SSRG International Journal of Agriculture and Environmental Science
ISSN: 2394 – 2568 /doi:10.14445/23942568/IJAES-V7I5P116

Volume 7 Issue 5,108-113, Sep-Oct 2020

Data analysis and modeling of *Pasteuria penetrans* spore attachment

Ioannis Vagelas

Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Fytokou St., N. Ionia, GR-38446 Magnesia, Greece

Abstract *- This paper discusses a process of developing data analysis and modeling of Pasteuria penetrans spore attachment in vitro (water and soil bioassay), based on the observation that the number of spores attaching to juveniles within a given time increased with increasing the time of exposure to spores. Based on that, P. penetrans spore attachment in vitro was modeled using the negative binomial distribution, considering that P. penetrans spores are clumped. But the most important step in this research is not in running the Negative binomial distribution model, but further predicted the P. penetrans spore attachment and J2s invasion to plant roots, with a Markov process when J2s are encumbered with clumps of P. penetrans spores (e.g., 4-7 or ≥8 spores). Predicted data show that a) the rate of parasitism by P. penetrans differs significantly among time of exposure to P. penetrans spores and b) successful parasitism (depends on the attachment of 4-7 spores per juvenile), which is sufficient to initiate infection without reducing the ability of the nematode to invade roots and probably P. penetrans spores multiplies in the body of the female of the plant-parasitic nematodes.*

Keywords - *Modelling, Markov chain, Biocontrol, Soilborne pathogens, Nematodes, Meloidogyne spp.*

I. INTRODUCTION

Pasteuria penetrans [36], is a mycelial, endospore forming bacterial parasite of plant parasitic nematodes [21], [17], showing promising results in a biocontrol strategy of root-knot nematodes (*Meloidogyne* spp.), [33], [29], [15].

The *P. penetrans* spores (endospores) attach to the outside nematode body wall (cuticle) of the infective stage, the second-stage juveniles (J2) of *Meloidogyne* populations [22]. After root-knot juvenile penetrates a plant root and begins to feed, *P. penetrans* spores penetrate the nematode body and begin to grow and develop in the developing nematode [23], [17], [30]. Eventually, the infected female nematode body becomes completely filled with *P. penetrans* spores [30], [33]. Based on that, each infected female may contain up to 2.5 million *P. penetrans* spores [9], which are released into the soil.

The potential of *P. penetrans* to control of root-knot has been widely studied [32], [10], [5], [13], including host range, distribution, specificity, biotic and abiotic factors [14], [12], [41], [26].

As literature mention, successful parasitism depends on the attachment of 5-10 spores per root-knot juvenile (J2), which is enough to initiate infection without reducing the ability of the juvenile to invade roots [11], [27]. Hence, there may be little or no root invasion if there are >15 *P. penetrans* spores attached/J2, presuming that spore attachment will affect the ability of a J2 to locate or to invade a root [11], [37], [38]. Based on that, these results imply high variances in the numbers of *P. penetrans* spores attaching, but no attempt has been made until now to examine this variability in detail, to model or to predict it.

Overall, in this paper, methods are provided to analyze and to model counts of *P. penetrans* spore attachment to root-knot juveniles (*Meloidogyne* spp.) Based on these methods, we presented in this study, and we examine in detail the variability of *P. penetrans* spore attachment and offer an explanation for it. Moreover, we used a Markov chain model [6], [39], to describe and predict the attachment ability of *P. penetrans* spores to root-knot juveniles (*Meloidogyne* spp.), on different time of exposure a) in water and in soil bioassay. Further, we provide evidence of good estimator models to describe nematode root invasion when J2s were encumbered with different numbers of *P. penetrans* spores.

II. MATERIAL AND METHODS

A. Root-knot nematode culture

A culture of *Meloidogyne javanica* [37] was maintained on tomato plants (cv Tiny Tim) in the glasshouse. Eggs were collected by dissolving the gelatinous matrix into a solution of 0.5% sodium hypochlorite (NaOCl) (10% commercial bleach), passing the solution through a 200-mesh (75 mm) sieve, nested over a 500-mesh (26 mm) sieve, and rinsing the eggs under slow running tap water to remove residual NaOCl [16]. Second-stage juveniles (J2) were then hatched using standard laboratory practices [31], [34], [42].

