Biological Control

Viability of *Nezara viridula* (L.) Eggs for Parasitism by *Trissolcus basalis* (Woll.), under Different Storage Techniques in Liquid Nitrogen

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Viabilidade de Ovos de *Nezara viridula* (L.) ao Parasitismo por *Trissolcus basalis* (Woll.), sob Diferentes Técnicas de Armazenamento em Nitrogênio Líquido

RESUMO - Diferentes técnicas de conservação de ovos foram avaliadas, sob condições de baixa temperatura, visando a multiplicação do parasitóide de ovos de percevejos *Trissolcus basalis* (Woll.) (Hymenoptera: Scelionidae). Massas de ovos frescos de *Nezara viridula* (L.) foram armazenadas, por 12 meses, em nitrogênio líquido (-196^{0}C), embaladas em papel alumínio, em tubos de microcentrífuga ("eppendorf") e em sacos plásticos duplos vedados a vácuo (500 ovos/técnica/mês). A cada 30 dias, 10 massas de ovos, conservadas sob as diferentes modalidades, foram retiradas, descongeladas imediatamente e submetidas ao parasitismo por *T. basalis*. Ovos do percevejo verde, estocados a -196^{0}C, apresentaram condições de conservação e viabilidade para o desenvolvimento de *T. basalis*, constatando-se elevados índices de parasitismo e emergência nos ovos armazenados sob as três diferentes técnicas. Os ovos conservados em papel alumínio (95,4%), em tubos (92,3%) ou a vácuo (93,7%) apresentaram parasitismo estatísticamente igual aquele verificado em ovos frescos (97,3%). A viabilidade dos ovos, expressa pela taxa de emergência dos adultos do parasitóide, indica que as três técnicas estudadas proporcionaram excelentes condições de desenvolvimento e emergência de *T. basalis*, não havendo diferenças significativas quando comparadas a ovos frescos. Ao longo do período total de armazenamento (12 meses), não houve redução no número de fêmeas geradas por postura, embora tenham ocorrido flutuações na razão sexual, para alguns períodos. Os resultados obtidos viabilizam a conservação dos ovos do percevejo verde, e posterior multiplicação do parasitóide *T.basalis*, por um período duas vezes mais longo ao que hoje é conhecido e utilizado.

PALAVRAS-CHAVE: Insecta, controle biológico, conservação de ovos de percevejos, baixa temperatura, parasitóide.

ABSTRACT - Different egg conservation techniques were assessed under low temperature conditions to multiply the parasitoid of stink bug eggs *Trissolcus basalis* (Woll.) (Hymenoptera:Scelionidae). Masses of fresh *Nezara viridula* (L.) eggs were stored for 12 months in liquid nitrogen (-196ºC), wrapped in aluminum foil, in microcentrifuge tubes ("eppendorf") or in vacuum sealed double plastic bags (500 eggs/technique/month). Every 30 days, 10 egg masses from each of the different storage techniques, were taken out, thawed immedi-

ately and subjected to parasitism by *T. basalis*. Green stink bug eggs, stored at -196ºC presented conservation conditions and viability for *T. basalis* development, with high parasitism rates and emergence in the eggs stored under the three different techniques. Parasitism of eggs stored wrapped in aluminum foil (95.4%), in tubes (92.3%), and under vacuum (93.7%) were statistically equal to parasitism in fresh eggs (97.3%). Egg viability, expressed by the rate of emergence of adult parasitoids, indicates that the three techniques studied provided excellent *T. basalis* development and emergence conditions with no significant differences when compared with fresh eggs. Throughout the total storage period (12 months) there was no reduction in the number of females hatched, although there were fluctuations in the sexual ratio for some periods. These results make green stink bug egg conservation, and later multiplication of the parasitoid *T. basalis* possible for a period twice as long as that known and used today.

KEY WORDS: Insect, biological control, stink bug egg conservation, low temperature, parasitoid.

In biological control programs which involve the release of a great number of parasites, it is extremely important to preserve the eggs of the hosts for prolonged periods to make better use of the production from a preestablished colony, and to have great quantities of beneficial agents available when field release is necessary.

