Title: USE OF A COLEOPTERAN ACTIVE *BACILLUS THURINGIENSIS* FOR CONTROLLING THE TASMANIAN EUCALYPTUS LEAF BEETLE *CHRYSOPHARTA BIMACULATA*

Abstract

This invention relates to the use of a coleopteran active *B. thuringiensis* or its delta endotoxin in controlling the Tasmanian eucalyptus leaf beetle *Chrysopharta bimaculata*. 
### FOR THE PURPOSES OF INFORMATION ONLY

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Title: Use of a coleopteran active *Bacillus thuringiensis* for controlling the Tasmanian eucalyptus leaf beetle *Chrysophtharta bimaculata*.

**FIELD OF THE INVENTION**

This invention relates to the use of a coleopteran active *B. thuringiensis* or its delta endotoxin in controlling the Tasmanian eucalyptus leaf beetle *Chrysophtharta bimaculata*, a method of controlling *Chrysophtharta bimaculata* by applying an effective amount of coleopteran active *B. thuringiensis* or its delta endotoxin to an area infested by *Chrysophtharta bimaculata*, and insecticidal compositions for controlling *Chrysophtharta bimaculata*.

**BACKGROUND OF THE INVENTION**

Commercial preparations of *Bacillus thuringiensis* are used worldwide for biological control of pest insects. The advantages of these bacterial insecticides are that they are highly selective for a very limited range of target insects and are biodegradable.

Commercial preparations of *Bacillus thuringiensis* can be applied right up to the time of harvest with no adverse effects.

*Bacillus thuringiensis* is a rod-shaped, aerobic, spore forming bacterium uniquely characterized by the production during the sporulation process of one or more inclusions, referred to as parasporal crystals. These crystals are composed of high molecular weight proteins, referred to as delta-endotoxins. The delta-endotoxins are the active ingredient in available commercial preparations of *Bacillus thuringiensis*.

Many *B. thuringiensis* strains with different insect host spectra have been identified. They are classified into different subspecies based on their flagellar antigens. Of particular interest is *Bacillus thuringiensis subspecies kurstaki* and *subspecies aizawai* used for the control of lepidopteran pest insects, *Bacillus thuringiensis subspecies israelensis* used for the control of dipteran pest insects and *Bacillus thuringiensis*
subspecies tenebrionis and Bacillus thuringiensis subspecies San Diego used for the
control of coleopteran pest insects.

The first isolation of a coleopteran toxic Bacillus thuringiensis was reported
0149162 A2).

The isolate, which was designated Bacillus thuringiensis subsp. tenebrionis,
has been deposited with the German Collection of Microorganisms under accession
number DSM 2803. Bacillus thuringiensis subsp. tenebrionis was isolated in 1982 from
a dead pupa of the mealworm, Tenebrio molitor (Tenebrionidae, Coleoptera). The strain
produces within each cell one spore and one insecticidal parasporal crystal which is of
flat plate-like form with an edge length of about 0.8 μm to 1.5 μm. It belongs to
serotype H8a,8b and pathotype C of Bacillus thuringiensis (Krieg et al.,

It is only toxic against certain leaf eating beetle larvae (Chrysomelidae), but
ineffective against caterpillars (Lepidoptera), mosquitoes (Diptera) or other insects.

Coleopteran active Bacillus thuringiensis has been shown to be an effective
control agent for the colorado potato beetle larvae. After uptake of crystals and
spores from coleopteran active Bacillus thuringiensis or isolated crystals larvae, and to
a certain extent adults, of the colorado potato beetle (Leptinotarsa decemlineata) stop
feeding. Larvae stages L1-L3 die within 1-3 days (Schnetter et al., in "Fundamental &
applied aspects of invertebrate pathology", eds. R.A. Samson et al., Proceedings of the
4th Int. colloquium of Invertebrate Pathology, p. 555, 1986).

The activity of B. thuringiensis subsp. tenebrionis and San Diego has further
been confirmed against the elm leaf beetle Xanthogaleruca luteola, and for B.
thuringiensis subsp. tenebrionis activity has also been demonstrated against the cereal
leaf beetle Oulema melanopa (Lema melanopa(L.)).

However, it has also been found that coleopteran active B. thuringiensis
does not exhibit activity towards all insects within the Chrysomelidae. It has for example
been found that it has no activity towards the small black flea beetle, Phyllotreta atra
and the small striped flea beetle, Phyllotreta undulata, and only minor activity against
the banded cucumber beetle, *Diabrotica balleata*, and the asparagus beetle, *Crioceres asparagi*.

