

Studies on Low-Toxic Sugar Ester Based Pesticides against *Rhizopertha Dominica*

A dissertation submitted in fulfillment of the requirement for the degree of
Master of Philosophy

by

Hsin-Yi Sheena Chen

August 2015



THE UNIVERSITY
of ADELAIDE

School of Chemical Engineering
Faculty of Engineering, Computer and Mathematical Sciences

TABLE OF CONTENTS

	Page
ABSTRACT	i
DECLARATION	iii
ACKNOWLEDGEMENT	iv
LIST OF PUBLICATIONS	v
LIST OF TABLES	vi
LIST OF FIGURES	viii

CHAPTER 1

Background and Literature Review

1.1 Post-Harvest Losses	1
1.2 <i>Rhyzopertha dominica</i>	3
1.3 Integrated Pest Management	5
1.3.1 Australian strategies and perspective for pest control of stored grain	8
1.4 Chemical Insecticides	8
1.5 Diatomaceous Earth	12
1.5.1 Effect of experimental conditions	14
1.5.2 Effect of insects	15
1.5.3 Effect of DE properties	16
1.5.4 Advantages and disadvantages	17
1.6 Sugar fatty acid esters	18
1.6.1 Insecticidal activity	18
1.6.2 Extraction from natural sources	22
1.6.3 Synthetic methods	24
1.6.3.1 Transesterification method	24
1.6.3.2 Acyl chloride esterification method	27

1.6.3.3 Regioselective chemical synthesis method	27
1.6.3.4 Regioselective enzymatic synthesis	32
1.6.4 Advantages and disadvantages	34
1.7 Summary: challenges in the control of stored grain pests	38
1.8 Aims and Objectives	38
1.9 Thesis Structure	39
References	41

CHAPTER 2

Experimental design and methodology

2.1 Chemicals and Materials	55
2.1.1 Diatomaceous earth	55
2.2 Equipment	56
2.3 Synthesis of Sugar Esters	56
2.4 Characterisation of Sugar Esters	57
2.4.1 FTIR	57
2.4.2 NMR	58
2.4.3 Contact Angle	59
2.5 Insecticide Bioassays	59
2.5.1 Preparation of grain	59
2.5.2 Selection of insects	60
2.5.3 Comparative bioassays	61
2.5.4 Lethal dose bioassays	62
2.5.5 Surface bioassay	63
2.6 Grain bulk density	63
2.7 Angle of Repose	64
2.8 Optical Microscopy	64
2.9 Scanning electron microscopy (SEM)	65
References	67

CHAPTER 3

Synthesis and characterisation of sugar esters

3.1	Introduction	68
3.2	Materials and methods	69
3.2.1	Synthesis and purification of sugar esters	69
3.2.2	Characterisation	69
3.2.2.1	FTIR	69
3.2.2.2	NMR	69
3.2.2.3	Contact angle	69
3.3	Results and Discussion	70
3.3.1	Product yield	70
3.3.2	FTIR	71
3.3.3	Proton NMR	74
3.3.4	Determine the HLB value	76
3.3.5	Contact Angle	77
3.4	Conclusions	82
	References	83

CHAPTER 4

Insecticidal activity of sugar esters against *Rhyzopertha dominica*

4.1	Introduction	85
4.2	Materials and methods	86
4.2.1	Sugar esters	86
4.2.2	Bioassay	86
4.2.3	HLB value calculation	86
4.2.4	Contact angle	86
4.3	Results and Discussion	87
4.3.1	Size of the sugar molecule, chain length of fatty acid	87

and their impact on insecticidal activity	
4.3.2 Purification and the impact on insecticidal activity	88
4.3.3 Investigation of the mode of action of sugar esters	90
4.2.3.1 Contact angle	90
4.2.3.2 Hydrophilic-lipophilic balance value	92
4.2.3.3 Mode of feeding	93
4.2.3.4 Electron microscopic study	93
4.4 Conclusion	96
References	98