Egg parasitoids can successfully develop in host eggs that have undergone freezing or heating (Wajnberg & Hassan 1994). Promising results for lepidopterous egg storage for later *Trichogramma* spp. multiplication and use in biological control studies have been reported in India (Dass & Ram 1983) and in China (Huai-Yi 1988, Morrison 1988, Zhenwei & Qiyao 1988).

Eggs from various Heteroptera species can be stored at low temperatures and still be parasited by Scelionidae species (Orr 1988). For stink bug eggs, Powell & Shepard (1982) found that *Trissolcus basalis* (Woll.) emergence was not reduced in *Nezara viridula* (L.) eggs stored in a freezer and they could be stored successfully for periods up to seven months. Similar results were obtained by Corrêa-Ferreira & Moscardi (1993) with eggs from this stink bug which, when wrapped in aluminum foil, were viable for *T. basalis* parasitism, and by Korneeva *et al.* (1981), cited by Orr (1988), for storage of *Dendrolinus pini* (L.) eggs parasited by *Telenomus tetratonus* Thomson. Popov (1974), cited by Orr (1988), found that the T*rissolcus grandis* (Thompson) species did not prefer fresh eggs of several Pentatomidae species in relation to eggs storaged at -20°C for more than 270 days, although the researcher found a decrease in the parasitism rate in eggs stored for periods longer than 180 days.

Low temperature storage of already parasited eggs or of adult parasites has also been studied, although the results obtained by Gautam (1986) at 10°C showed periods of up to a week without effect on the parasiting efficiency by *Telenomus remus* Nixon, when stored for 6-7 days after parasiting. Noble (1937) stored *N. viridula* eggs parasited by *T. basalis* for 18 days at this temperature. Adults developed and emerged only from eggs stored with larvae at the first stage, which were considered by Kamal (1937) as the most tolerant forms to refrigeration. Similar results were obtained by B.S. Corrêa-Ferreira (unpublished data) for the same host and parasitoid, with a greater adult emergence rate when the egg storage took place in the first phases of parasitoid development.

Studies of stink bug egg storage in liquid nitrogen were carried out by Genduzo (1979). This author demonstrated the viability of storing eggs from several Heteroptera species for periods of up to three years and the parasitism by *Gryon* and *Trissolcus* species were not affected. The usefulness of this method was confirmed by Gennadiev & Khlistovskii, cited by Orr (1988) also for parasitism by *Trissolcus simoni* (Mayr) in *Graphosoma lineatum* (L.) eggs stored for five years. M.L. Costa & F.G.V. Schmidt (personal communication), using the same method, obtained viability in *N. viridula* eggs after six months storage at -196°C with 83.3% parasitism and 79.0% *T. basalis* adult emergence, however, results for longer conservation periods were not mentioned.

As the production and conservation of host eggs is still a limiting factor in soybean stink bug biological control programs, different storage techniques in liquid nitrogen were assessed to allow a greater storage period and thus a better use of the *N. viridula* egg production for later multiplication and massive release of *T. basalis* in soybean fields.

Material and Methods

Three storage techniques were compared under laboratory conditions for *N. viridula* egg viability for parasitism by *T. basalis*, after different storage periods, at low temperatures. The eggs were collected daily from a host insect colony, set up in the laboratory following the methodology described by Corrêa-Ferreira (1985), and kept in liquid nitrogen at -196°C for 12 months, under different types of packaging: eggs wrapped in aluminum foil; eggs placed in microcentrifuge tubes ("eppendorf"), and eggs placed in vacuum sealed double plastic bags.

Approximately 500 non-parasited eggs / technique/month were used to assess the egg storage techniques. Every 30 days, ten egg masses stored under the different methods were taken out and thawed by immediate immersion in 30°C water for five seconds, and subjected to parasitism by *T. basalis*. Comparison with parasitism in *N. viridula* fresh eggs was used as a control. Parasitism of the stored eggs was assessed during 12 months, using glass tubes (8.0 x 2.5 cm) and two-day-old females, previously mated and fed on honey. Each group of ten egg masses was subjected to ten *T. basalis* females, for 24 hours. After this period, the egg masses were individualized in petri dishes with moist filter paper, and kept in incubators, under controlled conditions of temperature (25±2°C), relative humidity (65±10%) and photophase (14h), until emergence and death of the parasitoids. The parasited eggs were later counted and the number of male and females emerged from each egg mass recorded.