Four commercial products of coleopteran active *Bacillus thuringiensis* have been developed for the control of coleopteran pests. NOVODOR® from Novo Biokontrol, Novo Nordisk A/S, TRIDENT® from Sandoz, and DiTerra® from Abbott Laboratories Inc. all based on *Bacillus thuringiensis subspecies tenebrionis*, and the commercial product M-one® from Mycogen Corporation based on a closely related strain *Bacillus thuringiensis subspecies San Diego*.

The Tasmanian leaf beetle *Chrysophtharta bimaculata* is a major forest pest responsible for much damage caused by defoliation of eucalyptus trees.

15 **SUMMARY OF THE INVENTION**

It has now been found that coleopteran active *B. thuringiensis* delta endotoxin exhibits activity towards a Tasmanian eucalyptus leaf beetle *Chrysophtharta bimaculata*.

This invention consequently relates to the use of coleopteran active *B. thuringiensis* or its delta endotoxin in controlling the Tasmanian eucalyptus leaf beetle *Chrysophtharta bimaculata*.

The present invention also relates to a method of controlling the Tasmanian eucalyptus leaf beetle *Chrysophtharta bimaculata* by applying an effective amount of a coleopteran active *B. thuringiensis* or its delta endotoxin to an area infested by *Chrysophtharta bimaculata*.

The invention further relates to insecticidal compositions for controlling the Tasmanian eucalyptus leaf beetle *Chrysophtharta bimaculata*. 30
DETAILED DESCRIPTION OF THE INVENTION

As indicated above the present invention in its first aspect relates to the use of coleopteran active \textit{B. thuringiensis} products such as, NOVODOR\textsuperscript{®}, for controlling the Tasmanian eucalyptus leaf beetle \textit{Chrysophtharta bimaculata}.

In its second aspect the invention relates to a method of controlling the Tasmanian eucalyptus leaf beetle \textit{Chrysophtharta bimaculata} by applying an effective amount of a coleopteran active \textit{B. thuringiensis} product to an area infested with said Tasmanian eucalyptus leaf beetle. In this context the expression "effective amount" should be understood as single or repeated applications of from 0.5 l/ha to 8 l/ha of flowable preparations or 0.25 kg/ha to 5 kg/ha of powder or granular preparations.

The invention in its third aspect relates to pesticidal compositions or preparations comprising the coleopteran active \textit{B. thuringiensis} delta endotoxin product in admixture with an agriculturally acceptable diluent or carrier for use in controlling \textit{Chrysophtharta bimaculata}.

The compositions of the invention can take any form known in the art for the formulation of agrochemicals, for example, a suspension, a dispersion, an aqueous emulsion, a dusting powder, a dispersible powder, an emulsifiable concentrate or granules. Moreover they can be in a suitable form for direct application or as a concentrate or primary composition which requires dilution with a suitable quantity of water or other diluent before application.

The concentration of the insecticidally active \textit{B. thuringiensis} delta endotoxin in the compositions of the present invention when used alone or in combination with another pesticide, as applied to trees or other plants is preferably within the range from about 0.5 to about 25 per cent by weight, especially 1 to 15 per cent by weight.

The coleopteran active \textit{B. thuringiensis} preparation or the compositions of the invention can be applied directly to the tree or other plant by, for example, spraying or dusting at the time when the pest has begun to appear on the tree or plant. The preferred mode of application is by spraying. It is generally important to obtain
good control of pests in the early stages of pest development as this most effectively prevents defoliation or other crop damage.

The invention is illustrated in the following example showing a specific embodiment of this invention. The example should in no way be construed as limiting for the scope of the invention as defined in the appended claims.

EXAMPLES

In a number of field trials the product NOVODOR® FC was evaluated for its efficacy against the L1 & L2 larval stages of the Tasmanian eucalyptus leaf beetle *Chrysophtharta bimaculata*.

In the trial the insecticide was applied to various eucalyptus crops as indicated below:

The NOVODOR® FC product is an aqueous flowable concentrate formulated with microbial stabilizers, detergents and suspension aids.

