Laboratory bioassay to assess the efficacy of the Cryonite system against German cockroaches and Indian meal moths

Insect Investigations Ltd
Capital Business Park
Wentloog
Cardiff CF3 2PX
UK

Helena Dawe
October 2006

Certification

This report represents a true and accurate record of all data obtained.

Signed…….. Date……..
Helena Dawe
Study Director

Approved by…… Date……..
Dr Peter M^{c}Ewen
Managing Director

All raw data and a copy of the final report will be archived at I_2L for a period of five years. At the end of this period all data relating to this report will either be retained by I_2L for a further disclosed period of time, or passed on to the sponsor.

Report circulated to: Insect-o-cutor (1 copy)
Insect Investigations Ltd (1 copy)

Contents

Study Information

Laboratory bioassay to assess the efficacy of the Cryonite system against German cockroaches and Indian meal moths

i. Submitted by: Insect Investigations Ltd
Capital Business Park
Wentloog
Cardiff CF3 2PX

ii. Sponsor: Insect-o-cutor
Oakhurst Drive
Cheadle Heath
Stockport
Cheshire SK3 0XT

iii. I_2L Study code: 06/99

iv. Study start date: 12.10.06
Study completion date: DRAFT COPY

v. Study Director: Helena Dawe

vi. Principal personnel: Graham Small, Lucy Emery

vii. Certification: ORETO Number 175

viii. Test chemicals:

Test Product	I_2L code number
Cryonite system	06101913

Summary

A laboratory bioassay was conducted to assess the efficacy of the Cryonite system against German cockroaches and Indian meal moths. The Cryonite system was applied as four bursts into plastic tanks containing cockroach adults, nymphs and eggcases and meal moth larvae. At the time of treatment the cockroaches were on scrunched cardboard to simulate crack/crevices and the larvae in a thin layer of flour to simulate natural conditions. The number of dead adults, nymphs and larvae was assessed at 24 hours and the total number of first instar nymphs that hatched was assessed for a total of 7 days post treatment.

The Cryonite system was very effective against cockroach adults and nymphs and meal moth larvae; all insects were either moribund or dead at 24 hours post application. Although some cockroach nymphs hatched out of the eggcases treated with the Cryonite system, these were fewer than in the untreated control.

Aim

A laboratory bioassay was conducted to assess the efficacy of the Cryonite system against German cockroaches and Indian meal moths, in terms of mortality and egg hatching (cockroaches only).

Methodology

Test insects

German cockroaches (*Blattella germanica*) were obtained from a laboratory culture maintained at Insect Investigations Ltd, cultured according to SOP/226/04 (Appendix II). Mixed sex (50:50, male:female), mixed age adults, 4-5th instar mixed sex nymphs and heavily gravid females (see deviation 1) were used in the experiments.

Indian meal moths (*Plodia interpunctella*) were obtained from a laboratory culture maintained at Imperial College (London, UK). Mixed instar larvae, approximately 1 cm long, were used in the experiments.

Treatments and application

The Cryonite system was applied as four bursts for each replicate. An untreated control treatment for each species was also included for comparative purposes, giving a total of two treatments per species.

Experimental design

Twenty adult cockroaches (ten males and ten females), twenty mixed instar nymph cockroaches and ten heavily gravid female cockroaches or twenty mixed instar meal moth larvae (see deviation 2) were placed into one plastic tank (33 cm long x 21 cm wide x 21 cm high). The sides of the tanks containing the cockroaches were coated in Fluon (liquid PTFE) to prevent the insects from escaping. A piece of scrunched cardboard was placed in the cockroach tanks to simulate crack/crevices and a thin layer of flour was placed in the larvae tanks to simulate a natural substrate. The Cryonite system was then

applied to the tank. After application, cockroaches were provided with food (one bran pellet) and water. The number of dead insects was assessed at 24 hours post treatment with the Cryonite. The number of first instar cockroach nymphs that hatched was assessed for a total of 7 days post treatment application.

Replication

Three replicates were performed for each of the treatments for each species, each replicate consisting of one tank.

Trial conditions

Temperature ranged from 23.1 – 25.3 ºC and relative humidity was between 39 and 47%.

Protocol deviations

The study protocol is included in appendix I. The following deviations occurred:

Deviation 1: Instead of deposited eggcases, heavily gravid female cockroaches were used. This would be more realistic, as deposited eggcases usually hatch within minutes.

Deviation 2: Twenty meal moth larvae were used instead of forty. This was due to the number of larvae available from the supplier at short notice; therefore fewer larger larvae were used, which would be less susceptible than younger larvae.

Results and Conclusions

Treatment with the Cryonite system resulted in 100% cockroach mortality at 24 hours post application (Table 1). Although meal moth larvae mortality was 85%, the remaining larvae were severely affected at 24 hours post treatment (Table 2). Although some eggcases hatched during the 7 days post treatment, the number of 1st instar nymphs was lower compared to the untreated control hatch rate. It can be concluded that the Cryonite system is effective in controlling German cockroach adults and nymphs and Indian meal moth larvae.