The egg viability was assessed monthly for the parasitism rate, adult emergence and sexual ratio. A parasited egg was considered to be any whose adult completed development. All the adults which emerged, plus those which had some perforation in the parasited egg chorion were used to calculate the emergence percentage. The sexual ratio was calculated by the formula SR= females / males + females. Any egg without emergence was later dissected to confirm its contents.

The experiment layout of the treatments was a factorial completely randomized design, with ten replications. The normality of the errors, model design, constancy of variances for the treatments and error dispersion were assessed for the requirements of the analysis of variance by the W statistic test (Shapiro & Wilk 1965), F of the additivity model (Tukey 1949), Q for the homogeneity of variances (Burr & Foster 1972) and the error test (R.C.P. Parente, unpublished) and the means were compared by the Tukey test to 5% probability.

Results and Discussion

Fresh *N. viridula* eggs stored at -196°C for 12 months presented conservation condition and viability for *T. basalis* parasitoid development. Parasitism rates and emergence of the adults from the eggs stored under the different tested techniques were generally high

(> 90%) (Table 1).

Eggs wrapped in aluminum foil had mean parasitism percentages ranging from 89.3% to 99.8%, statistically equal to parasitism recorded in fresh green stink bug eggs, for every month. Similar results were obtained when the eggs were stored in microcentrifuge tubes, eggs submited to parasitism in a 12 month storage period (Table 1). These results show that liquid nitrogen storage conditions corrected distortions found up to now in previous studies on *T. minutum* (Lund 1934, Van Stenberg 1934), with *T. grandis* (Popov 1974 cited by Orr 1988) and with *T. basalis* (Corrêa-

Table 1. Viability of *Nezara viridula* eggs for parasitism by *Trissolcus basalis*, under different storage techniques[1], during 12 months, in liquid nitrogen (-196ºC).

Techniques[2]	Viability (X ± SE)[3]		
	Parasitism (%)	Emergence (%)	Sexual Ratio[4]
Control	97.3 ± 0.89	98.5 ± 0.41	0.82 ± 0.01
Aluminum	95.4 ± 1.01	97.4 ± 0.71	0.72 ± 0.03
Tube	92.3 ± 2.57	97.9 ± 0.50	0.73 ± 0.04
Vacuum	93.7 ± 1.07	98.2 ± 0.42	0.74 ± 0.02
C. V. (%)	5.51	1.90	12.29

[1]N= 500 eggs / months / technique.
[2]Control= fresh eggs; Aluminum= eggs wrapped in aluminum foil; Tube= eggs placed in microcentrifuge tubes, Vacuum= eggs placed in vacuum sealed double plastic bags.
[3]No significant differences were found when the different techniques were compared by Tukey test $P< 0.05$.
[4]Sexual Ratio= Females/ Males + Females.

which had a mean parasitism rate of 92.3%, during a year. Although the results had significantly different values from the control for some storage periods (3, 6, 7 and 10 months) with the exception of seven months, all the others had parasitism higher than 88% of the eggs offered.

Storing eggs in vacuum sealed double plastic bags also resulted in high mean rates of parasitism, ranging from 86.2% to 99.5%. No reduction was found in egg parasitism for this technique, nor for the others, as storage time at -196°C increased.

The viability of eggs stored at low temperatures as expressed by the emergence rate of *T. basalis* adults showed that the three tested techniques provided ideal conditions for development and emergence of the parasitoids, and no significant differences were found among the eggs stored at -196°C and fresh

Ferreira & Moscardi 1993), where these authors found a decrease in parasitoid emergence for periods greater than 180 days storage, probably due to the increased desiccation of the host eggs. Under nitrogen, stink bug egg storage in aluminum foil, besides being a very practical technique, gave parasitism (94.2%) and adult emergence (94.6%) higher than 83.3% and 79.0% respectively, obtained by M.L.Costa & F.G.V. Schmidt (personal communication) for storage for six months in double plastic bags at the same temperatures.

