Studies on Hatching of the Root-Knot Nematode, *Meloidogyne incognita* var. *acrita* CHITWOOD

I. The Relation between Hatching and the Original Egg Content of Egg Mass

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INTRODUCTION

We have as yet little information as to what is involved in hatching of root-knot nematode larvae (*Meloidogyne* spp.) except for the experiment on the effect of osmotic pressure on eggs (DROPKIN, MARTIN & JONSON, 1958). On the other hand, the emergence of cyst forming nematode larvae from cyst and various conditions bringing on it have been studied by many workers. Furthermore the relationship between the larval emergence and the variability has been discussed by many workers (BOYD, 1943; ELLENBY, 1946a, 1946b, 1955, 1956; GEMMEL, 1943; TRIFFITT, 1930; BISHOP, 1953; LOWNSEBURY, 1951; FERRIS & MAI, 1956; WINSLOW, 1956; WALLACE, 1959; HESLING, 1959).

According to their studies the aspects of the egg within a cyst have gradually been clarified. ELLENBY (1956) takes eggs in a cyst for a community and suggests that hatching of larvae is restricted by biotic and chemical factors arising from the community.

Recently WALLACE (1959) has shown the several factors related both to hatching within a cyst and to emergence of larvae of beet eelworm from cyst, and treated of the co-relation of their factors with a schema. He also suggested that the presence of active larvae within a cyst inhibits further egg hatch, so that the rate which the larvae emerge determines the rate at which the eggs hatch further. His suggestion is in agreement with the observations on the potato root eelworm by ELLENBY (1946a),

HELING (1959) and ONIONS (1955).

The studies on the cyst have been proceeded as above, while the studies on the egg masses of root-knot nematode have not so enough been carried out as to apply the conception for cyst to egg masses.

The authors have been engaged in the study of the root-knot nematode, *Meloidogyne incognita* var. *acrita* CHITWOOD, since 1958. In the present paper a few data obtained by the experiments on egg masses will be described.

EXPERIMENTAL

Two series of experiments are described; in one the hatching among egg masses, in the other the comparison of hatching between egg mass and free eggs.

1. The difference of hatching among egg masses

Material and method.—Egg masses were obtained from the roots of sweet potato of the variety Kanto-14 in July 1959. They were divided into two groups. One was packed in gauze and then immersed in flowing tap water at 17~18°C, while the other was kept in a refrigerator at 5~8°C. After this pre-treatment for 3, 6 and 12 days respectively, 5 egg masses were placed in about 1.0 ml of distilled water in a watch glass with 7 replicates. They were incubated at 26°C. The hatched larvae were counted at 24 hour intervals for 7 days. Distilled water was renewed at each time of counting. After one week the remaining eggs in egg masses were counted by separating them each other with

10% antiformalin.

Results.—The correlation between the original egg content of egg masses and the percentage of hatch is shown in Fig. 1. With increasing the egg content the

Original egg content per 5 egg masses

Fig. 1. Relation between the original number of egg content and the percentage of hatch. The pre-treatments for 3, 6 and 12 days are all included.

- : Egg masses immersed in flowing tap water.
○---○: Egg masses stored at low temperature.

percentage of hatch decreases. The formulae of regression line are $Y=60.4-128(X-2.99)$ for the egg masses in the flowing tap water, and $Y=35.5-82.7(X-3.30)$ for the store at the low temperature ($5\sim 8^{\circ}\text{C}$); the correlation coefficients are -0.97 and -0.87 respectively, and these are highly significant ($p<0.001$). The number of the larvae emerged more or less

Original egg content per 5 egg masses

Fig. 2. Relation between the original egg content and the number of hatched larvae. The pre-treatments for 3, 6 and 12 days are all included.

