# EFFECT OF BACTERIA ON THE BIOLOGY OF DIAMONDBACK MOTH (*Plutella xylostella*) ON CABBAGE (*Brassica oleraceae* VAR. *capitata*) CV. MIDORI

FLÁVIO HENRIQUE VASCONCELOS DE MEDEIROS
GIL SILVA
ROSA DE LIMA RAMOS MARIANO
REGINALDO BARROS

*Universidade Federal Rural de Pernambuco, Departamento de Agronomia Rua Dom Manuel de Medeiros, S/N, 52171900, Recife, PE, Brazil, Phone/Fax: 55 81 33021211 / 33021205, E-mail: flaviohvmedeiros@bol.com.br*

---

RESUMO

**EFEITO DE BACTÉRIAS SOBRE A BIOLOGIA DA TRAÇA DAS CRUCÍFERAS (*Plutella xylostella*) EM REPOLHO (*Brassica oleraceae* VAR. *capitata*) CV. MIDORI**

A traça das crucíferas (DBM) *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) é a praga mais importante do repolho no Brasil, podendo ser controlada pelo uso de químicos e *Bacillus thuringiensis*, individualmente ou combinados em programa de manejo integrado. Bactérias promotoras de crescimento de plantas (PGPR) podem ser uma importante nova alternativa para o controle biológico de *P. xylostella*. Este trabalho objetivou estudar os efeitos de PGPR na biologia de DBM em repolho durante dois períodos de plantio Maio a Julho de 2000 (estação chuvosa) e Fevereiro a Maio de 2001 (estação seca). Sementes de repolho cv. Midori foram imersas durante 30 min em suspensões bacterianas a $A_{580}$ = 0,52 correspondendo a $10^8$ unidades formadoras de colônias/mL, secas durante 12 h, semeadas em bandejas de poliestireno e transplantadas para 3 parcelas no campo em delineamento inteiramente casualizado. Em diferentes dias após o transplantio (DAT) folhas de cada tratamento foram coletadas ao acaso e levadas ao laboratório, onde discos de 8cm de diâmetro foram cortados e colocados em placas de Petri sobre papel de filtro. Dez larvas de DBM 1$^{st}$ instar, criadas em laboratório foram então colocadas para se alimentar sobre cada disco de folha. Após três dias, os discos foram trocados diariamente até a formação da pupa. Foram observadas mortalidade larval (LM) e viabilidade pupal (PV), duração larval (LD) e pupal (LP. Em ambos experimentos LM e PV foram significativamente elevadas e reduzidas pelos isolados ENF14 (*Enterobacter cloacae*), EN5 (*Alcaligenes piechaudii*) e EN4 (*Kluyvera ascorbata*). LM atingiu 60% comparada com 1,7% na testemunha. Na estação chuvosa, a proteção foi verificada entre 63 a 74

DAT. Na estação seca, o efeito foi menor e antecipado para o período entre 45 a 60 DAT.

**Termos para indexação**: controle biológico, bactérias promotoras de crescimento de plantas, *Plutella xylostella*

ABSTRACT

**EFFECT OF BACTERIA ON THE BIOLOGY OF DIAMONDBACK MOTH (*Plutella xylostella*) ON CABBAGE (*Brassica oleraceae* VAR. *capitata*) CV. MIDORI**

The diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is the most important cabbage pest in Brazil. Its control includes the use of chemicals and *Bacillus thuringiensis*, individually or combined in an integrated pest management program. Plant growth-promoting rhizobacteria (PGPR) may be an important and new alternative in biological control of *P. xylostella*. This work aimed to study the effects of PGPR on the biology of DBM on cabbage through two different periods May to July 2000 (raining season) and February to May 2001 (dry season). Cabbage seeds cv. Midori were immersed for 30 min in a $A_{580}$ = 0.52 or $10^8$ CFU/ml bacterial suspensions, dried overnight, planted in polystyrene trays and transplanted field to three plots in a randomized design. At different days after transplanting (DAT) leaves collected randomly were brought to the laboratory and 8cm Ø-discs were placed in a Petri dish over filter paper. Ten 1st instar DBM larvae grown in laboratory conditions were placed to feed on each leaf disc. Beginning on the third day, leaf disks were changed daily until pupation. It was analyzed larval mortality (LM) and pupal viability (PV), larval (LD) and pupal (LP) duration. In both experiments LM and PV were significantly increased and reduced by strains ENF14 (*Enterobacter cloacae*), EN5 (*Alcaligenes piechaudii*) and EN4 (*Kluyvera ascorbata*). Values for LM reached 60% compared with 1.7% in control. In the raining season the protection was observed from 63 to 74 DAT. In the dry season the effect was reduced and anticipated to the period from 45 to 60 DAT.

