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EFFECT OF SNA 415, STRAIN OF BACILLUS THURINGIENSIS AND SCHISTOSOME INFECTION ON THE SURVIVALNESS AND FECNDIUTY OF BIOMPHALARIA ALEXANDRINA SNAILS

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ABSTRACT

Effect of SAN415 strain of Bacillus thuringiensis and Schistosome infecion on snsil survivalness and fecundity was investigated, in a population of laboratory-bred Biomphalaria alexanderina. Both the survival rates and egg production capacity)assessed by determing number of egg masses / 10 snail / week ; number of eggs / egg mass and histological examination of the ovitestis) have been adversely affected by Schistosoma mansoni miracidial infection. The effect is inversly proportioal to the number of infecting miracidia and by treatments with a sublethal concentration of SAN 415 strain of Bacollus thuringiensis.

INTRODUCTION

Bacillus thuringiensis. in its commercial bacterial preparation is recently used as a biological agent to control insects (Armstrong

Mummigatti & Roghunathan, 1988; Nishiura, 1988 and Tottier et al., 1988); parasitic nematodes (Ignoffo and Dropkin, 1977; Bottjer & Bone, 1987; Osman et al. , 1987 and Osman, 1991) and mloouscs (Terytze & Hogman, 1986; Osman & Mohamed, 1991 and Osman et al., 1992).Previous trials with B. thuringiensis as a biological agent against the molluscan gastropode snail Biomphalaria alexandrina the vector of Schistosomiasis proved its high potentiality as a molluscicidal agent (Osman and Mohamed, 1991). The comparative molluscicidal action of 4 different preparations of B. thuringiensis was investigated. It has been concoluded that B. thuringiensis in its prparation SAN 415, has proved to be the most potent prepation against B. slexandrina (Osman et al., 1992) The authers reported that exposure of snails to 500 ppm of SAN 415 for 3 days caused complete sterilization.

Previous investigations have proved that Schistosome infection of snails lead to complete inhibition of the egg laying capacity (Mehleman, 1972; Cheng et al., 1975; Ishak & Mohamed, 1975 and Mohamed , 1978).

It was proved that develpment of miracidia to mother and daughter sporocysts in the hepatopancreas of the snails was accompaned by releasing toxic material called Schistosomine. This toxine (s) caused inhibition in spermatogenic and oogenic processes (El-Saadany & Mohamed, 1990)

The present work was planned to compare the effect of both

Schistome infection and pathogenic effect of B. thuringiensis on the fecudity of the hermaphrodite snail B. alexandrina. This was followed by histological studies on the hermaphrodite gland of treated snails.

MATERIAL AND METHODS

The snails used in the present study , B. alexandrina were collected from Abou-Rawash, Giza Governorate, Egypt the snails were examined for narural infection with trematode parasites. Negative snails were maintained in a glass aquaria, at room temprature.

All snails were maintained in dechlorinated water uneder standard conditions (photoperiod 16 L / 8 D, water temperature 24-25 C° and PH 7+O.4, Chu et al., 1966 b ; 10 snalils per one litre) . Snails were fed fresh and dried lettuce leaves added daily.

Laboratory snails were initiated by collecting fresh egg masses laid by wild snails on thin polyethylene sheets or a small pieces of foam placed on the surface of the aquaria (Oliver et al., 1962). The polyethlene and foam containing egg masses were removed from the aquaria to beakers (250 cc) containing dechlorinated tap water and fragments of chicken egg-schell as a calcium supplement since the local supply of water is soft (Thornhill et al., 1986). They were examined daily and emerging snsils were trnsfered with a fine brush, few days after hatching, to special rearing aquaria where they were kept until used for studying proposes.

Effect of infection on snail suvivalness and fecundity

Unifected laboratory-bred B. alexandrina snails were classified according to size of juvenile and adult groups 3 & 5 mm respectively) and both were exposed to various numbers of miracidia. The level of miracidial infection were , 4-6 and 8 miracidia for each snail in each experimental group. Survival rates and number of egg masses laid within each snail group were recorded twice a week over a period of eight weeks. A control uninfected group of snails of both sizes were inspected in parallel terms of both paramters.