CHAPTER 5

Insecticidal activity of sorbitol octanoate combining with diatomaceous earth against <i>Rhyzopertha dominica</i>	
5.1 Introduction	99
5.2 Materials and methods	101
5.2.1 Sorbitol octanoate	101
5.2.2 DE and Dryacide	101
5.2.3 Comparative bioassay	101
5.2.4 Lethal dose bioassay	101
5.3 Results and discussion	102
5.3.1 Efficacy of combined formulations of DE and sC8	102
5.3.2 Mode of insecticidal action	109
5.3.3 Effect of formulation treatments on grain properties	117
5.3.3.1 Grain colour	117
5.3.3.2 Bulk density	117
5.3.3.3 Angle of repose	119
5.4 Conclusion	121
References	123

CHAPTER 6

Conclusions and Future Perspectives

6.1 Conclusion	141
6.2 Future direction	144
Appendix 1 IRAC MoA Classification v 7.3, February 2014	146
Appendix 2 NMR spectra of sugar esters	153
Appendix 3 Supplementing bioassay data	159
Appendix 4 Contact angles of sugar esters	173
Appendix 5 SEM images showing insect parts including mandible, tibia, tarsal segments and abdominal sternites after treated by DE-SO combined formulation	175

ABSTRACT

Rhizopertha dominica is a destructive, cosmopolitan pest of major concern to post-harvest storage of grains and other products. Failing in control of primary insect pest such as *R. dominica* assist the attack of cereal commodities by secondary pests and thus greater damages and losses. Conventional treatments rely heavily on rapid eradication of pest insects using synthetic organic chemicals. However, serious trade-off was only learnt after the broad-spectrum toxicity threatens our eco-system as well as health and the emerging of insect resistance. Insecticide research during the past decades have shift focus to seek for safer alternatives, preferably from natural sources with low toxicity, to ensure the biosecurity of stored grains world-wide. Inert dusts such as diatomaceous earths (DE), the fossil sediments of marine algae mainly composed of silica dioxides, have been used as grain protectants. This biosilica with delicate microstructures has demonstrated control on several insect species including *R. dominica* without the development of resistance. The inhibition mechanism of these effects was believed to be the absorption of cuticle wax by the micro- and nano-pores on DE which leads to dehydration then death of target insects. However, application of DE on stored grains has not been widely adopted. This is mainly due to its negative influences on grain appearance and processing as a result of relatively large effective amounts required. Research in this field has been worked on ways to address this challenge and increase their market acceptance. One promising approach is to combine DE with other safe additives, such as sugar esters. Synthetic sugar ester is a group of low-toxic insecticides produced from natural sources. Previous studies have demonstrated this compound was capable in killing insects without causing any adverse effects to other organisms. Owing to the surfactant nature, these compounds were believed to desiccate target insects via interrupting the cuticular wax layer. In this research, sugar esters were for the first time assayed against *R. dominica*. The synergistic effect from combining with DE has also been evaluated. A series of esters of fatty acids with polyols have been synthesized via direct esterification using an environment-friendly solvent-less

method. These crude composites, namely sorbitol octanoate, sorbitol decanoate, sorbitol laurate, xylitol octanoate, xylitol decanoate and xylitol laurate were then purified using a two steps process. Qualitative analysis of the products was conducted using Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (NMR) spectroscopy methods. FTIR provided rapid and reliable identification for the characteristic functional group of sugar esters showing absorption bands. NMR spectra agree with the FTIR results, which have confirmed the formation of expected compounds. The insecticidal activity of these compounds was investigated where sorbitol octanoate was found to be the most effective at the concentration of 4000 ppm. No strong correlation of Hydrophilic-lipophilic balance values (HLB, calculated based on NMR spectra) associated with sugar ester's killing effect was observed as suggested by other research groups. It was also realized that purification was essential in order to enhance insect mortality. Further study has revealed that the mechanism of this compound on killing the insects is by contact rather than ingestion. Combination of sorbitol octanoate with DE was also studied. Data analysis showed that while there was an improvement in the quantity of DE required, the effect is not quiet synergistic where combined is less effective than expected. Lethal dosage of DE that were required to kill over 90% of insect population have been reduced from 700 ppm to 100 ppm when combined with 4000 ppm of sugar ester. Bulk density tests indicated that this formulation can increase grain hectoliter mass by 2-fold and reduced the angle of repose from 34 ° to 31 ° as opposed to 36 ° by 100ppm DE alone. Addition of sugar ester has effectively reduced the friction between DE treated grains which was proven to be a favourable strategy. Adherence of DE on *R. dominica*'s exoskeleton were investigated by both optical microscope and scanning electron microscope (SEM). Both techniques have shown a significant reduction of DE attachment on insect body when combined with sugar esters. Our finding supports the theory that inhibition mechanism may be due to penetration of the lipophile section of sugar ester in the cuticular layer.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time

