

Entomology

Efficacy of *Beauveria bassiana* Isolate against Pine Shoot Beetle *Tomicus piniperda* (L) (Coleoptera, Scolytidae) in Laboratory

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ABSTRACT. Investigation was carried out in 2009 at three different sites of pine forests of Eastern Georgia. The pine shoot beetles (PSB) *Tomicus piniperda* occurred on *Pinus eldarica* and *Pinus Sosnovskyi* throughout the investigation area. During a survey the entomopathogenic fungus *Beauveria bassiana* (BbTp) was identified and tested for adult beetles of PSB. The experiments showed that the isolated indigenous strain BbTp is of high virulence for the adults *T. piniperda*. The highest infection rate by mycosis and mortality were observed in variants of both concentrations 1×10^6 and 1×10^7 conidia /ml⁻¹, where mortality achieved 81.5 -100 %. © 2010 Bull. Georg. Natl. Acad. Sci.

Key words: *Tomicus piniperda*, *Beauveria bassiana*, bioassay.

The pine shoot beetle (PSB), *Tomicus* (= *Blastophagus*) *piniperda* L (Coleoptera, Scolytidae) is a widely distributed pest of *Pinus*, especially of the Scotch pine (*Pinus sylvestris*) throughout Europe, North Africa and Asia, which frequently infests unbarked pine timber in forests and other timber storage areas and spreads into the surrounding pine forests [1-3]. Pine plantations are used for reforestation of vast areas in Europe and Georgia as well, where it is often the predominant tree species in some forest areas. Environmental stress, such as forest fires, windstorms and defoliating insects, help and create favorable conditions for bark beetles for the breeding and infestation of mature stands and also for pine shoot beetles, which may lead to shoot damage in the surrounding pine forests [4].

Tomicus piniperda was first found in Georgia in the 1940s and its primary host plant is *Pinus sosnovskyi*, but is capable of colonising, developing, and shoot-feeding in other pine species, as Eldari pine (*Pinus eldarica* Medw.), European Black pine (*Pinus nigra*).

PSB may occasionally attack spruce (*Abies* spp.) and larch (*Larix* spp.) as well. Unlike most Scolytidae, bark beetles that feed exclusively on the inner bark of their hosts, PSB has two feeding stages. Adults emerge from over-wintering sites in early spring to seek new breeding material in the form of stumps, fresh logs, and broken limbs. During mass outbreaks, the beetles successfully attack living trees. Attacks are characterized by reddish-brown boring dust and resin holes on the bark surface and near the shoot. Pine shoot beetle completes one generation per year in Georgia [5].

The aim of the present investigation was identification of bioagents in the populations of *T. piniperda* from various locations in Eastern Georgia.

Material and Methods

The survey of *Tomicus piniperda* was carried out in 2009 (May, July, September) at three different sites and altitudes of pine forests - Shavnabada (700 m a.s.l., L=41° 39' 13" N=44° 50' 29") in Lower Kartli Region(1),

Khashuri (950 m a.s.l. L=41° 00' 55" N=43° 35' 21") Middle Kartli Region (2) and Patara Tsemi (1600 m a.s.l. L=41° 44' 12" N=43° 32' 14") Borjomi–Bakuriani gorge (3) in Eastern Georgia.

Adults of PSB were collected from pine trees and by directly taking out of their galleries in the phloem of pilled pieces and the bark with beetles was brought to the laboratory and kept at 15 ±1 °C in a refrigerator. Only living or slowly moving beetles were removed from their galleries every day and were examined first using a stereomicroscope (magnification 8-16-32-56x). Subsequently, the beetles were dissected according to the methods described in Wegensteiner and Weiser [6]. The whole gut, gonads, adipose tissue and haemolymph were inspected using light microscope (magnification 150-600x). After fixation with methanol, the smears were stained with Giemsa's dye and re-inspected in normal light microscope.

An inoculum of fungus obtained from an original isolate of *T.piniperda*. Isolated fungus was cultivated on PDA (Difco) - plates (Ø 90 mm) for 15 days at 25 °C. The fungus was identified by using generally accepted methods in insect fungal pathology [7]. An indigenous isolate tested for adult beetles of PSB.

Healthy beetles were collected by hand from infested trees or log section from pine trees and placed on pine-bark pieces (10x15 cm) treated with a fresh cultural suspension of *B.bassiana* (1.0 x10⁶ and 1.0 x10⁷ conidia/ml⁻¹). The beetles of each variant were placed in a container and incubated at 23 ±3 °C, without light and at 90% RH.

Dead beetles were removed daily and replaced on Petri dishes in moist environment for the conidia to develop.

Results and Discussion

Pine shoot beetles *Tomicus piniperda* occurred on *Pinus eldarica* and *Pinus sosnovskyi* at all investigation sites of pine forests of Eastern Georgia. Emerging beetles, both dark colored parental and pale colored "callow adult" beetles were detected in their gallery. The dates and sites of the collected beetles, mature trees with width, heights and diameters are given in Table 1.

In total, 239 beetles were collected during the survey. Only 95 beetles were dissected individually. Different microorganisms and nematodes were found in PSB.

Single findings of the "callow adults" in the population of *T.piniperda* under bark, with mycosis symptoms (covered with white hyphae) have been detected in the pine plantation of Patara Tsemi district. The identification shows that the original isolate of this fungus was *Beauveria bassiana* and given name BbTp. The efficacy of the isolates of this fungus was examined and their virulence against adults of PSB tested (Table 2).

The experiments showed that isolated indigenous strain BbTp is of high virulence for the adults *T.piniperda*. The highest infection rate by mycosis and mortality was observed in variants of both concentrations 1x10⁶ and 1x10⁷ conidia /ml⁻¹; where mortality reached 81.5 -100 %.