Effect of SAN 415 Strain of Bacillus thuringiensis on the survivalness and fecundity of snails

The tested commercial bacterial pathogen insecticide Bacillus thuringiensis Berliner was used as SAN 415 strain (32000 I.U. / mg.) A test solution was made up with dchlorinated tap water, PH 7.5-7.7. Stock solution of the experimental material was made on basis of weight volum (O.4 mg / L.) The effect of tested material was investigated for infected and uninfedted adult snails. The tested solution was changed weekly . Control snails maintained under the same experinental conditions were used.

The survival rate and number of egg-masses laid by the snails were recorded daily. Observations were extenede over a period of 21 days to determine the effect of prolonged exposure to SAN 415, on the oogenesis and spermatogeesis of B. alexandrina snails. At the end of experimental periods, the ovotestis was dissected out,

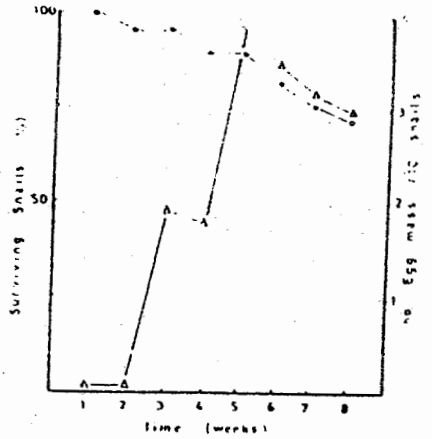
fixed in Bouin,s fluid, embedded in paraffin wax and sectioned at 5um. Sections were stained with haematoxylin and eosin and inspected and photographed by light microscopy.

RESULTS

A) Survivalness and fecundity of laboratory - bred snails

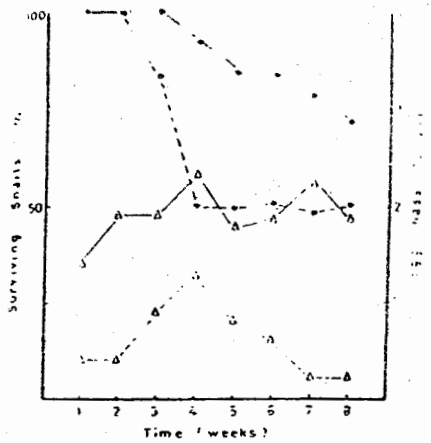
The survival rate and fecundity of sexually - mature, adult snails initiated and bred under laboratory conditions, were assessed by determing the number of dead snails , the number of egg masses and the number of eggs in each, twice a week for a period of eight weeks. Data collected from three groups, each consisted of fourty size and age - matched B. alexandrina reared separately and maintained under identical conditions, are given in (Fig. 1), Within the limits of the experimental setting , survival of snails maintained a fairly high rate and yet expressed a slow decline reaching a minimum of 70 % by the end of the experimental period. Over the same observation period, the total reproductive out put of fourty snails was 66 egg masses containing 607 eggs (92± 1.5 eggs / egg mass). The number of egg masses produced per 10 snaols per week (Fig. 1) showed, after an initial unproductive period of two weeks, a gradual increase with time, reaching a peak by fifth week.

Examination of histolgical sections of ovotestes, excised and fixed from about 10 snails at weekly intervals, indicated that the organ is composed of a number of intact acini supported by connec-



(Figure 1)

Srvival (°) and egg mass production / 10 snails / week (Δ) of normal, mature B. alexandrina. Each point in the cuves is the mean value of three separate series of experiments with SD 5.