Hsin-Yi Sheena Chen

Date: _____

ACKNOWLEDGEMENT

First and foremost, I would like to express my most sincere gratitude to my advisory team; Dr. Lucas Johnson, Prof. Dusan Losic, Dr. Patrick Collins and Prof. John Jennings, for their invaluable and continuous guidance of my MPhil research and study, without which it would be impossible to have these achievements during my M.Phil period. I would also like to give my thanks to Mr. Angus Netting, Ms. Ruth Williams, Mr. Ken Neubauer and the team from Adelaide Microscopy for their technical support during my research.

Besides, I would like to give my heartfelt thankfulness to my beloved family. Words cannot express my gratitude to my husband, Dr. Ji Liang, for his sacrifice, firm support and sustained encouragements during the last four years of my M.Phil. This thesis would not be possible without the support and love from him. Our son, Joshua Liang, was born during the time. It's the love and responsibility to this new life which then become my most important strength to carry on.

Moreover, I want to thank the members in my research group for their support in my research; A/Prof. Mahaveer Kurkuri, Dr. Abel Santos, Dr. Diana Tran, Mrs. Shervin Kabiri, Dr. Moom Sinn Aw, Mr. John Hayles, Dr. Krishna Kant, Mr. Tushar Kumeria, Mr. Karan Gulati, Mr. Ye Wang and all other team members.

Last but not least, I want to express my earnest appreciation to the ECMS Faculty for the Divisional Scholarship, which has financially supported my living costs during this period.

LIST OF PUBLICATIONS

Journal

1. **Chen, H. S.**, Johnson, L., and Losic, D. (2015) Synthesis of sugar esters and characterization their insecticidal properties (In preparation)
2. **Chen, H. S.**, Johnson, L., Collins, P. and Losic, D. (2015) Evaluation of sugar esters for stored grain protection in combination with diatomaceous earth (In preparation)

Conference Presentation

1. T. Kumeria, B. Manpreet, A. Tariq, **H. S. Chen**, K. Mahaveer, W. R. Yang and D. Losic (2012) Diatomite Silica - Graphene Oxide Based Nanohybrid Structures. In OzCarbon 2012. Adelaide
2. **H. S. Chen**, Johnson, J. T. Jennings, P. J. Collins and D. Losic (2014) Morphological study of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) using FIB/SEM. in ICONN.ACMM 2014. Adelaide

LIST OF TABLES

	Pages
Table 1.1 Common insect pests of stored grain	2
Table 1.2 Stored grain pest control strategies used in IPM	7
Table 1.3 Classification of major group of chemical insecticides and their modes of action	9
Table 1.4 Factors determining diatomaceous earth insecticide efficacy	15
Table 1.5 HLB values and the related functional properties	20
Table 1.6 The effect of synthetic sugar esters against insects of various species	36
Table 3.1. Products retained as to total weight of starting chemicals (w/w) after repeated washing	70
Table 3.2. Functional group characterisation using FTIR	71
Table 3.3. Ratio of reacted to unreacted fatty acids remaining in the purified sugar esters	75
Table 3.4. Fatty acid to sugar ratio for synthesized sugar esters	75
Table 3.5. Contact angles of grains with different concentrations of sorbitol octanoate	79
Table 3.6. Contact angles of grains treated with sugar esters at 1000 ppm concentration	80
Table 4.1 Toxicity of sugar ester on LGB prepared with polyol and fatty acids (4000ppm) having different chain length in 7, 14 and 60 days	87