B. Attachment process

Spore suspensions of *P. penetrans* (Nematech Co. Ltd Japan) were prepared a) in tap water [37] and b) in tap water mixed with a small amount of loam soil. All attachment tests on freshly hatched J2 were conducted in 2.5-cm Petri dishes using standard techniques observing individuals at high power (x200) under an inverted microscope [12].

For the first bioassay (only tap water treatment), data were recorded 1, 3, 6, and 9 h after placing nematodes in the spore suspensions and recording spore attachment on individual nematodes [5]. For attachment bioassays, fresh J2s of root-knot nematodes were exposed to 5000 spores

per Petri dish [9]. All dishes were placed in a 28^0C incubator. Nematodes were observed under an inverted microscope at x200 magnification, and numbers of *P. penetrans* spores attached per nematode were recorded.

For the tap water bioassay (Figure 1, 2, 3, and Table 1), a total of 36 nematodes were examined for *P. penetrans* spore attachment after incubation of the Petri dishes at 28^0C for 1, 3, 6, and 9 h.

For the second bioassay (Figure 4, Table 2), a total of 36 nematodes were examined for *P. penetrans* spore attachment after incubation of the Petri dishes at 28 ^{0}C for 12, 24, 48, and 96 h. For the second bioassay, treatments were contacted in tap water mixed with a small quantity of loam soil (added 1g of soil per Petri dish).

Table 1: Estimate of the best fit probability to observed counts.

Incubation period	**3h**		**6h**		**9h**	
Distribution	**Neg Bin**	**Poisson**	**Neg Bin**	**Poisson**	**Neg Bin**	**Poisson**
Test value*	12.869	897.315	28.258	3603.83	26.804	209.577
Confidence*	>0.37	Rejected	>0.04	Rejected	>0.03	Rejected

* estimated by Chi-Square (χ^2 distribution) test.

Table 2: Observed values in soil bioassay of *P. penetrans* attachment to root-knot juveniles' cuticle over time 12, 24, 48, 96h of incubation.

Incubation time (h)	**without *P. penetrans* spores**	**number of J2s encumbered with *P. penetrans* spores**		
		1-3 spore/J2	4-7 spores/J2	≥8 spores/J2
12h	22	13	1	0
24h	13	12	9	2
48h	4	17	9	6
96h	1	5	15	15

C. Fitting the Negative Binomial Distribution to Pasteuria penetrans attachment

All calculations and graphs were made on Excel spreadsheets. Using the computer program BestFit 3.0 for Windows, the best fit discrete distribution was estimated. The best estimate functions were the Poisson and to negative binomial.

Using the computer program, BestFit 3.0 for Windows, the chi-square test for goodness-of-fit was performed to measure how well the sample data (observed values= *Pi*) would fit a hypothesized probability density function (theoretical value= *pi*).

D. I am predicting the probability of spores' attachment to root-knot juveniles with a Markov chain.

For soil bioassay, a Markov decision process (Markov chain) was used to predict the random variable (*P. penetrans* spores attachment) changes thought time. Based to formula $v^{(t)}=v^{(t-1)}A$, (where A transition matrix and $v^{(0)}$= initial probability vector), we computed the future probability distribution vectors for time t (t=12, 24, 48 and 96h) using a Markov chain calculator (http://math.plussed.net/markov/markov_calcs.php). In this form $v^{(t)}=v^{(t-1)}A$, the ij^{th} element of A is the conditional probability, A_{ij} = P(System will be in state j at time t | It is in state i at time t-1) and each row of A, the sum of the elements to 1 (http://math.plussed.net/markov/).

E. Efficacy of Pasteuria penetrans spores in planta

Fresh J2s were encumbered with *P. penetrans* spores as described by [38], making the following treatments a) J2s without *P. penetrans* spores, b) J2s with 4-7 *P. penetrans* spores, and c) J2s with ≥8 *P. penetrans* spores. Further, 3 weeks old tomato plants var Tiny Tim, were inoculated with 550 ± 30 J2/plant Plants were maintained in a glasshouse at 26^oC, and after 28 days, tomato plants were uprooted, washed under tap water and number of root galls and nematode egg masses were recorded as shown in Table 3. Replicates were 12 per treatment.