(Figure 2)

Survival of 4-6 miracidia infedted (ø) and 8 miracidia-infected (ø) and egg mass production / 10 snails / week of 4-6 miracidia- infected (-Δ-) and 8 miracidia- infected (..Δ..) mature B. alexandina. Each point in the cuves is the mean value of three separate series of experiments with SD 2.5

tive tissue elements (Fig. 4). In average, each acinus is about 215 um in diameter, lined with germinal epithelium and cxhibits the various developmental stages of male and female sex cells along with the corresponding supporting cells. Male spermtogonia, primary and secondary spermatocytes, spermatids and sperm were generally located in clusters occupying a central position within an acinus (Fig. 5). Scattered along the periphery of the acinus, on the other hand, oogonia, primary and secondary oocytes along with mature ova surrounded by follicular cells, were located. Reflecting the data on egg production in (Fig. 1) the histological appearance of the ovotestis was indicative of active spermatogenesis as well as oogenesis within the group of snails observed.

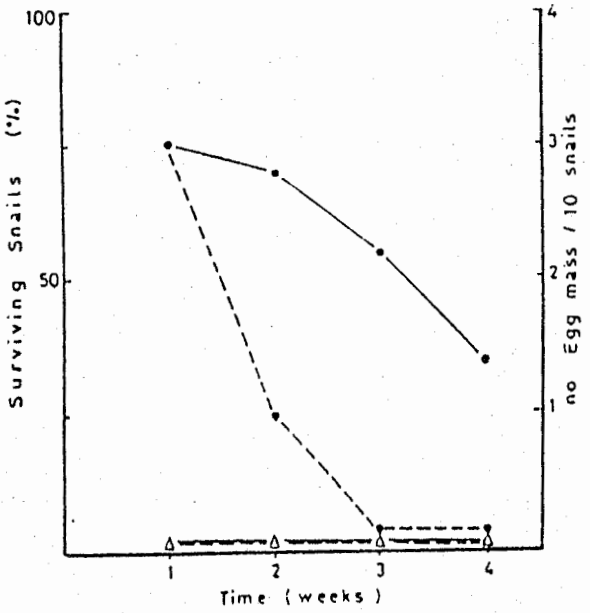
B) Suvivalness and fecundity of experimentally - infected snails

Groups consisting of fourty age-and size-matched snails were reared speparately, either infected by 4-6 miracida / snail or 8 miracidia/ snail after onset of maturity and inspected for survival rates and fecundity measures over an observation peroid and laboratory conditions identical to groups of uninfected, otherwise matched snails.

As depicted on (Fig 2), and as compared to (Fig. 1), it was evident that the effect of infection of suvivalness and oviposition was dependent on the number of miracidia to which an individual snail was exposed, Although infections with 4-6 mivacidia did vot

