한국농약과학회지 제2권 제2호(1998)
The Korean Journal of Pesticide Science
Vol. 2, No. 2, pp.113~118(1998)

Field evaluation of conidia of *Beauveria bassiana* (Balsamo) Vuillemin strain CS-1 against diamondback moth larvae

Cheol-Sik Yoon, Tae Joong Yoon[1], Hyun-Soo Park, Sang-Guei Lee, Jai-Ki Yoo and Jeang-Oon Lee*

Department of Crop Protection, National Institute of Agricultural Science and Technology, RDA, Suwon 441-707, Korea, and [1]Department of Agricultural Biology, College of Natural Resource, Korea University, Seoul 137-074, Korea

Abstract : The efficacy of conidia of *Beauveria bassiana* (Balsamo) Vuillemin strain CS-1 that showed high mortality in laboratory and net house was examined against diamondback moth larvae in the field. Conidia (4×10^7 conidia/ml) were applied to larvae on chinese cabbage in a 1.5% emulsifiable oil-emulsion amended with 4% clay at a volume of 0.55 L/3.3 m^2. There were no significant differences among plants and replicates in the deposition of spray droplets on water-sensitive papers or of conidia on leaves and larvae. Weather conditions were rainy and cool during first few days, and then hot and dry. Persistence of conidia was equally short on both leaves and larvae. Nevertheless, treatment of *B. bassiana* potentially reduced larval populations. But the field efficacy was lower than the laboratory efficacy. This reduced efficacy was ascribed to unfavorable environmental conditions. (Received June 13, 1998, accepted July 30, 1998)

Key words : *Beauveria bassiana* strain CS-1, field efficacy, diamondback moth.

Introduction

The diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Yponomeutidae) has become one of the most important insect pests of cruciferous plants in Korea due to the development of its resistance to the bacterial insecticide *Bacillus thuringiensis* as well as to various type of synthetic insecticides (Jones and Jones, 1981; Hama, 1992; Talekar and Shelton, 1993).

Increasing concern over adverse effects of these insecticides have brought the need for the development of new types of biological control agents including entomopathogenic fungi. Accordingly, much attempts are being made in many countries to isolate and select the most virulent indigenous fungal isolate for development of microbial control agent against DBM larvae.

Previous study (Lee *et al.*, 1997) indicated that the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin CS-1, isolated from silk worm larvae, showed potential for the management of DBM larvae in laboratory and net house trials.

In their study, corrected mortalities at 1×10^8 conidia/ml were 86.23% and 66.5% under laboratory and net house conditions, respectively. Because the virulence of a given *B. bassiana* isolate varies depending on environmental conditions (Fargues, 1972; Hall and Papierok, 1982), these mortalities observed under laboratory and net house conditions may not indicate field efficacy. In order to develop this strain into a practical DBM biocontrol agent, its field efficacy should be evaluated. The present study was designed to examine the efficacy and viability of the *B. bassiana* strain CS-1 as a function of temperature, moisture and UV radiation in a field trial.

Materials and Methods

Insect and inoculum preparation

Second instar DBM larvae were collected at an

* Corresponding author

insecticide-untreated chinese cabbage farm located at Wonju city. Conidia of *B. bassiana* strain CS-1 were produced on the Sabouraud dextrose agar plates supplemented with 1% yeast extract. Conidia were suspended in a 1.5% (v/v) emulsifiable soybean oil (Sigma) amended with 4% montmollinonite (w/v) for a target spore concentration of 4 x 10^7 conidia/ml.

Experimental field

The field experiment was conducted at experimental field of National Institute of Agricultural Science and Technology (NIAST) located at Suwon. The experimental field was consisted of two blocks (3 x 6 m) for a treatment of the strain CS-1 conidia and a carrier control. Two blocks were arranged at a distance of approximately 50 m. Each block was composed of three replicate plots (0.5 x 6 m) separated by a distance of 0.5 m. About 20 chinese cabbage plants were raised in each plot. Ten second instar DBM larvae were released to each plant.

Field application

Conidial suspension (4 x 10^7 conidia/ml) was sprayed at a rate of 0.55 L/3.3 m^2 with a sprayer on the late afternoon of 23 September 1997. The block of a carrier control was sprayed with a suspension of the same composition omitting conidia. To examine differences among replicates in the deposition of spray droplets, water-sensitive papers (TeeJet Spraying Systems Co.) were placed on the soil surface between plants in the block of a treatment. The number of living larvae was counted on 10 plants randomly chosen in each plot immediately after, and 1, 3, and 6 days post-application. Mean hourly total solar radiation (300-2800 nm), temperature, and relative humidity were recorded at the NIAST weather station during experiment period.