Example 1

<table>
<thead>
<tr>
<th>Product:</th>
<th>NOVODOR® FC</th>
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<tr>
<td>Pest:</td>
<td><em>Chrysophtharta bimaculata</em></td>
</tr>
<tr>
<td>Crop:</td>
<td><em>Eucalyptus regnans</em> 1 - 2 m tall</td>
</tr>
<tr>
<td>Location:</td>
<td>Westfield 100 Plantation site, Florentine Valley, Tasmania.</td>
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<tr>
<td>Objective:</td>
<td>To evaluate the efficacy of NOVODOR® FC against the larval stages of <em>Chrysophtharta bimaculata</em> under Tasmanian conditions</td>
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<tr>
<td>Application:</td>
<td>Tractor mounted mistblower. 380 l/ha</td>
</tr>
<tr>
<td>Trial design:</td>
<td>50 ha block: half sprayed with cypermethrin and half with NOVODOR® FC</td>
</tr>
</tbody>
</table>
Dose:  Product  Dose/ha  
NOVODOR® FC  5.0 liters  
cypermethrin  10 g ai/ha  
control  

Timing:  Application took several days so some NOVODOR® treated trees may have received the treatment later than optimal  

Assessments:  Random trees: eggs and larvae on 10 shoots/tree, precount and 25 shoots/tree post spray counts  

Results:  Product  Dose/ha  % reduction  
eggs and larva  
NOVODOR® FC  5.0  77.8  
cypermethrin  10 g  90.1  
control  

Comments:  Surviving L3 and L4 NOVODOR® FC treated larvae appeared healthy, but their gregarious behaviour was disturbed. Treated areas were less defoliated than untreated control areas  

Conclusion:  NOVODOR® FC gave a reasonable control of early instar C. bimaculatus larvae  

Example 2  

Product:  NOVODOR® FC  

Pest:  Chrysophtharta bimaculata  

Crop:  Eucalyptus delegatensis established in 1983  

Location:  Smith Plains Plantation, Devonport District, Tasmania  

Objective:  To evaluate the efficacy of NOVODOR® FC applied from the air against the larval stages of Chrysophtharta bimaculata under Tasmanian conditions  

Application:  Helicopter application:  20 - 100 l/ha  

Trial design:  Single block treatments
Dose: Product Dose/ha
NOVODOR® FC  5.0 liters
cypermethrin  10 g ai
control

5 Timing: late december 1989 (20/12/89)
Assesments: larval mortality

Results: Product Dose/ha % mortality
NOVODOR® FC  5.0 l 87
cypermethrin  10 g  .97

10 Conclusion: NOVODOR® FC gave good control of C. bimaculata larvae, when aerial application was adequately done

Example 3

15 Product: NOVODOR® FC
Pest: Chrysophtharta bimaculata
Crop: Eucalyptus regnans 1-2 m tall
Location: Westfield 100 Plantation site, Florentine Valley, Tasmania.

20 Objective: To evaluate the efficacy of NOVODOR® FC against the larval stages of Chrysophtharta bimaculata under Tasmanian conditions

Application: Motorised knapsack sprayer  930 l/ha
Trial design: Randomise plot design: part rows with 2 rows between treatments
Dose: Product Dose/ha
25 NOVODOR® FC  5.0 liters
cypermethrin  10 g ai/ha
cypermethrin  20 g ai/ha
control

Timing: early L2 on 30th November 1989

Assessments: The number of larval masses on each of 10 marked trees per plot. Unmarked trees were destructively sampled to determine the average number of larvae per mass at both pre spray (29/11/89)
and post spray counts (4/12/89). The average increase in growth was assessed on the destructively sampled trees.

Results:

<table>
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<th>% population reduction</th>
<th>av. increase in growth cm</th>
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<tr>
<td>NOVODOR® FC</td>
<td>5.0 l</td>
<td>74.0</td>
<td>43</td>
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<td>cypermethrin</td>
<td>10 g</td>
<td>99.9</td>
<td>35</td>
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<td>cypermethrin</td>
<td>20 g</td>
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<td>42.0</td>
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Comment: The assessment was probably too early to note the full effect of the treatment, which is apparent at 10 - 14 days.

Conclusion: NOVODOR® FC gave a reasonable control of *C. bimaculatus* larvae, and a worthwhile advantage growth over chemical treatments.
PATENT CLAIMS

1. Use of at least one coleopteran active *Bacillus thuringiensis* or its delta endotoxin for controlling the Tasmanian eucalyptus leaf beetle *Chrysophtharta bimaculata*.