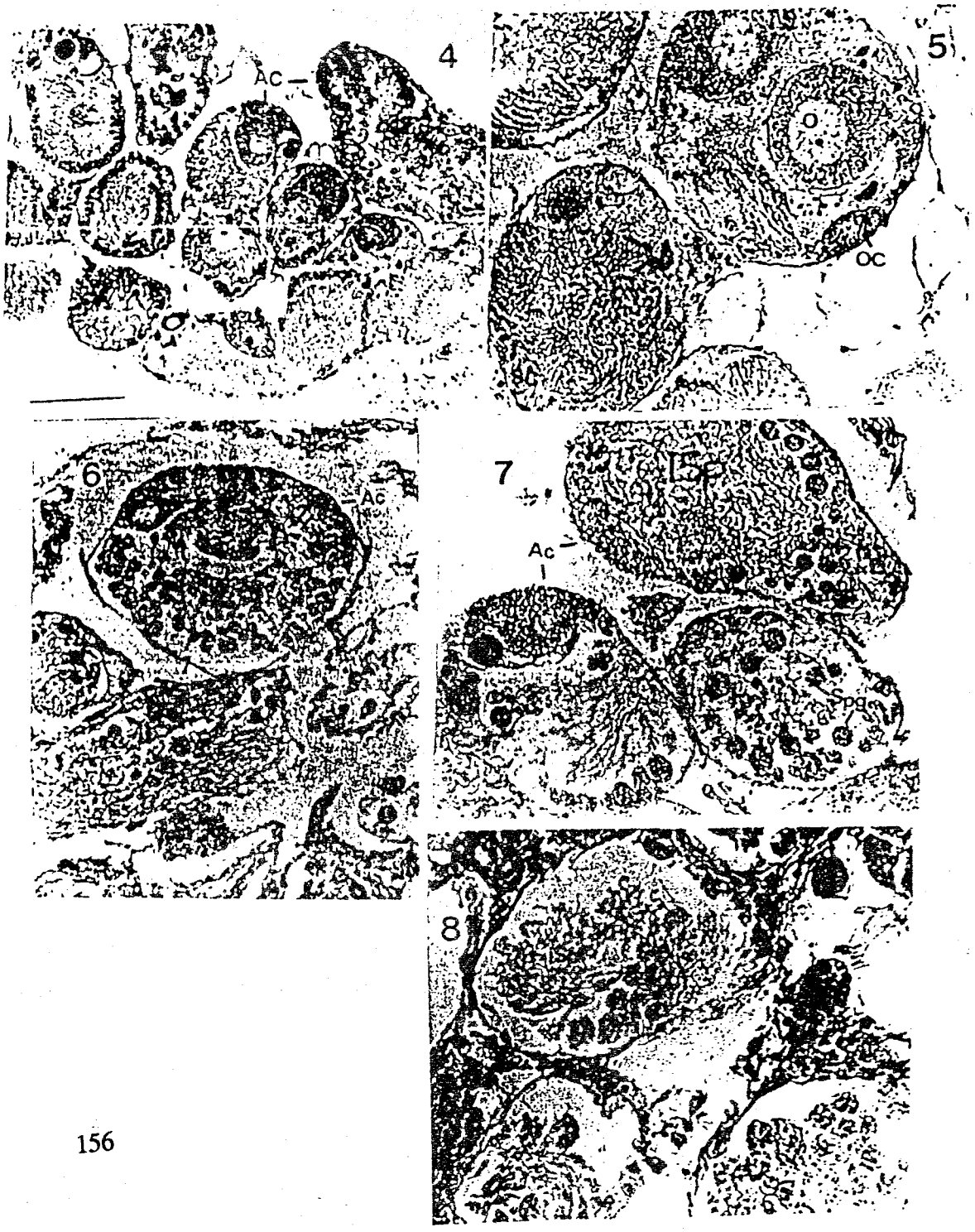
alter significantly the suvival rate of snails, infection by 8 miracidia per snail led to the death of about 50 % of snails by four weeks postionfections, yet with no further mortalities recorded thereafter. Over the same observations, period, the total reproductive out put per fourty snails infected with 4-6 miracidia / snail and 8 miracidia / snail was 61 egg masses containing 616 eggs (10.1 ± 1.2 eggs / egg mass) and 19 egg masses containing 116 eggs (6.1 ± 1.3 eggs / egg mass), respectively. It is noteworthy that while among snails exposed to 4-6 miracidia the number of eggs produced per 10 snails per week (Fig. 2) was maintained more or less constant throught the inspection period, the same numbers varied considerably among snalils exposed to 8 miracidia. In addition to the marked redcution in the overall prodcutivity among snails exposed to 8 mracidia, egg prodution showed a gradual increase during the first four weeks post - infection and there after declined shraply and almost disappeared by the end of the observation period.

In a direct correlation to the above observation while ovitestest of snails exposed to 4-6 meracidia were essentially similar to uninfected snails (Fig. 4 &5), the same organ in snails exposed to 8 miracidia was characterized histlolgically by the presence of a considerable number of degenerated acini (Fig. 6). Apperantaly, intact acini of reduced diameter (173. 13 ± 20.13 um: 10 snails inspected still however, persisted, and these exhibited signs of marked reduction in sperm production as well as complete inhibition of oogenesis, which was represented by few scattered degenerative oocytes.