Conidial persistence on leaves

Three chinese cabbage leaves were collected immediately after, and 1, 3, and 6 days post-application from each of the three replicate plots, and transported to the laboratory in plastic bags on ice. Each leaf was cut into three pieces with a cork borer (5 mm diameter), and pieces were vortex-mixed together with 5 ml of 0.01 M buffer-Tween (Inglis et al., 1997). Suspensions were diluted, and, three aliquots (0.2 ml each) were taken and spread on three replicate oatmeal-dodine agar plates (Inglis *et al.*, 1993). Number of colonies on each of the nine plates/leaf was counted after 7 days' incubation at 25℃; representative colonies were examined microscopically to confirm the identity of *B. bassiana*. The mean numbers were compared among three replicate plots, and treatment mean numbers, calculated from replicative means, were subjected to regression analysis.

Conidial populations on larvae

Five larvae per replicate were collected in an Eppendorf tube immediately after, and 1, 3, and 6 days post-application. After weighing larvae in an Eppendorf tube, they were homogenized with a sterile glass rod in 0.5 ml of 0.01 M buffer-Tween, and vortex-mixed for 30 seconds. Larval suspensions were diluted, and three aliquots (0.2 ml each) were taken and spread on three replicate oatmeal-dodine agar plates. Colony-forming units (cfu) of *B. bassiana* were enumerated at 25℃. The mean cfu populations were calculated per mg to account for variation in larval size. A method for data analysis was followed as described above.

Results

Application rates and weather conditions

There were no significant differences among replicate plots in the deposition of spray droplets because water-sensitive papers were evenly stained to blue color. The deposition of conidia was not significantly different among leaves and larvae which were randomly sampled from each replicate plot immediately after application. From leaves and larvae, 8.065 x 10^3 (SD = 3.219 x 10^3) cfu/19.625 mm^2 and 1.330 x 10^3 (SD = 0.525 x 10^3) cfu/mg were recovered, respectively. Standard deviation of