Table 4.2 Effect of sugar ester purification (4000ppm) on LGB mortality in 7 and 14 days and progeny formation in 60 days.	89
Table 4.3 Contact angles and their relative morality and progeny suppression of Katana grains coated with sugar esters at varied concentration (100 to 4000ppm)	91
Table 4.4 HLB value of sugar esters	92
Table 4.5 Morality of insect feeding on wheat granule with and without sugar ester coating (4000ppm) in 18 days	93
Table 5.1 Toxicity of sorbitol octanoate, DE and their combinations against LGB expressed in mortality (7 and 14 day) and progeny suppression (60 th day)	103
Table 5.2 Lethal doses (LD ₅₀ / LD ₉₀) and relative potency (vs. Dryacide) of the optimal formulation and its component ingredients in 14 days	104

LIST OF FIGURES

	Pages
Figure 1.1 Images of adult (left), larvae, pupae and eggs (right) of <i>R. dominica</i> .	3
Figure 1.2. Damaged grains by <i>R. dominica</i>	4
Figure 1.3 Microstructure of arthropod cuticle	5
Figure 1.4 Development of insecticide resistance	12
Figure 1.5 Examples of different morphology and microstructures of diatom frustules that comprise diatomaceous earth	13
Figure 1.6 Examples of mono, di and poly-form of sugar ester	19
Figure 1.7 Schematic representation of a cross section of tracheoles with liquid inside	22
Figure 1.8 Molecular structures of naturally produced sugar esters	23
Figure 1.9 Transesterification reaction	24
Figure 1.10 Transesterification reaction of sucrose forming sugar ester	25
Figure 1.11 Acyl chloride esterification of sucrose forming sugar ester	27
Figure 1.12 Molecular structure of sucrose	28
Figure 1.13 Mitsunobu esterification process	29
Figure 1.14 Selective acylation of sucrose using 3-acyl-5-methyl-1,3,4- thiadiazole-2(3H)-thiones	30
Figure 1.15 In-situ isomerization of sucrose with DBU or Et ₃ N.	30

Figure 1.16 Process of dibutylstannylene-acetal condensation forming dibutylstannylene-acetal	31
Figure 1.17 Process of sucrose ester formation by dibutylstannylene-acetal condensation	31
Figure 1.18 Synthesis of sugar esters using enzymes as catalysts	32
Figure 1.19 Lipase catalysed synthesis of sugar ester using vinyl ester as starting chemicals	33
Figure 1.20 Protease catalysed formation of sucrose	34
Figure 2.1 FTIR spectrophotometer equipment with attenuated total reflection system	58
Figure 2.2 Schematic setup of NMR	58
Figure 2.3 Photo of the jar for the bioassay	60
Figure 2.4 Setup of BK Plus Imaging System	65
Figure 2.5 Schematic illustration of insect body parts that are prompted to attacks by surface active insecticides such as DE	66
Figure 3.1 FTIR spectra of the raw materials and synthesized esters (a) fatty acids and (b) polyols, and end products (c) sorbitol esters and (d) xylitol esters	72
Figure 3.2 FTIR spectra of products obtained from each purification step (a) sorbitol octanoate; (b) sorbitol decanoate; (c) sorbitol laurate; (d) xylitol octanoate; (e) xylitol decanoate; (f) xylitol laurate	73
Figure 3.3 Normalized NMR spectra of sorbitol octanoate	76
Figure 3.4 HLB values and associated water solubility	77