III. RESULTS

A. Fitting the Negative Binomial Distribution to Pasteuria penetrans attachment

In studying *P. penetrans* spores attachment, a juvenile of root-knot nematode (J2) may be encumbered with one or more spores over a fixed period of time. Counts can be summarized in a frequency distribution, showing the number of units containing χ = 0, 1, 2, 3, ….. individuals of an observed J2. If every J2 were exposed equally to the chance of being encumbered with *P. penetrans* spores over a fixed period of time, the distribution would follow the Poisson series, and the expected variance (s^2) is equal to a mean, that means its J2 will have the population mean. As Figure 1 shows, the observed variance (s^2) is significantly larger than the mean when recorded at 3, 6, and 9h incubation. And that (success events of *P. penetrans* attachment) can't fit a Poisson process as the parameter s^2 is not small or equal to the mean (Figure 1).

As shown in Figure 1, the means, e.g., at 6 or 9 h, of nematode exposure to a *P. penetrans* spore suspension are twice less than the variance, indicating a strong overdispersion. This suggests that *P. penetrans* spores are clumped, and more than one spore sticks on each J2.

As described above, *P. penetrans* attachment does not follow the Poisson distribution. Based on this, the data show a better fit for the negative binomial distribution than to Poisson (Figure 3). The chi-square test of the hypothesis, in cases of *P. penetrans* spores/J2 attachment 3, 6, and 9h after application, shows that only the negative binomial model was the most appropriate to fit the observed counts, and in all cases, Poisson distribution model was rejected (Table 1). This phenomenon is characterized as an "overdispersion" [4], indicating that *P.*

penetrans spores are clumped, and more than one spore attaches to a J2 cuticle over a fixed period of time.

Also, the results in Figures 2, 3 suggest that the model for estimating probabilities of *P. penetrans* attachment depends on the time of J2 exposure to *P. penetrans*, e.g., 6 or 9h.

Moreover, the results of Table 1 show that the negative binomial is the more appropriate distribution fitting all observation. Explanations for the negative binomial describing better the *P. penetrans* attachment area) because the observed variance (s^2) is larger than the mean (Figure 1) and b) as time increases, the over-dispersion was clearly too large for the Poisson distribution (Figure 3).

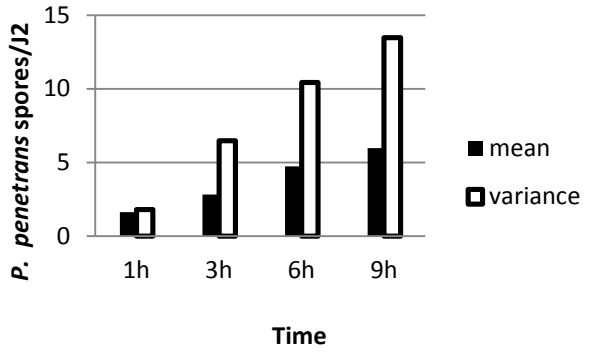

Fig. 1: Mean and variance estimates for *P. penetrans* attachment to root-knot juveniles (J2s).

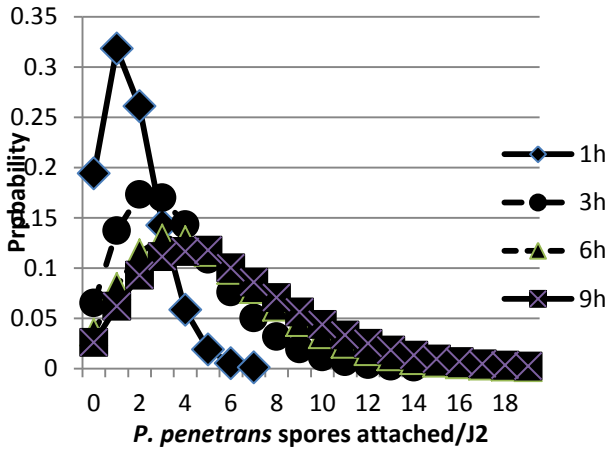

Fig. 2: Fitting the theoretical values of *P. penetrans* spore attachment per root-knot juvenile, based on the negative binomial distribution model of 3, 6, and 9 h exposure and Poisson distribution model of 1 h exposure.