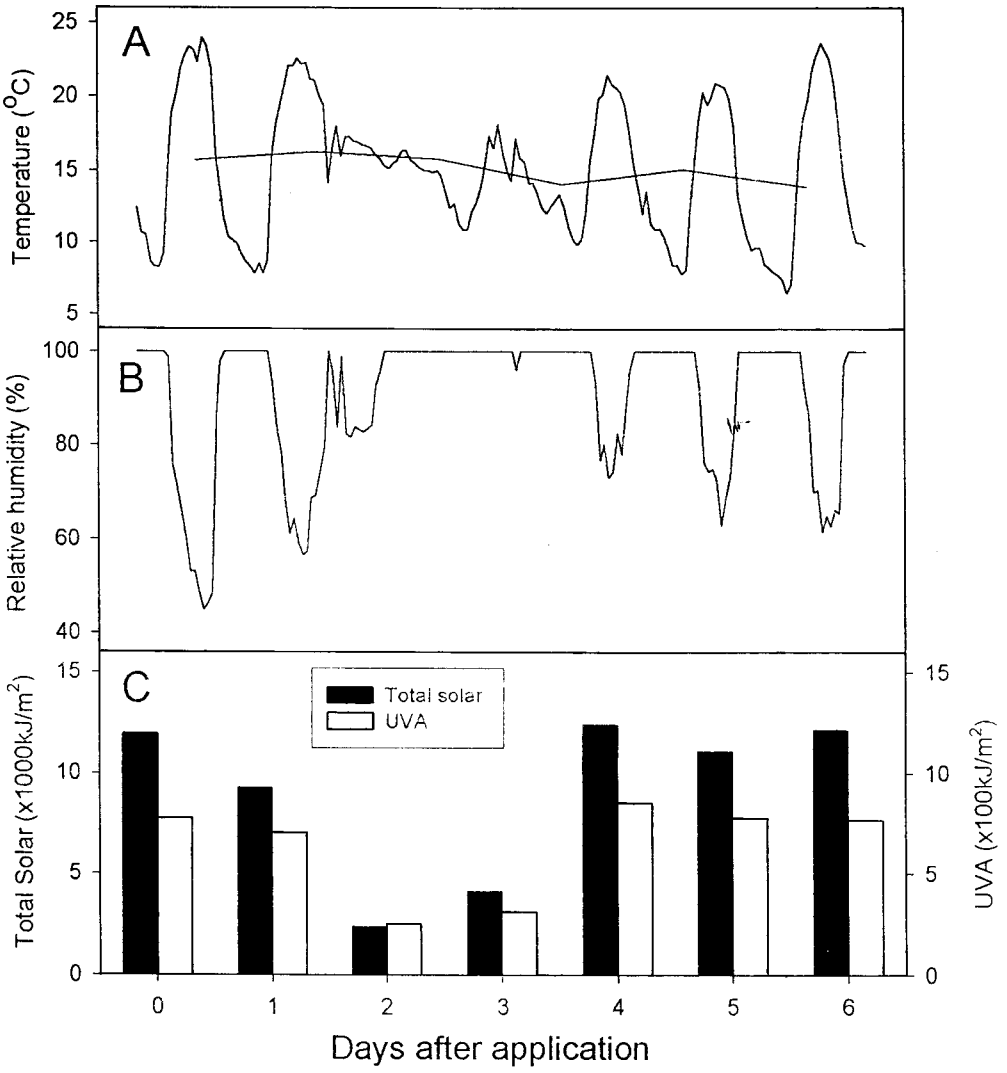

Fig. 1. Weather during the field experiment. Hourly and mean temperature (A), relative humidity (B), and total daily solar radiation (C) were recorded at the NIAST weather station.

the former was relatively higher than the later due to difficulty in equal application to all leaves. In general, weather conditions were unfavorable for fungal infections due to rainy weathers on 2 and 3 days after application (Fig. 1). After rain, dry and hot weathers were followed.

Conidial persistence

Figure 2 shows conidial survival on leaves and larvae immediately after, and 1, 3, and 6 days post-application. Conidial survival on leaves and larvae declined significantly over the four sampling dates. In Duncan's multiple range test, conidial persistence on leaves exhibited significant differences among four sampling dates (PROC GLM; $F = 67.62$, $df = 3$, $P \leq 0.05$). Conidial survival on larvae was significantly reduced on the second sampling date but small differences were observed thereafter (PROC GLM; $F = 23.66$, $df = 3$, $P \leq 0.05$).

Field efficacy

At the time immediately after application, population densities ranged from 6 to 10 larvae per plant in the blocks for a treatment of the conidia and a carrier control. As shown in Fig. 3, corrected mortality was gradually accumulated up to 54.37% until 6 days after application,

and LT_{50} was 5.37 days. This mortality was lower than that in the previous laboratory study (Lee *et al.*, 1997) which exhibited 86.23% mortality with LT_{50} of 1.63 days. Nevertheless, this results indicated that *B. bassiana* strain CS-1 has capability to lower the DBM populations even under unfavorable field conditions.

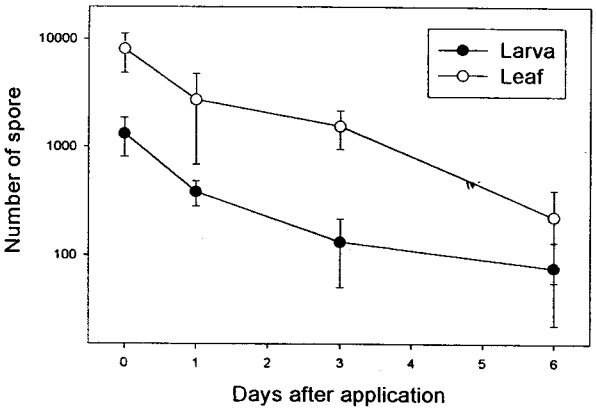

Fig. 2. Persistence of conidia of *Beauveria bassiana* CS-1 on leaves and larvae. The mean cfu populations on leaves and larvae were calculated per 19.625 mm^2 and per mg, respectively. Vertical lines represent standard deviation of means.