Throughout the total storage period (12 months) no sharp reduction in the number of females from egg mass was found with increased egg storage time for the different techniques compared to offspring from fresh eggs (Table 1). Normally the *T. basalis* offspring , hatching originating in fresh *N. viridula* eggs,

had, in absolute values, mean sex ratios higher than those from eggs stored at low temperatures, although real differences were only found for some storage periods and in certain techniques, varying from 0.58 to 0.91 in the 12 month period.

Although packing eggs in aluminum foil has been used under freezer conditions by the *T. basalis* multiplication laboratories, development and emergence obtained in liquid nitrogen in 12 months storage were twice as great as those obtained in the freezer. Throughout the storage time, high mean rates were found which were always greater than 90% for parasitism and emergence, which were not statistically different from the control (Fig 1). There was, however, a greater variation in the sexual ratio of the generation developed in these eggs, with values statistically different from the control for the 5, 6, 8 and 10 month periods. There was no reduction in the proportion of females generated with increased storage time, but fluctuations throughout the period, differences which may be explained by the influence of other factors which are known to affect the sexual ratio of egg parasitoids.

However, there have been advances in Brazil in *N. viridula* egg conservation methodology, with viability for multiplication of the *T. basalis* parasitoid, the recommended storage methodology of the biological control program (Corrêa-Ferreira 1993) did not allowed the use of the egg production for a greater storage time than six months, whether in a freezer (Corrêa-Ferreira & Moscard 1993) or in liquid nitrogen (M.L. Costa & F.G.V. Schmidt, personal communication).The lack of alternative for egg storage has hindered the use of the stink bug egg production in periods when these insects are most abundant in the field, at the end of the soybean harvest (March-April). This use can now be viabilized with the results obtained in liquid nitrogen for 12 months, providing egg conservation for double the period, and the possibility of releasing a greater quantity of *T. basalis* in soybean fields in the following year.

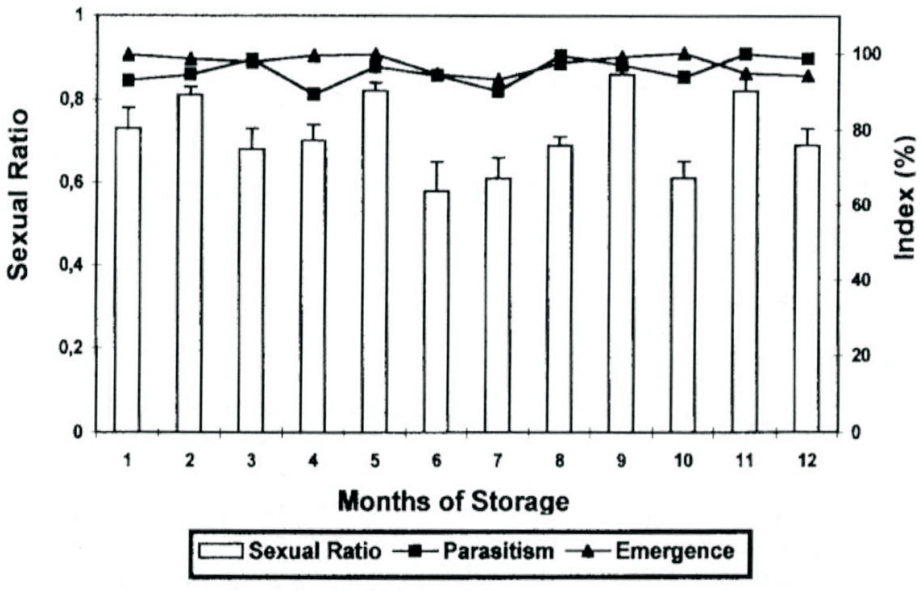

Figure 1. Parasitism, emergence and sexual ratio of *Trissolcus basalis* developed in stink bug eggs stored in aluminum foil by 12 months in liquid nitrogen (-196ºC).