Figure 4.1 SEM images showing the antenna, sternites, and pronotum and elytra of <i>Rhyzopertha dominica</i> , before (a, c, e) and after (b, d, f) exposure to grains treated with 4000ppm sorbitol octanoate	95
Figure 5.1 Insect mortality of LGB placed in DE treated grains in 14 days	105
Figure 5.2 Insect mortality of LGB placed in sorbitol octanoate treated grains in 14 days	106
Figure 5.3 Insect mortality of LGB placed in Dryacide® treated grains in 14 days	107
Figure 5.4 Insect mortality of LGB placed in DE / sorbitol octanoate treated grains in 14 days	108
Figure 5.5 Images of insects treated with DE dust at different concentration	111
Figure 5.6 Images of insects treated with sC8 at different concentration	112
Figure 5.7 Images of insects treated with DE (varied in concentration) combined with 4000ppm sC8	113
Figure 5.8 Images of insects treated with 2000ppm and 4000ppm sC8 combined with DE at varied levels	114
Figure 5.9 Pronotum of <i>R. dominica</i> treated by DE-sC8 combined formulation displayed at 1000x and 5000x magnification	115
Figure 5.10 Elytra of <i>R. dominica</i> treated by DE-sC8 combined formulation displayed at 1000x and 5000x magnification	116
Figure 5.11 Images of Katana grains dusted with DE at varied concentration.	118

Figure 5.12 Bulk density (kg/hL) of Katana treated with sorbitol octanoate (4000ppm) and DE (100, 400, 700 and 1000ppm)	119
Figure 5.13 Angle of repose of Katana treated with sorbitol octanoate (4000ppm) and DE (100, 400, 700 and 1000ppm)	120

Chapter 1

Background and Literature Review

1.1 Post-Harvest Losses

United Nations (UN) have projected the global population growth of humans to be peaking at 9 billion by 2050 (UN, 2009). By the middle of the this century, we will need to produce at least 70 % more food from the same size of (or even smaller) available available land to ensure even the poorest dwellers are food-secure and can be environmentally sustained (FAO, 2009). Although new technologies have increased total annual food production up to three fold for crops and five folds for animals (Godfray et al., 2010), further advances are needed to continue to meet this challenge.

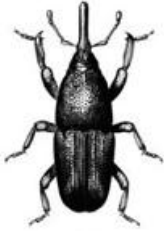
One major source of food is grain, where maize (or corn), rice and wheat are the major crops, accounting for 89 % of annual grain production. These crops provide over 50 % of all food calories consumed, and will be very likely remain the mainstay of human nutrition (FAOSTAT, 2013a). Apart from agricultural intensification to increase production, closing the yield gap via saving post-harvest losses from poor storage management offers an ideal solution to help feed the world (Godfray et al., 2010).

The utilisation of global food production was estimated to be below 45 % on average, where annual losses of stored crops from insect attack contributed significantly to the low-utilisation (Lorini and Beckel, 2006, Oerke, 2006, Godfray et al., 2010, Upadhyay and Ahmad, 2011, FAOSTAT, 2013a, FAOSTAT, 2013b). Insect infestation is induced by two types of pest: primary and secondary. Primary pests cause damage through direct-feeding on intact grains, which may involve boring inside the grains. This allows secondary pests to feed on damaged and broken grains and causing further loses. The principal insect species that commonly infest cereals and grain pulses are illustrated in **Table 1.1**. The presence of insect damaged kernels (IDK) with visible holes, undesirable remains of body parts and characteristic odours from metabolic by-products deteriorates grain crop values for

Table 1.1 Common insect pests of stored grain (WFP, 2013).

Primary pests

Sitophilus zeamais



Rhyzopertha dominica



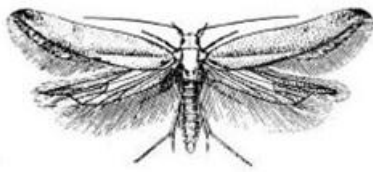
Prostephanus truncatus



Acanthoscelides obtectus



Sitotroga cerealella



Callosobruchus spp.