Comparison of beetle mortality generally demonstrated the superiority of both concentrations and no significant difference was observed (Fig. 1).

Table 1.

Pine shoot beetles collected from different sites of Eastern Georgia in 2009
(GPS= Global Positioning System; L= Latitude; N= Longitude)

#	Data collection	Location, a.s.l	GPS		Tree species	H* (M)	D* (cm)	P* (year)	Number of collected beetles
			L	N					
1	15.05.09	Shavnabada, 700	41° 39' 13"	44° 50' 29"	<i>Pinus eldarica</i>	10-12	25-28	45-50	68
2	22.07.09	Khashuri, 750	42° 00' 55"	43° 35' 21"	<i>Pinus sosnovskyi</i>	~10	23-25	40	42
3	17.10.09	Patara Tsemi, 1600	41° 44' 12"	43° 32' 14"	<i>Pinus sosnovskyi</i>	~10	27-30	45-50	129

* H – Tree height; D – Tree diameter; P – Age

Table 2.

Efficacy of indigenous entomopathogenic fungus isolates of *Beauveria bassiana* (BbTp) against adults of pine shoot beetles in laboratory

Concentration (conidia /ml ⁻¹)		Beetles mortality in days																Total (by Abbot)	
		1		2		3		4		5		6		7		8			
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
BbTp	1x10 ⁶	-	-	-	-	4	8	13	26	24	48	33	66	38	76	41	82	41	81.5
	1x10 ⁷	-	-	1	2	6	12	18	36	32	64	41	82	46	92	50	100	50	100
Control	Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2.5	40	2.5

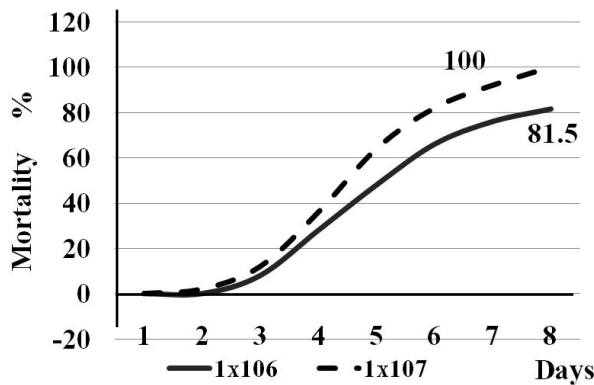


Fig. 1. Mortality by mycosis (%) of *Tomicus piniperda* after inoculation with indigenous strain of *Beauveria bassiana* (BbTp) at two concentrations (1×10^6 - 1×10^7 conidia/ml⁻¹) in laboratory

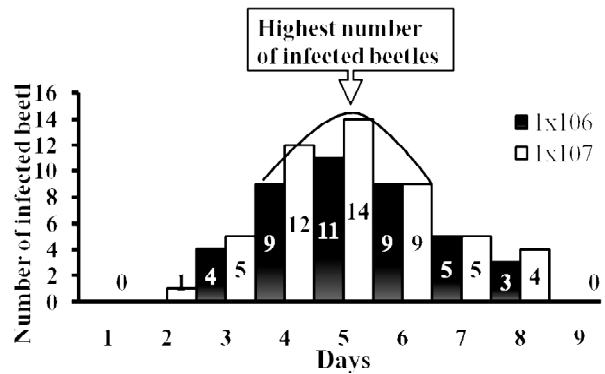


Fig. 2. The most susceptible time (in days) for mycosis infection to appear among the beetles of *Tomicus piniperda* caused by indigenous strain of *Beauveria bassiana* (BbTp)

A high number of infected beetles and their mortality by mycosis were observed between 4-7 days. Maximum rate of mortality was achieved within 8 days of specific treatment (Fig. 2).

The relatively high *B.bassiana* infection rates in some of the control groups, and the high prevalence of *B.bassiana* in bark beetles population[8] is characteristic

of this entomopathogenic fungus species. Selection and isolation with highest virulence of this species against *T.piniperda* is presumed to have a good chance of control not only of this but other bark beetles as well.

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ენტომოლოგია

***Beauveria bassiana*-ს ადგილობრივი შტამის ეფექტურობის დადგენა ფიჭვის დიდი ლაფნიჭამიას *Tomicus piniperda* L (*Coleoptera, Scolytidae*) მიმართ ლაბორატორიაში**

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2009 წელს აღმოსავლეთ საქართველოს ფიჭვნარებში ჩატარებული გამოკვლევების შედეგად გამოვლინდა ფიჭვის ღეროს მკვნიველი ფიჭვის დიდი ლაფნიჭამია *Tomicus (=Blastophagus) piniperda* L (*Coleoptera, Scolytidae*). აღნიშნული მკვნიველის პოპულაციებში გამოვლინდა პათოგენური სოკო *Beauveria bassiana*. გამოვლენილი პათოგენის ვირულენტობის დადგენის მიზნით აღნიშნული სოკოს მონოკულტურის სუსპენზია, ორი სხვადასხვა კონცენტრაციით 1×10^6 და 1×10^7 კონიდა/მლ⁻¹ გამოცდილი იქნა ფიჭვის დიდი ლაფნიჭამიას ზოტოების წინააღმდეგ ლაბორატორიაში. დადგინდა, რომ *Beauveria bassiana*-ს ადგილობრივი შტამი

ეფექტურია აღნიშნული მავნებლის მიმართ. მწერის მაქსიმალური სიკვდილიანობა დაფიქსირდა მე-4-7 დღეების შუალედში და იგი შესაბამისად 81,5-100% შეადგენს.

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