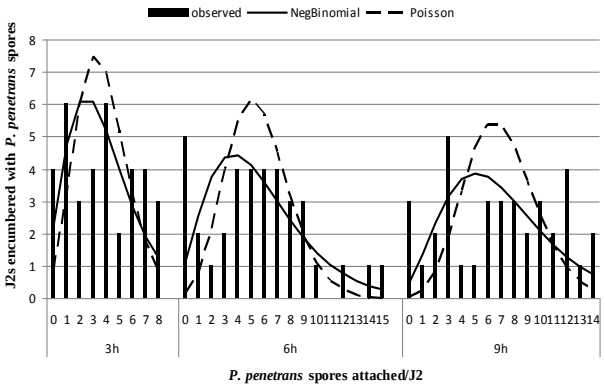

Fig. 3: Tab water bioassay. Fitted Poisson and Negative Binomial (NegBinomial) distribution to observed counts.

The same was concluded for nematodes encumbered with spores or without *P. penetrans* spores for soil bioassay conducted at different times of application, e.g., 24, 48, and 96h (Figure 4). These data (Figure 4) show that the best fit is obtained with the negative binomial distribution for *P. penetrans* spore attachment per juvenile at 24, 48, and 96h after application suggested (a) the observed variance (s^2) being larger than the mean and (b) as time increases the overdispersion was clearly too large for the Poisson distribution.

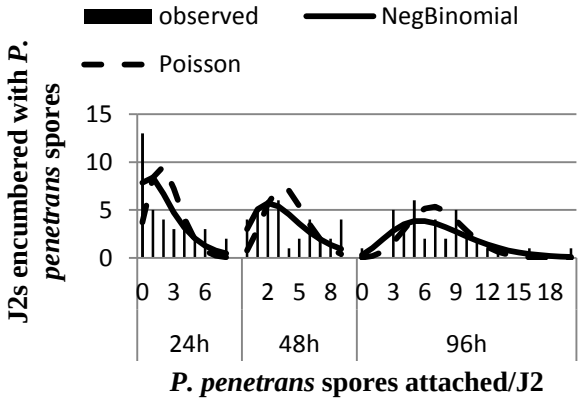

Fig. 4: Tab water mixed with loam soil bioassay. Fitted Poisson and Negative Binomial (NegBinomial) distribution to observed counts.

B. I am predicting the probability of spores' attachment to root-knot juveniles with a Markov chain.

The probabilities of *P. penetrans* attachment to root-knot juveniles' cuticle over a time period, given the per cent of J2s, encumbered with or without *P. penetrans* spores as presented in Table 2, can be presented by the following transition matrix:

$$P = \begin{vmatrix} 0.611 & 0.361 & 0.027 & 0.0 \\ 0.361 & 0.333 & 0.250 & 0.055 \\ 0.111 & 0.472 & 0.250 & 0.166 \\ 0.027 & 0.138 & 0.416 & 0.416 \end{vmatrix}$$ Transition matrix (1)

Matrix 1 represents the probabilities of J2's encumbered with *P. penetrans* spores, showing the probabilities of transitions for encumbered with no spores, after 12 h at 0.611 followed by a probability for encumbered with 1-3 spores, after 12 h at 0.361, a probability for encumbered with 4-7 spores, after 12 h at 0.027 and probability for encumbered with ≥8 spores, after 12 h as zero. After 96 h the corresponding probabilities are 0.027, 0.138, 0.416 and 0.416, respectively.

Solving Transition matrix (1), gives the steady state distribution P^{12}:

[q1 q2 q3 q4] = [0.384 0.352 0.178 0.084]

or, the steady state distribution P^{100}:

[q1 q2 q3 q4] = [0.384 0.352 0.178 0.084]

Based on the results obtained from the steady-state distribution P^{12} (q1 q2 q3 q4) or P^{100} (q1 q2 q3 q4), we note that the last result q4 (0.084) is <0.416 in the original matrix (Transition matrix 1) for ≥8 spores after 96 h suggesting that spores detached, e.g., after 96 h.