- : Egg masses immersed in flowing tap water.
○---○: Egg masses stored at low temperature.

increases as the egg content increases, as shown in Table 1. But there is no marked positive relationship between them (Fig. 2); $Y=64.8+431(X-3.06)$, $r=0.578$, $p<0.02$ for the flowing tap water, $Y=700.3+22.0(X-3.20)$, $r=0.02$, $p<0.01$ for the store at the low temperature. The variabilities of both per cent hatch and number of emergence became greater with an increase in egg content (Table 1). The number of hatched larvae remaining in egg mass was counted at the end of a 7-day incubation, at which the emergence of larvae from the egg mass almost ceased, then plotted against the original egg content, and is shown in Fig. 3. It shows monomodal distribution having a peak in the range of $300\sim 400$ original egg content per one egg

Table 1. Egg content and hatchability of egg masses of *Meloidogyne incognita* var. *acrita*

Number of egg masses	Original egg content of egg masses	Mean original egg content	Egg content S. D.*	Per cent mean hatch	Per cent hatch C. V.**	Mean number of emergence	Emergence C. V.**
55	100~200	149.7	21.39	83.4	0.0790	125.9	0.1992
75	201~300	253.8	28.27	52.7	0.2081	132.0	0.2278
85	301~400	336.5	29.05	36.1	0.3445	129.6	0.3434
65	401~500	434.4	26.5	38.7	0.3705	167.2	0.3571
30	501~600	556.9	38.1	31.6	0.5073	174.8	0.4735
1	655			11.5		75.0	
1	725			18.7		148.0	

*: Standard deviation

** : Coefficient of variance

Fig. 3. Showing the number of hatched larvae remaining in egg masses plotted against the number of original egg content, when emergence of larvae from egg masses ceased at the end of 7 days of incubation

Original egg number per 5 egg masses
Fig. 4. The probit of per cent hatch plotted against the original egg content in egg masses

mass.

By transforming the percentage of hatch into probit, it is possible to calculate that the original egg content in an egg mass

required for a 50% hatch for 7 days at 26°C (Fig. 4); the probit equation is $Y = 5 - \frac{1}{0.3}(X - 3.165)$, $p < 0.001$, and the egg con-

Table 2. The relationship between the number of original egg content of egg masses and the percentage of hatch in each period of pre-treatments

The percentages of hatch are cumulative values during 7-day count after pre-treatment.

Immersion in flowing tap water			Store in refrigerator		
Period of pre-treatment (day)	Original egg content per 5 egg masses	Per cent hatch	Period of pre-treatment (day)	Original egg content per 5 egg masses	Per cent hatch
3	1028	57.4	7	928	81.6
	1201	55.6		1688	41.4
	1411	45.8		1731	60.4
	1428	35.5		2141	61.0
	1804	47.9		2505	55.7
	2146	21.6		2998	46.5
	2252	33.5	12	291	92.4
6	1690	49.5		760	87.7
	1773	52.9		790	88.4
	1793	49.4		877	73.5
	2002	46.1		1104	81.1
	2113	48.7		1290	60.4
	2225	46.5		1319	65.4
	2301	42.6		1456	54.1
12	571	94.5		1476	54.2
	608	85.5		1982	51.8
	684	73.5			
	735	88.5			
	945	68.1			
	1229	65.8			

tent for 50% hatch with a confidence limit is 292 ± 7 for one egg mass. The pre-treatments of both immersing in flowing tap water and storing at low temperature were carried out in order to obtain egg masses with varying degrees of egg content, especially the former for obtaining egg masses having smaller egg content. However, the general features, i. e. higher percentage of hatch in smaller egg masses than in larger ones, were observed in both treatments (Table 2).

In summary, with increasing the egg content, the percentage of hatch decreases, whereas the variabilities for the percentage of hatch and the number of emergence increase.

2. Hatching from intact-whole egg masses, halved egg masses and from free eggs

The experiment mentioned above was extended to Experiment 2, whose aim was to further clarify the hatching from another approach.

Material and method.—In January 1960, egg masses were collected from the test tube with sterilized soil, which had been buried in 20 cm underground in late November. They were divided into three groups, and in each group three egg masses were put in a watch glass with 10 replicates; the first group was left intact, the second cut into halves, and the third separated each other with dissecting needles. Then about 1.0 ml of distilled water was added to each of them. The coun-

ting of hatched larvae was made as in the Experiment 1. After 7 days the remaining eggs were counted.