**Index terms**: biological control, plant growth-promoting rhizobacteria, *Plutella xylostella*.

## 1. Introduction

The cabbage (*Brassica oleraceae* var. *capitata* (L.) is among the most economically important vegetables belonging to the cruciferous family (Silva Júnior, 1987).

Among the factors that limit cabbage production worldwide (Adams *et al.*, 1990), the diamondback moth *Plutella xylostella* (L.) is the one that causes higher yield losses, which are explained by the high percent of injured heads (Barros *et al.*, 1993), what renders cabbage production unsustained, unless integrated management strategies are employed.

To date, chemical products are the most largely employed method to manage this pest (Castello Branco & Guimarães, 1990), however it implies in high costs and ecosystem misbalance from larger usage. Furthermore, works reported the selection pressure exerted by insecticides (Campos *et al.*, 1997) and by the enthomopatogenous *Bacillus thuringiensis* (Castello Branco & Gatehouse, 1997), which results in the increasing number of resistant populations.

Hence, new control alternatives for diamondback moth have been searched. One of them is the induction of systemic acquired resistance, mediated by jasmonic acid, which is launched after caterpillar feeding or pathogenesis and has been considered one of the advances in pest control (Felton & Korth, 2000). Plant growth-promoting rhizobacteria (PGPR) can also mediate induction of systemic resistance (ISR), being important for the biocontrol of pests (Zehnder *et al.*, 1997) and diseases (Mariano *et al.*, 1998). Zehnder *et al.* (1997) demonstrated the efficacy of PGPR in the control of cucumber wilt, caused by *Erwinia tracheiphila*. Treated plants showed lower levels of cucurbitacin which reduced feeding of the vectors: *Diabrotica undecimpunctata hawardii* (Barber) and *Accalymma vittatum* (F.) and thus, decreased the wilt incidence and beetle populations during the season. Tomczyk (1999) also observed that PGPR reduced susceptibility of cucumber to *Tetranychus cinnabarinus* (Boisd).

The presented work aimed to verify the potential of plant growth-promoting rhizobacteria in the biological control of *P. xylostella* in cabbage cv. Midori during the plant life cycle.

## 2. Material and Methods

This works was carried out in the Núcleo de Resistência de Plantas a Insetos of the Departamento de Agronomia at the Universidade Federal Rural de Pernambuco (UFRPE). The laboratorial conditions were temperature 27ºC±1, relative humidity 60%±5 and photophase of 12 h.

A total of two experiments were performed, one in a raining season, from May to June 2000 (experiment 1) and the other in a dry season, from February to April

2001 (experiment 2). Temperatures for the two seasons were 25±5 ºC and 27±5.5 ºC and average monthly rainfall 518.4 mm and 186.8 mm, respectively.

Three strains of PGPR, previously tested (Silva *et al.*, 1999) ENF14 (*Enterobacter cloacae* (Jordan) Hormaeche & Edwards), EN4 (*Kluyvera ascorbata* Farmer *et al.*) and, EN5 (*Alcaligenes piechaudii* Kiredjian *et al.*) were used. In the experiment 1, it was also used strain E8 (not identified), all obtained from the Collection of the Laboratório de Fitobacteriologia - UFRPE. In the lab, strains were grown in medium nutrient yeast dextrose agar (NYDA) for 36h, when suspensions were prepared in sterile distilled water (SDW) containing 0,05 % of Tween 80 to obtain a concentration of $A_{580}$ = 0,52 or $10^8$ CFU/ml, adjusted in photocolorimeter. The suspensions were used to bacterize seeds of cabbage cv. Midori, by immerging them for 30 min, rinsing and letting dry out over night under lab conditions. Seeds were sowed in polystyrene trays containing a mix of commercial substrate Plantmax® and sterile soil (1:1).