2. Use according to claim 1, wherein said coleopteran active *Bacillus thuringiensis* is *Bacillus thuringiensis subspecies tenebrionis* or *Bacillus thuringiensis subsp. San Diego*, preferably *Bacillus thuringiensis subspecies tenebrionis*.

3. Use according to any of claims 1 or 2 in trees, shrubs or plants belonging to the family Myrtaceae.

4. Use according to any of claims 1 to 3 on eucalyptus trees.

5. A method for controlling the Tasmanian eucalyptus leaf beetle *Chrysophtharta bimaculata*, wherein an effective amount of a coleopteran active *Bacillus thuringiensis* product is applied to trees or plants infested with *Chrysophtharta bimaculata*.

6. The method of claim 5, wherein said coleopteran active *Bacillus thuringiensis* product originates from *Bacillus thuringiensis subspecies tenebrionis* or *Bacillus thuringiensis subsp. San Diego*, preferably *Bacillus thuringiensis subspecies tenebrionis*.

7. The method of claim 6, wherein a liquid product is applied in an amount of 0.5 l/ha to 10 l/ha, preferably 2 l/ha to 7 l/ha.

8. The method of claim 6, wherein a flowable powder or granular product is applied in an amount of 0.25 kg/ha to 5 kg/ha, preferably 0.5 kg/ha to 2 kg/ha.

9. The method of claim 6, wherein a wetable powder product is applied in an amount of 0.25 kg/ha to 5 kg/ha, preferably 0.5 kg/ha to 2 kg/ha.
10. The method of claim 6, 7, 8, or 9, wherein the product is applied repeatedly with intervals.

11. A pesticidal composition for controlling the leaf beetle *Chrysophthara bimaculata* comprising the delta endotoxin of a coleopteran active *Bacillus thuringiensis* in admixture with an acceptable carrier.

12. The composition of claim 11, wherein said coleopteran active *Bacillus thuringiensis* is *Bacillus thuringiensis* subspecies *tenebrionis* or *Bacillus thuringiensis* subsp. *San Diego*, preferably *Bacillus thuringiensis* subspecies *tenebrionis*. 
INTERNATIONAL SEARCH REPORT

International Application No: PCT/DK 91/00150

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: A 01 N 63/00, 63/02, C 12 N 1/20//(C 12 N 1/20, C 12 R 1:07)

II. FIELDS SEARCHED

Classification System Classification Symbols

IPC5 A 01 N; C 12 N

Documentation Searched other than Minimum Documentation to the extent that such Documents are Included in Fields Searched

SE, DK, FI, NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>Environmental Entomology, Vol. 19, No. 2, 1990 L.S. Bauer: &quot;Response of the Cottonwood Leaf Beetle (Coleoptera: Chrysomelidae) to Bacillus thuringiensis var. san diego&quot;, see page 428 - page 431</td>
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IV. CERTIFICATION

Date of the Actual Completion of the International Search: 4th September 1991

Date of Mailing of this International Search Report: 1991-09-11

International Searching Authority: SWEDISH PATENT OFFICE

Signature of Authorized Officer: Gerd Wranne
### III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

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| P,X      | EP, A1, 0382990 (PLANT GENETIC SYSTEMS, N.V.)  
22 August 1990,  
see claim 4; page 2, lines 1-2, 8-10 | 1-12                |
| X        | US, A, 4797276 (C. HERRNSTADT ET AL.)  
10 January 1989,  
see table 1; the claims | 1-12                |
| P,X      | WO, A1, 9013651 (IMPERIAL CHEMICAL INDUSTRIES PLC)  
15 November 1990,  
see claims 1-3, 10, 11; page 6, line 17 - page 7, line 10 | 1-12                |
| X        | EP, A1, 0309145 (MYCOGEN CORPORATION)  
29 March 1989,  
see the claims | 1-12                |
| P,X      | EP, A1, 0411582 (PRESIDENZA DEL CONSIGLIO DEI MINISTRI -UFFICIO DEL MINISTRO PER IL COORDINAMENTO DELLE INIZIATIVE PER LA RICERCA SCIENTIFICA E TECNOLOGICA)  
6 February 1991,  
see claims 3-5; page 6, lines 12-14, lines 29-31 | 1-12                |
ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. PCT/OK 91/00150

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the Swedish Patent Office EDP file on 91-07-31. The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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