07 February 2015/2016	*76	2094
27 January 2016/2017	*109	2458
04 February 2017/2018	*112	1998

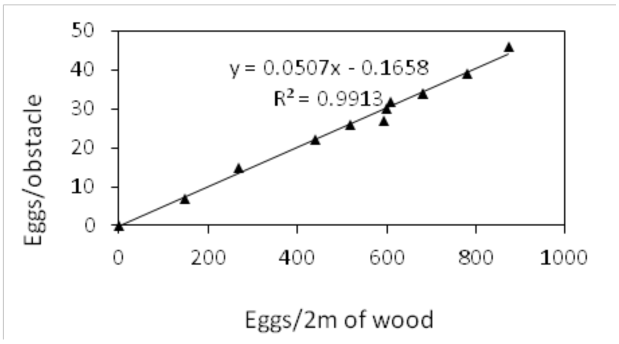

Figure 2 Mean density-variance relationship using the Taylor power law for *P. ulmi* winter eggs.

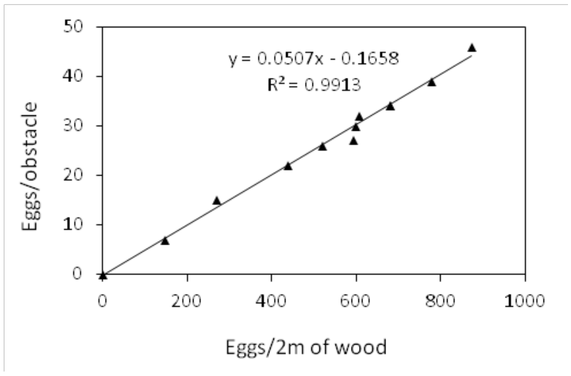

Figure 3 Relationship between average number of eggs per obstacle and total number of eggs per 2m of wood.

Discussion

The populations encountered in this study deposited considerable numbers of wintering eggs on the wood. Previous studies of *P.ulmi* populations have not considered the factors simulating oviposition on the fruits.[11] Wintering eggs of most species *Tetranychidae* are laid in autumn on two-year-old wood crevices with higher numbers generally around of obstacles.[12] Before Temperature and sufficient late trigger the hatching of wintering eggs, an expected number of losses due to abortion of eggs under the natural effect of ultraviolet rays.[13] At a temperature of 20°C, about 50% of wintering eggs hatch.[14] Low temperatures in the coldest nights of winter can differ from one area to another, this has an important bearing on winter survival of the pest mites. Low temperature of -37°C is required to kill diapausing eggs of *P. ulmi*.[15,16] however, the upper temperature tolerance of these mites is unknown. Overwintering sites of crop mites range from exposed situations which remain at air temperature to well protected ones on the ground where temperatures rarely go below -5°C.[17] The results showed that an average lost number was 13 eggs per obstacle. However, the effect of temperatures below 0°C on winter eggs of the pest mite was discussed regarding super cooling in freezing temperatures,[18] Nevertheless, early season mite feeding is more detrimental than late season feeding,[16] because until fruit set, the trees are under tremendous physiological stress to produce the necessary nutrients to develop foliage, flowers and young fruit. The total number of eggs deposited in early winter is a practical challenge in integrated pest management, although this number is falling during the winter months, the whitish eggs detected on the obstacles and on the wood surface correspond to the aborted forms under the effect of cold extremes.

Drop observed in average number of eggs can be explained by cold and lower temperatures.[19] To this natural factor, is added the effect due to predators several species (*Coccinellidae*, *Phytoseiidae*, *Miridae*…) feed on the spider mite eggs.[20–22] Females of *Phytoseiidae* destroy a proportion of winter eggs before starting of diapause and contribute to the regulation of the *P. ulmi* population.[23] In our study, Typhlodromus (Typhlodromus) setubali consumes between 10 to 20% of winter eggs, result of four seasons of biological control against the red mites by this phytoseiid in the apple orchard. The results obtained by using empirical method and the method proposed by the International Organization for Biological Control are similar. Although the procedures are different, the result is based on the number of obstacles that can contain 2 meters of wood. The relationship between the mean number of eggs per obstacle and total count of eggs per 2m of wood, is properly linear with a significant correlation between the two calculations (r2= 0.99) (Figure 3). For example, the averages of 35 and 22.28 eggs per obstacle obtained using counting method correspond respectively to 700 and 400 eggs per 2m/wood in the IOBC method.

The ovicidal effect of spraying dose of 2(l.hl-1) on codling moth eggs in apple trees has been established.[24] By its action on eggs and larvae, the white oil provides a high level of efficiency and responds to the resistance problems induced in most of the pests. Oil applications reduce between 80 to 95% of the total eggs as showed in Table 4. In some studies, mortality rates from the same or diluted doses may reach up to 97% mortality of codling moth eggs with lubricating oil [2%(vol:vol)] and 75% with a 1%(vol:vol).[25] Perhaps the best use of mineral oil in apple pest control programs is for management of secondary pests. Oil is moderately effective with relatively few applications against spider mites, leafhoppers, and aphids, primarily because a lower level of suppression is economically acceptable. This use pattern minimises risk of phytotoxicity and provides a low-cost alternative to conventional insecticides that is not disruptive of biological control. Overall, horticultural oil is a valuable, selective component of an IPM program in apple orchards.[26]

Conclusion

Binomial sampling plan established to control the wintering eggs of *P.ulmi* or other species of the family *Tetranychidae*, is a true tool facilitating the control of infestation level. Monitoring protocol based on counting a sample size of 100 obstacles makes it possible to accurately assess the level of risk. This method seems an effective alternative to the method proposed by the international organization of biological control. Practical applications in terms of sampling evolve with improved methods, which is one of the objectives of integrated control. The reliability of the method presented allows managers to better optimize the management of phytophagous mites as part of a management consistent with the principles of reasoned management.

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Conflict of interest

The author declare that no conflict of interest.

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