After 22 to 31 days, when most plants displayed 4 to 7 leaves, seedlings were transplanted to 11 x 1m plots, located in the Departamento de Agronomia - UFRPE and distributed in a 0,5 x 0,5m design. To avoid cross contamination in the extremities of plots and in between treatments, boarder plants were placed but not included in evaluations. Plants were distributed in three randomized plots, fertilized with matured manure at 5 Kg/$m^2$ 10 days before transplant and manual weed control was performed as needed. Evaluations of treatments' efficacy were performed in the Núcleo de Resistência de Plantas a Insetos at 30, 45, 60, 75 and 90 days after transplant (DAT). In each evaluation, leaves were collected and 8cm-disks were transferred to 9cm-Petri dishes, over an 8cm-filter paper. Ten 1$^{st}$ instar caterpillars of *P. xylostella* (obtained from the referred Núcleo) were placed on the adaxial leaf surface of each disk. Beginning on the third day, leaf disks were changed daily until pupation. The obtained pupae were placed individually in Elisa®-plate-wells and daily observed for emergence of adults. The variables analyzed were larval mortality (LM), determined as the percent of caterpillars that did not originate pupae compared to the initial larval population; pupal viability (PV), percent of pupae that originated adults compared to the initial number of pupae; duration of larval (LD) and pupal (PD) cycles, determined as the timeframe from eclosion to pupation and from pupation to adult emergence, respectively. Data were submitted to variance analysis and means compared by the Tukey test (P=0,05) using SANEST®.

## 3. Results and Discussion

In experiment 1, differences were observed for LM and PV. At 63 DAT strains EN5, ENF14, EN4 and E8 augmented larval mortality (60, 60, 35 and 26.7%) compared to the control (1.67%) while at 74 DAT only strain EN5 (33.3%) differed from the control (10%) (Figure 1A). At 31 DAT pupal viability was significantly reduced compared to the control (100%) by strains E8 (72.1%) and EN5 (62.4%), while at 63 DAT strains EN4 (77,7%) and E8 (77,3%) produced the same reducing effect (Figure 1B).

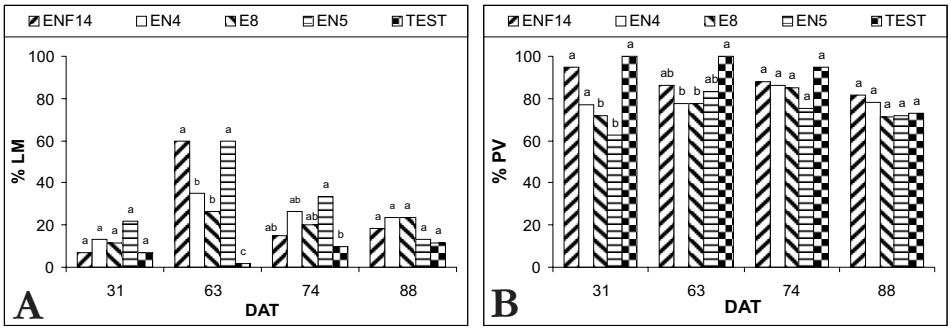


**Figure 1. —** Effect of bacteria on larval mortality (LM) (A) and pupal viability (PV) (B) of *Plutella xylostella* at 31, 63, 74 and 88 days after transplanting (DAT) of seedlings obtained from seeds bacterized by immersion for 30 min in suspensions of ENF14 (*Enterobacter cloacae*), EN5 (*Alcaligenes piechaudii*), EN4 (*Kluyvera ascorbata*) and E8 (not identified). Experiment performed in May-July 2000 (raining season).

In experiment 2, differences were also observed for larval mortality and pupal viability. At 45 DAT strain EN4 augmented (28.3 %) *P. xylostella* larval mortality compared to control (10 %) (Figure 2A) while at 60 DAT ENF14 significantly reduced the pupal viability (90 %) compared to the control (100 %) (Figure 2B). Significant differences were not observed for the variables LD and PD (data not presented).