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References Cited

Burr, I.W. & L.A. Foster, 1972. A test for equality of variances. Mimeo series 282. University of Purdue, West Lafayette, 26 p.

Corrêa-Ferreira, B.S. 1985. Criação massal do percevejo-verde *Nezara viridula* (L.). Londrina, Embrapa-CNPSo, Documentos 11, 16 p.

Corrêa-Ferreira, B.S. 1993. Utilização do parasitóide de ovos *Trissolcus basalis* (Wollaston) no controle de percevejos da soja. Londrina, Embrapa-CNPSo, Cir. Téc. 11, 40 p.

Corrêa-Ferreira, B.S. & F. Moscardi. 1993. Técnicas de armazenamento de ovos do percevejo-verde visando à multiplicação do parasitóide *Trissolcus basalis* (Wollaston). Pesq. Agropec. Bras. 28: 1247-1253.

Dass, R. & A. Ram. 1983. Effect of frozen eggs of *Corcyra cephalonica* Staiton (Pyralidae: Lepidoptera) on parasitism by *Trichogramma exiguum* (Pinto & Platner) (Trichogrammatidae: Hymenoptera). Indian J. Entomol. 45: 345-347.

Gautam, R.D. 1986. Effect of cold storage on the adult parasitoid *Telenomus remus* Nixon (Scelionidae: Hymenoptera) and the parasitized eggs of *Spodoptera litura* (Fabr.) (Noctuidae: Lepidoptera). J. Entomol. Res. 10: 125-131.

Genduso, P. 1979. Crioconservazione di uova di eterotti in azoto liquido per l'allevamento di parasitoidi oofagi. Atti Cong. Naz. Ital. Entomol. 11: 365-370.

Huai-Yi, M. 1988. Studies on long-term storage of hosts for propagating *Trichogramma*. *Trichogramma* and other egg parasites International Symposium, 2. Les Colloques de l'INRA 43: 369-371.

Kamal, M. 1937. The cotton green bug, *Nezara viridula* L., and its important egg-parasite, *Microphanurus megacephalus* (Ashmead), (Hymenoptera: Proctotrupidae). Bull. Soc. Entomol. d'Egypte 21: 175-207.

Lund, H.O. 1934. Some temperature and humidity relations of two races of *Trichogramma minutum* Riley (Hymenoptera:Chalcididae). Ann. Entomol. Soc. Am. 27:324-340.

Morrison, R.K. 1988. Methods for the long-term storage and utilization of eggs of *Sitotroga cerealella* (Olivier) for production of *Trichoramma pretiosum* Riley. *Trichogramma* and other egg parasites, International Symposium, 2.Les Colloques de l'INRA 43: 373-377.

Noble, N.S. 1937. An egg parasite of the green vegetable bug. Agric. Gaz. New South Wales 3094: 337-341.

Orr, D.B. 1988. Scelionid wasps as biological control agents: a review. Fla. Entomol. 71: 506-527.

Powell, J.E. & M. Shepard. 1982. Biology of australian and United States strains of *Trissolcus basalis*, a parasitoid of the green vegetable bug *Nezara viridula*. Australian J. Ecol. 7: 181-186.

Shapiro, S.S. & M.B. Wilk . 1965. An analysis of variance test for normality. Biometrika 52: 591-611.

Tukey, J.W. 1949. One degree of freedom for non-additivity. Biometrics 5: 232-242.

Van Stenburg, W.E. 1934. *Trichogramma minutum* Riley as a parasite of the oriental fruit moth (*Laspeyresia molesta* Busck.) in Ontario. Can J. Res. 10: 287-314.

Wajnberg, E. & S.A. Hassan. 1994. Biological control with egg parasitoids. IOBC, CAB International, 286pp.

Zhenwei, H. & X. Qiyao. 1988. Studies on frozen storage of eggs of rice moth and oak silkworm. *Trichogramma* and other egg parasites, International Symposium, 2.Les Colloques de l'INRA 43: 327-338.

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