Results.—The outstanding feature of emergence, shown in Fig. 5, is a large

Fig. 5 Emergence from intact-whole egg masses (●), halved egg masses (◐) and from free eggs (○)

emergence from free eggs even on the first day of experiment; in the case of the free eggs the emergence on the first day is about 28% of the total emergence, for the halved egg masses it is about 9%, and it is only 2% for the intact-whole egg masses. The percentage of hatch and

Table 3. Hatching from free eggs, halved egg masses and intact-whole egg masses, and variation of data

	Per cent mean hatch	Coefficient of variability of per cent hatch (%)	Mean number of hatched larvae per 3 egg masses	Coefficient of variability of number of larvae emerged (%)	Per cent emergence during the first 24 hours to total emergence for 7 days
Intact-whole egg masses	19.01	72.11	257.0	68.32	2.14
Halved egg masses	27.70	42.23	316.6	42.94	8.92
Free eggs	41.10	29.26	412.0	27.45	27.90

number of emergence increase in the order of free eggs, halved egg masses and intact-whole egg masses. On the other hand, the coefficients of variance of them decrease in that order (Table 3).

DISCUSSION

According to the present experiments, the following facts have been clarified:

- 1) The percentage of hatch tends to decrease with an increase in egg content of egg masses.

- 2) The percentage of hatch decreases with an increase in variability of both the per cent hatch and the number of emergence of larvae from egg masses.

- 3) The existence of critical point of egg content for hatchability.

The fact (1) is in agreement with HESLING (1959) who suggested the same phenomena in cyst. Many reports have been published on the cause of the decrease in per cent hatch. ELLENBY (1956) suggested that a 'hatching inhibitor' produced by the larvae might be a factor reducing hatching from cysts. ONIONS (1955) suggested that the stop of hatching is caused by oxygen deficit and increased acidity due to the hatched larvae. GILLARD, D'HERDE & VAN DEN BRANDE (1958) showed that carbon dioxide inhibits emergence. These suppositions might also be applied to the egg masses of *Meloidogyne*.

According to the author's observation, there is no difference in the velocity of egg development between egg masses and free eggs. Therefore, the factors which operate to reduce the percentage of hatch might affect mostly larval stages. This has some bearing upon the experiment by DROPKIN *et al.* (1958) that osmotic pressure does not affect egg development but larval motion.

ELLENBY's experiment (1956) showing that the factors operating on the reduction of hatching also operate on the production of variability is also supported by the fact (2) above. It is considered that the increase in variability of hatching is due to the fluctuation of the quantity of eggs

tending to hatch sooner than others as ONIONS' suggestion (1955) that hatching from a cyst is not random. Whether these eggs abound or not in an egg mass is probably due to the mutual interaction of the egg community in an egg mass. ELLENBY (1956) suggested the interaction in a cyst. The mucoid substance of egg mass is not considered to be a direct factor preventing the larvae from hatching. The mucoid substance only constitutes the boundary of the egg community, so that the decrease of hatching or the increase of variability may arise from the egg mass being bound up with a so called 'closed system' as reported by ELLENBY (1956) in cyst.

With regard to the fact (3) above, it is very interesting that the number of hatched larvae within an egg mass shows a monomodal distribution when plotted against the original egg content in egg mass as shown in Fig. 3. The authors refer the peak of the distribution to a critical point, that is, the egg masses having egg content larger than the critical point are qualitatively different from those having less eggs. It seems that any factor to reduce hatching acts more strongly with increasing the egg content. The authors could not find bimodal distribution of hatching response as shown by ELLENBY (1956) in cysts.

From the Experiment 2, it is considered that there are differences in hatchability between egg mass and free eggs. Furthermore the differences in the susceptibility to stimulus between the two are conceivable from the Figs. 6 and 7. These figures show that free eggs generally reach to higher percentage of hatch than egg masses. The accumulated per cent hatch from the egg masses treated with phenol reached 38% with the emerged larvae dead, whereas it reached only 3% in free eggs with the emerged larvae dead also. The egg masses treated with phenol became black-brown in colour, so that it is possible to suppose the presence of polyphenol oxidase in the mucoid substance of egg mass. ELLENBY (1946c) demonstrated the presence

Fig. 6. Showing the effect of chemicals on the hatch of egg masses

- : 1×10^{-4} M picric acid
- : Distilled water
- : 0.001% formalin
- : 1×10^{-4} M phenol

Fig. 7. Showing the effect of chemicals on the hatch of free eggs

- : 1×10^{-4} M picric acid
- : distilled water
- : 0.001% formalin
- : 1×10^{-4} M phenol

of polyphenols and polyphenol oxidase in the cyst wall. Supposing the hatching as an indicator to stimulus, the differences in susceptibility as seen between egg masses and free eggs might be expected also among egg masses.