The protective effect was not observed before 45 DAT. Similarly, Wei *et al.* (1991) only obtained control of *Colletotricum orbiculare* (Berk.& Mont.) Arx. at 27 days after sowing, using cucumber seeds treated with *Pseudomonas aureofaciens* Kluyver strain 25-33. Mahafee & Kloepper (1997) studying colonization of cucumber by *Enterobacter* sp., observed that the timeframe necessary for the bacterium to produce any host modification is an indicative the induction of systemic resistance (ISR). Another element that attests for ISR is the large spectrum of protection, obtained in

plants bacterized with *K. ascorbata* EN4. Besides the protection against diamondback moth, this strain has been reported to protect cabbage from black rot infections, caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson (Assis *et al.*, 1998). Furthermore, the peak of action of tested bacteria, occurred from 40 to 60 days, a period where plants are more susceptible to *P. xylostella* (Silva Jr., 1987). This will ensure a suitable integrated pest management under field conditions.

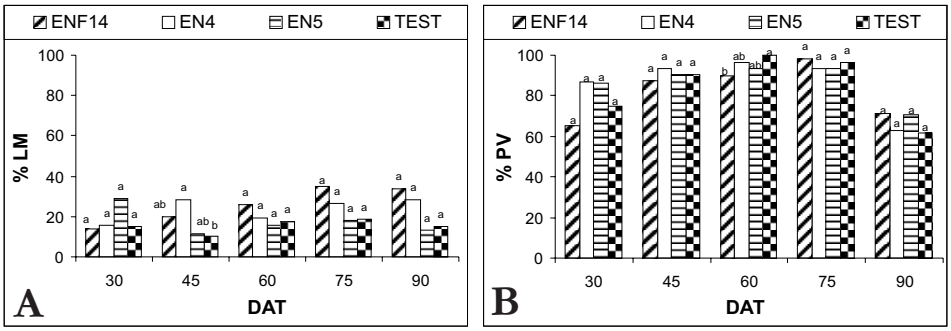


**Figure 2. —** Effect of bacteria on larval mortality (ML) (A) and pupal viability (VP) (B) of *Plutella xylostella* at 30, 45, 60, 75 and 90 days after transplanting (DAT) of seedlings obtained from seeds bacterized by immersion for 30 min in suspensions of ENF14 (*Enterobacter cloacae*), EN5 (*Alcaligenes piechaudii*) and EN4 (*Kluyvera ascorbata*). Experiment performed in February-May 2001 (dry season).

The differences in the efficiency of PGPR seed treatments between experiments and the insect mortality observed in the control plants could be attributed to variations in environmental conditions. The low pluvial precipitation (186.8 mm) in the dry period (compared to 518.4 mm in the raining season), may have negatively affected colonization and multiplication of PGPR in the host, lessening but not ceasing their beneficial effect. According to Nowak (1998), bacterized plants become more resistant to environmental stresses, due to morphological and physiological changes in the host.

The data obtained in this work suggests that specificity is not an important trait to be observed in selecting bacterial strains for biological control of *P. xylostella*, since absence of specificity was observed in ENF14, isolated from seeds of bean, and specificity was observed for strain EN4, obtained from cabbage leaves.

The use of PGPR is suitable and recommended for an integrated pest management and can be combined with resistant cultivars (Dickson *et al.*, 1990),

sterility of F1 generation (Bloem & Carpenter, 2001) and cultural control (Tabashnick *et al.*, 1986; McHugh *et al.*, 1995) for improving control of *P. xylostella*.

Complementary studies are required in order to determine the mechanisms involved in the biocontrol of *P. xylostella*, from the wide range of possibilities that may be exerted from PGPR treatments reported (Mariano & Kloepper, 2000).

## 4. Aknowlegments

The authors would like to thank the Fundação de Amparo a Ciência e Tecnologia do Estado de Pernambuco - FACEPE and the Conselho Nacional de Desenvolvimento Científico - CNPq for financial support. They also would like to thank the Instituto Nacional de Meteorologia for temperature and precipitation data.

## 5. References

ADAMS, A.J., HALL, F.R. & HOY, C.W. Evaluating resistance to permethrin in *Plutella xylostella* (Lepidoptera: Plutellidae) populations using uniformly sized droplets. Journal of Economic Entomology 83:1211-1215. 1990.

ASSIS, S.M.P., SILVEIRA, E.B., MARIANO, R.L.R. & MENEZES, D. Bactérias endofíticas: método de isolamento e potencial antagônico no controle da podridão negra do repolho. Summa Phytopathologica 24:216-220. 1998.