Table 1. Effect of the Cryonite system on mortality of *B. germanica* 24 hours post treatment and subsequent hatching of eggs at 7 days post treatment

	Treated				Control			
Replicate	No dead adults	No dead nymphs	No 1st instar nymphs	No eggcases hatched	No dead adults	No dead nymphs	No 1st instar nymphs	No eggcases hatched
1	20	20	171	7	0	0	274	9
2	20	20	26	3	0	1	158	6
3	20	20	52	3	0	1	212	8
Mean	**20**	**20**	**249***	**13****	**0**	**0.7**	**644***	**23****
SE	0	0	N/a	N/a	0	0.3	N/a	N/a

* total number

** total number out of 30

Table 2. Effect of the Cryonite system on mortality of *P. interpunctella* 24 hours post treatment

	Treated			Control
Replicate	No moribund larvae	No dead larvae	Total affected	No dead larvae
1	3	17	20	1
2	3	17	20	0
3	3	17	20	0
Mean	**3**	**17**	**20**	**0.3**
SE	0	0	0	0.3

Appendix I Study Protocol

Proposal submitted by Insect Investigations Ltd to Insect-o-cutor

Submitted by: Helena Dawe, Head of Laboratory Studies
11th October 2006

Protocol

Test insects. German cockroaches, *Blattella germanica*, will be obtained from a laboratory culture maintained at Insect Investigations Ltd. Mixed sex adults, nymphs and deposited eggcases (ready to hatch) will be used in the experiment.

Indian meal moths, *Plodia interpunctella*, will be obtained from a specified laboratory culture. Mixed instar larvae will be used in the experiment.

Treatments. The Cryonite system will be tested on cockroaches and moths. An untreated control will also be set up for each species, giving a total of two treatments.

Experimental design. Twenty adult cockroaches (ten male and ten female), twenty mixed instar nymph cockroaches and twenty deposited eggcases (ready to hatch) or forty mixed instar meal moth larvae will be placed into one plastic tank, measuring approximately 33 cm long x 21 cm wide x 20 cm high. The tank will be coated in Fluon (liquid PTFE) to prevent the insects from escaping. Creased cardboard will be placed in the cockroach tank to simulate crack/crevices and a thin layer of flour will be placed in the moth tank to simulate a natural substrate. The Cryonite system will then be applied to the tanks according to the sponsor's instructions. The number of live and dead cockroaches and moth larvae will be assessed at 24 hours post treatment application. The number of 1st instar nymphs that hatch from the treated eggcases will be assessed at an interval determined by the hatching period of the control eggcases. If no treated eggs

hatch following a specified period after the final control eggs have hatched, it will be assumed that the eggs are dead.

Three replicates will be performed for each treatment, each replicate consisting of one tank.

Temperature and humidity will be monitored throughout the experimental period and detailed in the final report.

Appendix II SOP/226/04 Culturing of cockroaches

Insect Investigations Limited
standard operating procedure

Issued: 01.04.04

Fourth Edition: This replaces the 3rd edition produced on 24.01.03

Third Edition: This replaces the 2nd edition produced on 14.12.00

Second Edition: This replaces the 1st edition produced on 5.7.00

TITLE: Culturing of Cockroaches

INTRODUCTION:

Cockroaches are reared under the correct conditions to maintain a steady supply of materials for experimental procedures.

AUTHOR: Helena Dawe

AUTHORISED BY: Dr P K McEwen

Signature:…….. Date:…….

DISTRIBUTION: Managing Director's office, Staff Office, Main Laboratory, Back Laboratory

INSTRUCTIONS

1. **Maintenance conditions**

    1.1 The cockroach colonies are maintained in a controlled temperature (CT room 1) normally set at 25 ± 3 °C, with a 16:8 light:dark cycle.

1.2 A daily (working week) record is kept of the maximum and minimum temperatures in the CT room. These records are displayed on the door of the CT room. Completed record sheets will be retained (SOP/202/04).

1.3 The cockroaches are housed in plastic, lidded tanks (34 cm long x 20 cm wide x 24.5 cm deep). Fluon (liquid PTFE) is painted around the rim of each tank to prevent escape.

1.4 All species of cockroaches are provided with:

- **Water.** Provided in a water pot, comprising an upturned half-pint pot on top of an upturned petri dish lid lined with filter paper, which acts like a wick.

- **Bran pellets.** (B & K, Bristol). Placed on a plastic petri dish in the cage.

1.5 Water pots are cleaned and replenished fortnightly. Bran pellets are replenished as necessary.

1.6 Every 2 months the tanks are cleaned thoroughly.

2. Disposal

2.1 Any cockroaches to be disposed of are frozen for 24 h before disposal (see SOP/222/04).

3. Other

3.1 The following species of cockroaches can be kept in colony: German cockroach (*Blattella germanica*); Oriental cockroach (*Blatta orientalis*); American cockroach (*Periplaneta americana*), Madagascan hissing cockroach (*Gromphadorhina portentosa*). The actual species kept at any one time may vary, depending on the requirements of experiments.

Appendix III **ORETO certification**

Certificate of

Official Recognition of Efficacy Testing Facilities or Organisations in the United Kingdom

This certifies that

Insect Investigations Limited

complies with the minimum standards laid down in Commission Directive 93/71/EEC for efficacy testing.

The above Facility/Organisation has been officially recognised as being competent to carry out efficacy trials/tests in the United Kingdom in the following categories:

Agriculture/Horticulture

Date of issue	6 January 2004
Effective date	1 January 2004
Expiry date	31 December 2008

Signature _____

Authorised signatory

Certification Number

ORETO 175

Department of Agriculture and Rural Development

PSD
SAFETY FOR PEOPLE AND THE ENVIRONMENT
an Executive Agency of DEFRA

ORET 3 (Rev. 4/01)