In conclusion, in the long term, (e.g., after 96h of incubation) 38.4% of J2s are without *P. penetrans* spores, 35,2% of J2s are with 1-3 *P. penetrans* spore, 17,8% of J2s are encumbered with 4-7 *P. penetrans* spores and 8,4% of J2s are encumbered with ≥8 *P. penetrans* spores.

C. Efficacy of Pasteuria penetrans spores in planta

The evaluation of *P. penetrans* in planta resulted in a lower rate of nematode invasion and development in tomato roots compared to controls, especially in treatment where J2s were encumbered with ≥8 *P. penetrans* spores (Table 3). Further, results indicate that the bacterium can exert a "nematostatic effect" when J2s are encumbered with high numbers of ≥8 of *P. penetrans* spores.

Proportions (%) of nematode invasion and the Proportions (%) of egg-masses were observed less in treatment were J2s were encumbered with ≥8 *P. penetrans* spores (Figure 5) indicate that *P. penetrans* spores probably multiplies in the body of the female of plant-parasitic nematodes.

Moreover, based on results obtained from steady-state distribution P^{12} and Table 3, the proportions of the observed J2s invaded tomato roots was fitted (Figure 5) to predicted data of *P. penetrans* spores attachment received from the steady-state distribution P^{12}, indicating that *P. penetrans spores* disturbed the nematode forward movement as presented by [39]. This observation provides more evidence that a high number of ≥8 spores of *P. penetrans* attached to the nematode cuticle have a significant impact on that movement, which plays a role in nematode locomotion and root invasion as described by [38].

Based on that, Figure 5 shows that *P. penetrans* spores caused successful parasitism to J2s encumbered with 4-7 of *P. penetrans* spore, which is sufficient to initiate infection without reducing the ability of the nematode to invade roots and probably some encumbered with the *P. penetrans* spores multiplies bacterial spores inside the female body (Table 3, Figure 5).

Overall, when *P. penetrans* spores attached more than 8 spores/J2s, *P. penetrans* significantly suppress nematodes invasion (Table 3) confirmed that clumps of spores significantly change nematode locomotion in the soil as reported by [37].

Table 3: Effect of *Pasteuria penetrans* on J2s invasion and development in planta.

Treatment	Invaded J2s/tomato root (Mean ± SEM)	egg masses/root (Mean ± SEM**)
Absolute Control	0^{a}* ± 0	0^{a} ± 0
Control (J2s without *P. penetrans* spores)	197^{d} ± 21	104^{d} ± 11
J2s with 4-7 *P. penetrans* spores	81^{c} ± 13	19^{c} ± 3
J2s with ≥8 *P. penetrans* spores	52^{b} ± 7	4^{b} ± 1,5
P value	*P<0,001*	*P<0,001*

* Values within a column followed by the same letter do not reflect different significantly according to Tukey's tests (*P*=0.05). Values are based on 12 replicates per treatment.

** where SEM, the standard error of the mean.

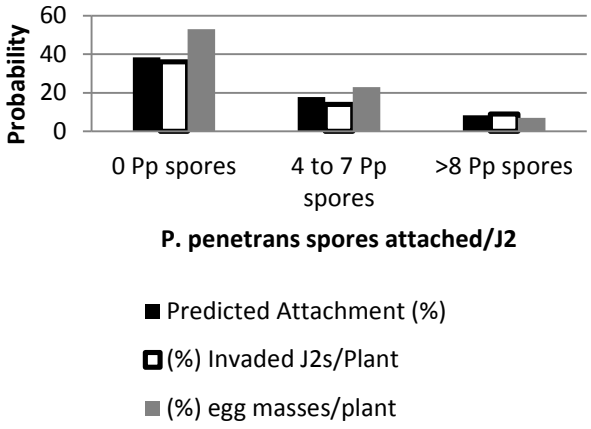

Fig. 5: Predicted probabilities and effect of *Pasteuria penetrans* on J2s invasion and development in planta. (Predicted attachment data were based on Transition matrix 1).

IV. DISCUSSION

This paper is concerned with fitting Poisson, Negative Binomial, and Markov chain models to *Pasteuria penetrans* attachment.