BARROS, R., ALBERT JR, I.B., OLIVEIRA, J.A., SOUZA, A.C.F. & LOGES, V. Controle químico da traça das crucíferas *Plutella xylostella* (Lepidoptera: Plutellidae) em repolho. Anais da Sociedade Entomológica do Brasil 22:463-469. 1993.

BLOEM, S. & CARPENTER, J.E. Evaluation of population suppression by irradiated Lepidoptera and their progeny. Fla. Entomology 84:165-170. 2001.

CAMPOS, L.C.A., CASTELLO BRANCO, M. & JUNQUEIRA, A.M.R. Susceptibilidade de três populações de traça das crucíferas a *Bacillus thuringiensis*. Horticultura Brasileira 15:40-42. 1997.

CASTELLO BRANCO, M. & GATEHOUSE, A.G. Insecticide resistance in *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) in the Federal District, Brazil. Anais da Sociedade Entomológica do Brasil 26:75-79. 1997.

CASTELLO BRANCO, M. & GUIMARÃES, A.L. Controle da traça das crucíferas em repolho. Horticultura Brasileira 10:24-25. 1990.

DICKSON, M.H., SHELTON, A.M., EIGENBRODE, S.D., VAMOSY, L.M. & MORA, M. Selection for resistance to diamondback moth (*Plutella xylostella*) in cabbage. HortScience 25:1643-1646. 1990.

FELTON, G.W. & KORTH, K.L. Trade-offs between pathogen and herbivore resistance. Current Opinion in Plant Biology 3:309-314. 2000.

MAHAFEE, W.F. & KLOEPPER, J.W. Bacterial communities of the rhizosphere and endorhiza associated with field-grown cucumber plants inoculated with a plant growth-promoting rhizobacterium or its genetically modified derivative. Canadian Journal of Microbiology 43:344-353. 1997.

MARIANO, R.L.R., SILVEIRA, E.B. & ASSIS, S.M.P. Indução de resistência sistêmica mediada por rizobactérias promotoras de crescimento de plantas. In: Garcia, E.S., Rabinovitch, L. & Tigano, M.S. (Eds.) Proceedings, VI Simpósio de Controle Biológico – SINCONBIOL, Rio de Janeiro, RJ. 1998. pp.345-360.

MARIANO, R.L.R. & KLOEPPER, J.W. Método alternativo de biocontrole: resistência sistêmica induzida por rizobacterias. Revisão Anual de Patologia de Plantas 8:128-137. 2000.

MCHUGH JR., J.J. & FOSTER, R.E. Reduction of diamondback moth (Lepidoptera: Plutellidae) infestation by overhead irrigation. Journal of Economic Entomology 88:162-168. 1995.

NOWAK, J. Benefits of *in vitro* "biotization" of plant tissue cultures with microbial inoculants. In Vitro Cellular Development Biology 34:122-130. 1998.

SILVA JR., A.A. Repolho: fitologia, fitotecnia, tecnologia alimentar e mercadologia. 1st ed. Florianópolis. EMPASC. 1987.

SILVA, G., ARAÚJO, D.V., COSTA, V.S.O., MARIANO, R.L.R., BARROS, R. & GOMES, A.M.A. Potencial de bactérias endofíticas para controle biológico da traça das crucíferas (*Plutella xylostella* L., 1758) (Lepidoptera: Plutellidae) em repolho. In: Mota, R.A., Faustino, M.A.G. & Almeida, M.G.A. (Eds.). Proceedings, IX Congresso de Iniciação Científica, Recife, PE. 1999. p.70

TABASHNICK, B.E. & MAU, R.F.L. Suppression of diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) oviposition by overhead irrigation. Journal of Economic Entomology 79:189-191. 1986.

TOMCZYK, A. The use of plant growth promoting rhizobacteria (PGPR) to decrease the susceptibility of cucumber to spider mites. Integrated Control in Glasshouses IOBC Bulletin 22:251-254. 1999.

WEI, G., KLOEPPER, J.W. & TUZUN, S. Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria. Phytopathology 81:1508-1512. 1991.

ZEHNDER, G., KLOEPPER, J., CHANGBIN, Y. & WEI, G. Induction of systemic resistance in cucumber against cucumber beetles (Coleoptera: Crysomelidae) by Plant-Growth-Promoting-Rhizobacteria. Journal of Economic Entomology 90:391-396. 1997.