(Figure 3)

Survival of SAN 415 treated , uninfected (ø) and 4-6 miracidia-infected (..ø..) mature B. alexandrina. Also shown is the egg mass production / snail / week of SAN 415 treated, uninfected (-Δ-) and 4-6 miracidia - infected (..Δ..) snails. Each point in the curves is the mean value of three seperate series of experiments with SD 4.5.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EXPLANTION OF FIGRUES

Figure 4 & 5

Light micrograph of an eosin - haematoxlin- stained normal ovotestis of mature snails showing acini (Ac) of normal size (4, x250) and active spermatogenesis and oogenesis (5, x400). S, sperm; Sc, spermartocyte; O, ovum; Oc, oocyte; F, follecular cells , Bar = 200 um.

Figure 6

Light micrograph of an eosin - haematoxlin - stained ovotestis of 8 miracidia- infected, mature snails showing acini (AC) of rduced size, lack of oogensis and markedly reduced spermatogenesis. Spg, stages of spermatogenesis. x400.

Figre 7 & 8

Light micrograph of eosin - haematoxlin - stained ovotestes of SAN 415 - treated , uninfected (7) and 4-6 miracidia - infected (8), mature snails showing acini (Ac) of reduced size, lack of oogensis and abrogation of spermatogenesis. Isp, irregrlar sperm; Spg, stages of spermatogenesis. x400.

C) Survivalness and fecundity of infected and SAN 415 treated snails

Snails treated with SAN 415 and then exposed to 4-6 miracidia / snail, since by the first week about 75 % of snails survived these treatments and thereafter started to decline, so that by the third week no survivors were observed (Fig. 3), On the other hand, a slower decline in survival rates was observed among snails treated with SAN 415 and left uninfected, since after an initial 25 % mortalities in the first week, additional 40 % mortalities were recorded by the end of four weeks.

At any given time of the observation period , snails treated with SAN 415 exhibited no oviposition activities, and thus was true in either uninfected or in snails infected with 4-6 miracidia / snail. Inspected histologically, ovotestes of uninfected snails or snails exposed to 4-6 miracidia / snail equally expressed signs of degneration. In both snail grorps, the acini were of reduce diameter (142.8± 18.2 um, 10 snails inspected or totally disrupted, spermatogenesis represented by clusters of spermatozoa whereas other stages were less prominent and ooginesis totally inhibited and represented by denucleated oocytes of unedfined shape and reduced size (Fig. 7 & 8).

DISCUSSTION

Considering several aspects of snail physiology as growth, fecundity and mortality, data based on field population have long suf-

fered uncertainties due to seasonal fluctuations, random infections as well as variations in climatic factors (Anderson et al;, 1982 ; woolhouse, 1989). In the present study , these variations have been rendered largly uneffective in populations of laboratory bred snails, which were initiated and maingained according to standard procedures (Thornhill et al ., 1986) Results recorded here regarding survival rates, egg-laying capacity as well as the integrity of the reprocuctive organs in uninfected mature B. alexandrina are in accordance with abservation reported for B. glabrata (Minchella, 1985), B. pfeifferi (Mrkanga, 1981), Lymnaea catascopium (Loker, 1979) and Lymnaea stagnalis (Sluiters et al ., 1980) reared under comparable conditions. Interestingly, S. mansoni infections seemed to be well tolerated by this population of laboratory - conditioned B. alexandrina. since over 70 % of snails originally exposed to 4-6 miracidia each, were still alive eight weeks post exposure. Increasing the exposure dosage from 4-6 to 8 miracidia per snail, subsequently decreased survivorship. yet still 50 % of the snails were survived throughout the same observation period. In relation to this observation, several investigators have reported that Schistosome infections adversely affect the survival of the molluscan host (Chu et al ., 1966 a; Sturrock, 1970; Lo, 1972; Meuliman, 1972; Mohamed and Ishak 1982), although others observed increased survival in infected snails (MsClelland and Bourns, 1969).These conflicting observations are directly dependent upon variations in the host-parasite combination studied and may prove to be linked to specific host genetic counter adaptation mechanisms to parasitism (Minchella,

1985).