The application of the Poisson and the Negative Binomial distributions approaches for modelling count variables of plant, and natural organisms were first presented by [4]. In Bliss's paper, clear evidence is provided to show that the biological models (mainly

natural organisms) are characterized by a significantly larger variance than the mean a phenomenon called "overdispersion." In this research [4], it was concluded that in analyzing natural organism counts for which the variance is significantly larger than the mean, besides the Poisson distribution, the model of the negative binomial distribution is the most appropriate.

Moreover, in this paper, data is presented of the observed counts of *P. penetrans* spore attachment to root-knot juveniles and the predicted values using both the Poisson and the negative binomial distribution. The *P. penetrans* spore attachment was modelled at one concentration (5,000 spores), in water and soil bioassay, at four times of exposure 1, 3, 6 and 9h and 12, 24, 48, and 99h respectively. Besides that, in water bioassay, counts confirmed that the Poisson distribution is a satisfactory model for *P. penetrans* spores attachment to root-knot juveniles but only for the 1h exposure. Interestingly the variance is equal to the mean suggesting 'under-dispersion' results, and the Poisson distribution is considered the most appropriate model to fit the data sets. Similar results on natural organisms were presented by [4] and [28], who used both the Poisson and the negative binomial distribution. Further, our data show that after 3h of exposure, the negative binomial model is the more appropriate model to fit the counts 'over-dispersion' [3], [4], [7], [28], [24],[40]. Further, we concluded that the negative binomial model is also the preferred model as a time of exposure increased (e.g., 6 or 9h). The same results were observed for the soil bioassay where the negative binomial model proved the most appropriate model to predict *P. penetrans* spore attachment, especially when the time of exposure increased (e.g., 48 or 96h).

In this research, data showed that after 6 or 9h (water bioassay) and 48 or 96h (soil bioassay) of J2s exposure to *P. penetrans* spores, high numbers of *P. penetrans* spores per nematode were observed, and the negative binomial model provided a more efficient means of describing attachment. We assume this is evidence of uneven distribution of *P. penetrans* spores in the suspension, and some J2s may encounter clumps of spores. We suppose that the above is an explanation in the study reported by [11], where when root-knot J2s encumbered with greater than 15 spores per juvenile, reduced invasion by >70%. The same was concluded by [37], [38], where the authors showed that the *P. penetrans* spores attached to the nematode cuticle have a significant impact on nematode turns, which plays a significant role in nematode locomotion (forward movement), affected nematodes invasion and establishment significantly on tomato root systems when encumbered with high numbers, e.g., 20–30 of *P. penetrans* spores, compared with unencumbered nematodes [38].

Exposure time, e.g. 3h or 6h (water bioassay) and 48 or 72h (soil bioassay), probably is an important factor to study the *P. penetrans* attachment process. To study this, time could be an important factor in developing a mathematical model for *P. penetrans* attachment.

As discussed above, many models have been used to estimate the 'over-dispersion' exhibited among natural organisms [2]. In this research, it was noted that the negative binomial distribution is the most appropriate model to describe *P. penetrans* 'over-dispersion', leading to the hypothesis that the *P. penetrans* spores are clumped under natural conditions.

Further, in this research, the Markov chain proved a good tool for predicting the *P. penetrans* spores attachment process even when root-knot J2s are encumbered with clumps of bacterial spores.

Data showed that with the Markov process, it is possible to estimate *P. penetrans* spore attachment related to time (soil bioassay) if the attachment process depends only on the distribution of the previous stage. However, based on the results of our previous studies [39], the Markov chain and Cochran probability model proved good estimators to describe the effect of clumps of *P. penetrans* spores attachment on root-knot juveniles locomotion. Overall, in this study, Markov chain model showed that even a low number of 4-7 spores of *P. penetrans* attached to the nematode cuticle have a significant impact on that movement, which plays a role in nematode locomotion and invasion to plant roots as described by [38].

Generally, as our data show, the Markov chain model proves an easy computation method to predict the observation function of the counts, we conclude that this is a useful point to estimate *P. penetrans* spores process based on other parameters such as soil properties and plant root invasion. Several authors have proposed the idea of Markov chains [19], [20], [1], [25], [35], [8], [18], as a statistical efficient estimator tool for many applications in biological modelling where future outcomes (output values) will predict from observed counts. Markov chain analysis is employed in algorithms, particularly in software programs such as Mathematica or Matlab and probably a Markov chain model needs to be constructed to such mathematical computation programs to produce output values based on the *P. penetrans* spores attachment process.