The supposition of the existence of the differences among egg masses to stimulus sets to thinking ELLENBY's (1956) suggestion that the cyst must be regarded as responding to stimulation as a unit to be capable of applying to egg masses.

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摘 要

ネコブセンチュウのふ(孵)化に関する研究

I. ふ化と卵のう蔵卵数の関係

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シストセンチュウのふ化あるいはシストからの幼虫遊出については、温度・水分などの物理的因子ばかりでなく、シスト内における生物的・化学的因子が大きく作用しているとされている。筆者らは、ネコブセンチュウ幼虫のふ化と卵のうからの遊出について二、三の知見を得たので報告する。

1) 卵のうの蔵卵数が少なくなるにしたがい、幼虫のふ化遊出率は高まる傾向がある。

2) 蔵卵数の大なる卵のうグループは、ふ化遊出率ならびに遊出数の変異係数は大になる傾向がある。

3) 蔵卵数が 240~300 のものは、26°C, 1 週間の incubation で約 50% のふ化率を示す。

4) 卵のうからの幼虫遊出が終えん状態にはいったころ、卵のう内ふ化幼虫数は蔵卵数 300~400 のものが最大値を示す。

5) 実験的に卵のうを完全、1/2、遊離卵とした場合のふ化遊出率および遊出数はともに、

完全卵のう < 1/2 卵のう < 遊離卵

となり、両者の変異係数はともにこの逆である。

抄

録

キバガ科一種の生命表と個体群動態

STARK, R. W. (1959) Population dynamics of the lodgepole needle miner, *Recurvaria starki* FREEMAN, in Canadian Rocky Mountain Parks. Can. J. Zool. 37: 917~943.

カナディアン・ロッキーの針葉樹林の一部 (*Pinus contorta* ssp. *latifolia* が主体) に 1940 年ごろから lodgepole needle miner, *Recurvaria starki* (キバガ科) と呼ばれる潜葉性のガが大発生した。この研究は大発生がそのピークを過ぎた 1954 年から 1958 年にかけて行なわれた、生命表の作製を中心とした生態学的研究の総括である。

生命表によると、本種は成熟に満 2 年を要し、(1) 卵期およびふ化直後の 1 齢幼虫期、(2) 2 齢で行なう最初の越冬期、(3) 3 齢で行なう第 2 回目の越冬期、および (4) 老熟幼虫からさなぎにかけての時期、の 4 回の critical period がある。このうち、前の 3 回はほとんど気候の影響によると想像され、最後の死亡期の

みが、寄生・捕食などの生物学的要因によるのである。年によって死亡率が著しく違うのは最初の 3 つの時期であって、特に卵から 1 齢幼虫の定着までの死亡率の違いは大きい。これは卵が産まれた葉の落葉と、悪天候によるふ化幼虫の死亡によるらしい。天敵の働きは率の上でも低く、密度依存性も認められない。ただし、大発生がすっかり終わって、本種が通常のごく近い密度に戻ったときには、寄生と捕食の割合は相当高くなる。今回の大発生では、食樹の衰弱や食物不足の兆候はない。結局、本種の大発生は気候によって起こされて気候によって終わったものと思われる。天敵は通常の低い密度を維持するうえでは役割を果たしているだろうが、大発生を抑えるうえでは 2 次的である。長年の気候変動を調べると、大発生は冬の気温が暖かくなり始めの年に始まり、温暖であった 1940~46 年の間続き、1949~50 年の著しい寒冷を機として衰退したものと考えられる。

(農技研 伊藤嘉昭)