Discussion

The data presented in the present study indicated that *B. bassiana* CS-1 was potentially able to suppress the DBM larval population even though the efficacy was lower than that in the previous laboratory experiment (Lee *et al.*, 1997). The lower field efficacy of entomopathogenic fungi is generally ascribed to various factors including poor viability of conidia, reduced virulence, slow germ tube formation, and combinations thereof (Inglis *et al.*, 1997). We examined the activity of conidia of *B. bassiana* strain CS-1 in field environment, and compared virulence observed under field conditions with that in the previous laboratory study (Lee *et al.*, 1997). This study determined whether virulence difference in field and laboratory trials could be explained by aspects of the pathogen - DBM larvae - environment interaction.

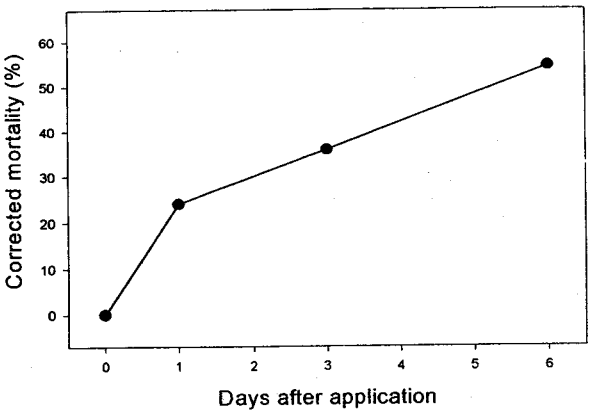

Fig. 3. Corrected mortality of DBM larvae after treatment of 4 x 10^7 conidia/ml suspension of *B. bassiana* CS-1 in field trial. Vertical lines represent standard deviation of means.

The ability of conidia of *B. bassiana* to be persistent against environmental conditions, and germinate rapidly and synchronously is considered an important aspect of the infection process (Perkula and Grula, 1979). For mycosis to occur, conidia of *B. bassiana* must be deposited on larvae in a suitable environment. After application, weather conditions were rainy and cool for a few days, and then hot and dry until the end of experiment. During first few days, relative humidity reached to almost 100% due to rainy weathers. Although there may be some beneficial effect of relative humidity on infection of insect by entomopathogenic fungi (Bateman *et al.*, 1993), infection by *B. bassiana* readily occurs at low humidities (Marcandier and Khachatourians, 1987). This aspect may be one of reasons for reduced efficacy of the *B. bassiana* CS-1. Another possibility is that conidia germination was inhibited by low temperature during experiment. Although temperature requirements may vary between species, and also at the intraspecific level, most entomopathogenic deuteromycetes show optimal germination *in vitro* at around 25℃. Thus, providing optimal temperature is an important factor to increase germination rate and consequently

virulence (Quedraogo, 1993). During rainy days, temperatures fluctuated, ranging from 12℃ to 19℃. Consequently germ tube formation, growth and enzymatic activities of the *B. bassiana* CS-1 were delayed (Hajek and St. leger, 1994). Combination of these factors or alone possibly contributed to slow and low infectivity of the fungus, explaining why *B. bassiana* CS-1 in field was less virulent than in laboratory.

In addition, less virulence in field seems to be stemmed from application of lower conidial concentration (4×10^7 conidia/ml) than that in the laboratory study (Lee *et al.*, 1997), reduced viability of conidia on leaves or larvae, and combination thereof. A threshold of inoculum is required to cause sufficient infections (Inglis *et al.*, 1997). But this study employed less than half of the concentration (1×10^8 conidia/ml) which showed 86.23% mortality at the laboratory experiment (Lee *et al.*, 1997). This aspect suggested that application of more concentrated conidial suspension could cause higher mortality.

We observed the similar deposition of spray droplets, and recovered populations from leaves and larvae immediately after application. However, quantities of viable conidia of *B. bassiana* CS-1 that we recovered from leaves and larvae at later sampling dates would not normally be sufficient to cause mycosis. For example, conidial populations on larvae were reduced by approximately 71% on the second sampling date. This result was similar to that on leaves, suggesting that UV-A radiation limits conidial survival on leaves and larvae. This assumes that most of the conidia on nymphs remained exposed to sunlight until rainy days. As a result, conidia did not initiate sufficient infections due to harmful effect of UV-A radiation.