REFERENCES

[1] Bailey NTJ. 1975. "*The Mathematical Theory of Infectious Diseases and Its Applications"*. Charles Griffin, London.

[2] Barbou, AD, Kafetzaki, M., 1991. "*Modelling the overdispersion of parasite loads*". Mathematical Biosciences, 107, 249-53.

[3] Binet FE. 1986. "*Fitting the negative binomial distribution*". *Biometrics*, 42, 989-992.

[4] Bliss CI. 1953. "*Fitting the Negative Binomial Distribution to Biological Data*". Biometrics, 9, 176-200.

[5] Channer AGDE R, Gowen SR. 1992. "*Selection for increased host resistance and increased pathogen specificity in the Meloidogyne - Pasteuria penetrans interaction*". Fundamental and Applied Nematology, 15, 331-339.

[6] Choo KH, Tong JC, Zhang L. 2004. "*Recent Applications of Hidden Markov Models in Computational Biology*". Genomics Proteomics Bioinformatics, 2, 84-86.

[7] Cox DR. 1983. "*Some remarks on overdispersion*". Biometrika, 70, 269–274.

[8] Daley DJ, Gani J. 1999. "*Epidemic Modelling: An Introduction"*. Cambridge University Press, Cambridge.

[9] Darban DA, Pembroke B, Gowen SR. 2004. "*The relationships of time and temperature to body weight and numbers of endospores in Pasteuria penetrans-infected Meloidogyne javanica females"*. Nematology, 6, 33-36.

[10] Davies KG, Danks C. 1992. "*Interspecific differences in the nematode surface coat between Meloidogyne incognita and M.*

arenaria related to the adhesion of the bacterium Pasteuria penetrans". Parasitology, 105, 475-480.

[11] Davies KG, Kerry BR., Flynn CA. 1988. "*Observations on the pathogenicity of Pasteuria penetrans, a parasite of root-knot nematodes". Annals of Applied Biology*, 112, 491-501.

[12] Davies KG, Laired V, Kerry BR. 1991. "*The motility development and infection of Meloidogyne incognita encumbered with spores of the obligate hyperparasite Pasteuria penetrans". Revue de Nematologie*, 14, 611-618.

[13] Gowen SR, Davies KG, Pembroke B. 2008. "*The potential use of Pasteuria spp. in the management of plant-parasitic nematodes, in Integrated management and biocontrol of vegetable and grain crops nematodes, eds".* A. Ciancio and K.G. Mukerji, Dortrecht. The Netherlands: Springer, 197-210.

[14] Hatz B, Dickson DW. 1992. "*Effect of temperature on attachment, development, and interactions of Pasteuria penetrans and Meloidogyne arenaria".* Journal of Nematology, 24, 512-521.

[15] Hewlett TE, Dickson DW. 1994. "*Endospore attachment specificity of Pasteuria penetrans from a peanut field in Florida*". Journal of Nematology, 26, 103_104.

[16] Hooper DJ. 1986. "*Extraction of nematodes from plant materials".* In J. F. Southey (Ed.), *Laboratory methods for working with plant and soil nematodes* (6th ed., pp. 51_58). London: Her Majesty's Stationary Office.

[17] Imbriani JL, Mankau R. 1977. "*Ultrastructure of the nematode pathogen Bacillus penetrans". Journal of Invertebrate Pathology*, 30, 337-347.

[18] Kot, M., 2001. "*Elements of Mathematical Ecology".* Cambridge. Cambridge University Press.

[19] Leslie PH. 1945. "*The use of matrices in certain population mathematics." Biometrica*, 33, 183-212.

[20] Leslie PH. 1948. "*Some further notes on the use of matrices in population mathematics*". *Biometrica*, 35, 213-245.

[21] Mankau, R., 1975. Bacillus penetrans n. comb. "*Causing a virulent disease of plant-parasitic nematodes.*" Journal of Invertebrate Pathology, 26, 333-339.

[22] Mankau, R., 1980. "*Biological control of Meloidogyne populations by Bacillus penetrans in West Africa*". Journal of Nematology, 12, 230.