Another physiological measure that is repeatedly reported to be modulated by Schistosome infection, is fecundity. In respones to infection, sharp reduction and eventual cessation of egg production among infected snails in one hand, has been a widely accepted observation (Meuleman, 1972; Baudoin , 1975; Mohamed and El Fiki, 1980). On the other hand, there has been evidence of a significant increase in egg-laying in either mature or immature infected snails (Minchella, 1985; Thornhill et al., 1986). Two brust of egg-laying immmedately following parasite exposure has been interpreted as a mlluscan adaptation in respones to trematode parasitism, and has been termed fecundity compensation (Minchella and Loverde, 1981). AN observation that could be correlated to this adaptation phenomenon was obtained in the present study. Mature snails infected with 4-6 miracidia seemed , in terms of fecudity measures to tolerate such infection and produced an overall number of egg masses and eggs per egg mass comparable to uninfected controls , yet without a pronounced burst in egg-laying at any given time post infection. Increasing the miracidial load, both egg production activity as well as the integrity of the ovotestis were markedly affected, suggesting that fecundity in B. alexandrina may after all be inversely proportional to the number of infecting miracidia as observed earlier with other related moullusks (Pan, 1965; Loker, 1979; Makanga, 1981; Crews and Each, 1986)

In addition to S. mansoni miracidia which represent (natural)

burdens on snail survivalness and fecundity, the effect of a sublethal concentration of a SAN 415 strain of the bacterium B. thuringiensis on theses parameters was investigated. Although previous reports screening natural bacterial pathogens among moribund snails have ruled out the presence of such bacterium (Cheng, 1986), its adverse effect on survivalness was remininscent of heavy natural infection. Interestingly treatment of either uninfected or infected snails have also resulted in complete abrogation of egg proeduction and yielded what seamed to be a state of castration. Total cessation of egg laying as early as two days posttreatment was paralleled by elevated sings of atrophy in the ovotestis, where retaradtion of spermatogensis and a complete absence of oogenesis was apparent. To our knowledge snail castration has been always attributed to infection by direct mechanical bolckage of neutrient transport due to the pressure exerted on gonadal tubules by invasive larval stages (James, 1965), or indirectly via the active secretion of cytolytic chemicals of hormones by the developing larvae (Chen 1983), In this study, the observation that no sporocyts were detected in the gonadal tissue of infected snails, farvous the suggestion that in B. alexandrina, gonadal elements may be selectively -susceptible targets to chemical modulations. In uninfected snails , these modulations seemed to be mimced in resposne to sublethal dose of Bacillus treatments indicating that possibly other toxic compounds may have similar effects . Although these suggestions demand futher investigations, our findings may have important implications in the biological control of B. alexandrina.

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تأثير البكتريا باسلس ثيرينجينسس سلالة سان٤١٥ والعدوى بالبلهارسيا على بقاء وخصوبة قواقع بيومفلاريا الكسندرينا

جمالات يوسف عثمان محمد أحمد مصطفى

هدى ابراهيم نجم عزة حسن محمد

قسم علم الحيوان - كلية العلوم -

جامعة المنوفية - شبين الكوم

تم دراسة تأثير كل من البكتيريا باسلس ثيرنجينسس سلاله سان ٤١٥ والعدوى بميراسيديا الشستوسوما مانسونى علي معدل بقاء وخصوبة السلالة المعملية لقوقع بيومفلاريا الكسندرينا أوضحت النتائج مايلى:

أن كل من معدل البقاء على الحياة وكفاءة إنتاج البيض (والتى حددت بمعدل وضع القوقع لكتل البيض وعدد البيض فى كتلة البيض الواحدة) وكذا دراسة الفحص النسيجى للمنسل الخنثوى للقوقع تتأثر بعدوى القوقع بميراسيديا الشستوسوما والتأثير هنا يتناسب تناسباً عكسياً مع كل من عدد الميراسيديا والمعاملة بالتركيز تحت المميت من بكتريا باسلس ثيرنجينسس سلالة سان ٤١٥.