In conclusion, the *B. bassiana* CS-1 that we tested was potentially efficacious against DBM larval population despite lower efficacy compared to laboratory experiment (Lee *et al.*, 1997). This reduced efficacy suggested that environmental conditions and the quantity of inoculum were responsible for the lower efficacy of *B. bassiana* CS-1 in field. In particular, this study indicated that a major obstacle to the utilization of this fungus for DBM larvae control will be its requirement for optimal moisture and temperature conditions, and reduced UV-A radiation for a substantial part of each day. Through additional studies designed to gain a more thorough understanding of *B. bassiana* dynamics, it may be possible to design application/introduction strategies integrated with cultural management practices to maximize expression of the inherent epizootics potential of this pathogen.

Literature cited

Bateman, R. P., M. Carey, D. Moore, and C. Prior (1993) The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. Annals of Appl. Biol. 122:145～152.

Hajek, A. E. and R. J. St. leger (1994) Interactions between fungal pathogens and insect hosts. Annu. Rev. Entomol. 39:293～322.

Hama, H. (1992) Insecticide resistant characteristics of the diamondback moth. pp.455～463, In Management of Diamondback Moth and Other Crucifer Pests (ed. N. S. Talekar), Proc. of the Second International Workshop. Shunhua, Taiwan: Asian Vegetable Research and Development Center.

Inglis, G. D., M. S. Goettel, and D. L. Johnson (1993) Persistence of the entomopathogenic fungus, *Beauveria bassiana*, on phylloplanes of crested wheatgrass and alfalfa. Bio. Cont. 3:258～270.

Inglis, G. D., D. L. Johnson, and M. S. Goettel (1997) Field and laboratory evaluation of two conidial batches of *Beauveria bassiana* (Balsamo) Vuillemin against grasshoppers. Can. Entomol. 129:171～186.

Jones, F. G. W. and M. G. Jones (1981) Pest of Field Crops. 3rd ed. pp.82～83, Edward Arnold Ltd, London.

Lee, J.-O, C.-S. Yoon, J.-K. Yoo, Y.-W. Lee, S.-G. Lee, K.-H. Moon, and G.-H. Sung (1997). *Beauveria bassiana* strain CS-1 isolated from domestic silkworm larvae and its utilization for DBM larvae control. Republic of Korea Patent 51035.

Marcandier, S., and G. G. Khachatourians (1987)

Susceptibility of the migratory grasshopper, *Melanoplus sanguinipes* (Fab.) (Orthoptera: Acrididae), to *Beauveria bassiana* (Bals.) Vuillemin (Hyphomycetes): Influence of relative humidity. Can. Entomol. 119:901~907.

Pekrul, S. and E. A. Grula (1979) Mode of infection of the corn earworm (*Heliothis zea*) by *Beauveria bassiana* as revealed by scanning electron microscopy. J. Invert. Pathol. 34:238~247.

Quedraogo, R. M. (1993) Investigations on the Use of the Fungus, *Beauveria bassiana* (Hyphomycetes: Moniliales) for Control of the Senegales Grasshopper, *Oedaleus senegalensis* (Orthoptera: Acrididae). M.P.M. thesis. Simon Fraser University, Burnaby, BC. Canada.

Talekar, N. S., A. M. Shelton (1993) Biology, ecology, and management of the diamondback moth. Annu. Rev. Entomol. 38:275~301.

포장에서 배추좀나방에 대한 백강균 CS-1의 방제효과

윤철식, 윤태중[1], 박현수, 이상계, 유재기, 이정운* (농업과학기술원 작물보호부, [1]고려대학교 농생물학과)

요 약 : 실내와 망실에서 배추좀나방에 대하여 높은 살충력을 보인 백강균 CS-1의 포장에서의 방제효과를 검정하였다. 1.5% 실험용 대두유, 4% 점토, 4 x 10^7 conidia/ml이 함유된 포자현탄액을 0.55 L/3.3 m^2의 양으로 살포하였으며 살포된 양이 배추간 또는 유충간에 큰 차이 없이 균등하게 살포되었음을 water-sensitive paper 실험으로 확인하였다. 포장실험 중 기상조건은 살포 후 2일째부터 이틀간 비온 후 덥고 건조하였으며 잎과 유충표면에서의 포자 지속성은 주위환경에 의하여 많이 단축됨을 알 수 있었다. 이러한 불리한 환경조건에서도 본 균주는 약 54%의 방제효과를 보여주었다. 따라서 본 실험을 통하여 백강균 CS-1을 이용한 미생물살충제의 개발 가능성을 확인 할 수 있었다

---

* 연락저자