[23] Mankau R, Imbriani JL. 1975. *"The life-cycle of an endoparasite in some tylenchid nematodes."* Nematologica, 21, 89-94.

[24] Morel GJ, Nagaraj KJ. 1993. "*A finite mixture distribution for modelling extra multinomial variation*". Biometrika, 80, 363–371.

[25] Nisbet RM, Gurney WSC. 1982. "*Modelling Fluctuating Populations".* John Wiley & Sons, Chichester, and New York.

[26] Our Y. 1997. "*Effect of spore sonication on attachment and host attachment range of Pasteuria penetrans to the root-knot nematode*". Applied Entomology and Zoology, 32, 101-107.

[27] Rao, MS, Gowen SR, Pembrok, B, Reddy PP. 1997. "*Relationship of Pasteuria penetrans spore encumbrance on juveniles of Meloidogyne incognita and their infection in adults*". *Nematologia Mediterranea*, 25, 129-131.

[28] Ross GJS, Preece DA. 1985. "*The negative binomial distribution*". *The Statistician*, 34, 323-336.

[29] Sayre RM, Starr MP. 1988. "*Bacterial diseases and antagonism of nematode*". In G. O. Poinar & H. B. Jansson (Eds.), Diseases of nematodes (pp. 69_101). Boca Raton, FL: CRC Press.

[30] Sayre RM, Wergin WP. 1977. "*Bacterial parasite of a plat nematode: morphology and ultrastructure*." Journal of Bacteriology, 129, 1091-1101.

[31] Southey JF. 1986. "*Laboratory methods for work with plant and soil nematodes."* London: MAFF/ADAS, Her Majesty's Stationary Office.

[32] Stirling GR. 1985. "*Host specificity of Pasteuria penetrans within the genus Meloidogyne".* Nematologica, 31, 203-209.

[33] Stirling GR. 1991. "*Biological control of plant-parasitic nematodes. Progress, Problems, and Prospects".* Wallingford: CAB International.

[34] Talavera M, Mizukubo T. 2003. "*Influence of soil conditions, spore densities, and nematode age on Pasteuria penetrans attachment to Meloidogyne incognita".* Spanish Journal of Agriculture Research, 1, 57-63.

[35] Taylor HM, Karlin S. 1998. "*An Introduction to Stochastic Modeling".* 3rd ed. Academic Press, New York.

[36] Thorne, G., 1940. *Dubosqia penetrans* n. sp. (Sporozoa, Microsporidia, Nosematidae), a parasite of the nematode Pratylenchus pratensis (De Man) Filipjev. Proceeding of the Helminthological Society of Washington, 7, 51-53.

[37] Vagelas IK, Pembroke B, Gowen SR. 2011. "*Techniques for image analysis of the movement of juveniles of root-knot nematodes encumbered with Pasteuria penetrans spores*". Biocontrol Science and Technology, 21, 239_250.

[38] Vagelas IK, Dennett MD, Pembroke B, Gowen SR. 2012. "*Adhering Pasteuria penetrans Endospores Affect Movements of Root-Knot Nematode Juveniles*". *Phytopathologia Mediterranea*, 51, 618-624.

[39] Vagelas IK, Dennett MD, Ipsilandis P, Pembroke B, Gowen SR. 2013a. "*Understanding the movement of root-knot nematodes encumbered with or without Pasteuria penetrans". Biocontrol Science and Technology*, 23:1, 92-100.

[40] Vagelas I, Dennett MD, Pembroke B, Gowen SR. 2013b. "*Fitting the negative binomial distribution to Pasteuria penetrans spore attachment on root-knot nematodes and predicting the probability of spore attachments using a Markov chain model*". *Biocontrol Science and Technology*, 23:11, 1296-1306.

[41] Verdejo-Lucas, S., 1992. "*Seasonal population fluctuation of Meloidogyne spp. and the Pasteuria penetrans group in Kiwi orchards*"', Plant Disease, 76, 1275-1279.

[42] Whitehead AG, Hemming JR. 1965. "*A comparison of some quantitative methods of extracting small vermiform nematodes from soil.*" Annals of Applied Biology, 55, 25_38.