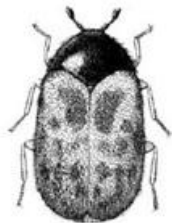


Zabrotes subfasciatus

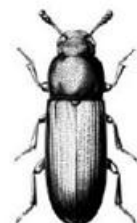


Secondary pests

Trogoderma granarium



Tribolium castaneum



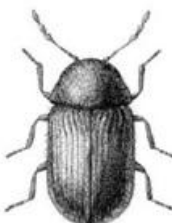
Cryptolestes spp.



Oryzaephilus surinamensis



Stegobium paniceum



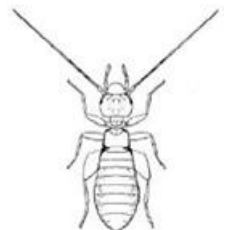
Cadra cautella



Plodia interpunctella



Psocids



Lasioderma serricorne



marketing and consumption (CGC, 2013, PulseAus, 2013). Insects feeding on the germ of a cereal can also result in poor germination or loss of viability for planting, while boring through the endosperm causes significant weight and quality loss, and creates elevated susceptibility to microorganism contamination (Hill, 2008, Teena et al., 2013). Impact on the economy has been estimated to be in the order of billions of dollars annually in developed countries, and are expected to be even higher in developing countries, although this is harder to quantify due to poor reporting systems (Oerke et al., 1999, Hodges et al., 2011, Prusky, 2011).

1.2 *Rhyzopertha dominica*: a major pest for stored grain

Rhyzopertha dominica (F.) (*R. dominica*) is a member of the *Bostrichidae* (*Coleoptera*) wood-boring family. They are primary pests that are capable of infesting to a depth of 12 metres in bulk grain stores and feed inside the kernels (Flinn et al., 2010), making early detection of *R. dominica* challenging.

R. dominica are described as being reddish- to dark-brown with average lengths of 2 to 3 mm. The head of *R. dominica* is located under the thorax when viewed from above (Potter, 1935). The optimal living conditions for *R. dominica* include temperature between 20 to 34 °C, grain moisture content (m.c.) between 12 to 14% and relative humidity (r.h.) of 50 to 60%. It is generally believed that *R. dominica* originated from the Indian subcontinent, and is now widely spread across the region spanning a latitude of 40 degrees north and south of the equator (Edde, 2012).



Figure 1.1 Images of adult (left), larvae, pupae and eggs (right) of *R. dominica*.

A single female *R. dominica* is capable of laying around 300 to 400 eggs on average (which are scattered loosely throughout the grain) over a period of 3 or more months. An average ontogenesis from egg to adult is about 58 days, depending on temperature. (Edde, 2012). The eggs hatch to produce curved white larvae with brown heads and three pairs of legs (**Figure 1.1**). The larvae burrow into slightly damaged grains and eat out the starchy interior. After pupating, the adults emerge from the grain leaving large irregular exit holes (**Figure 1.2**). The life-span of an adult insect is up to 240 days (Potter, 1935).



Figure 1.2. Damaged grains by *R. dominica* (taken with permission from wikipedia.org)

R. dominica's exoskeleton is an important multi-functional structure that supports locomotion, embed sensory organs, serves as a water-proof external covering and also as a protective lining for the gut and tracheal system that excludes toxins and helps retain internal moisture (Chapman, 2013). **Figure 1.3** illustrates the hierarchical structure and the main regions of the cuticle (Wigglesworth, 1957, Fabritius et al., 2011). It is composed of three distinct sections; non-chitinous epicuticle and chitinous procuticle, which is further divided into the exo- and endo-cuticle. The epicuticle layer is responsible for retaining water, and is comprised of an oriented monolayer of lipids which are mainly composed of long-chain hydrocarbons and wax esters, along with some polymers and bio-minerals. Underneath the lipids is the innermost cuticulin lipoproteins stratum, then the hard cement layer (Beament, 1964, Hackman, 1964). Alteration of the epicuticle hydrocarbon profile and their molecular configurations can

lead to uncontrolled loss of water (desiccation), and is most often lethal (Alexander et al., 1944a, Beament, 1964).

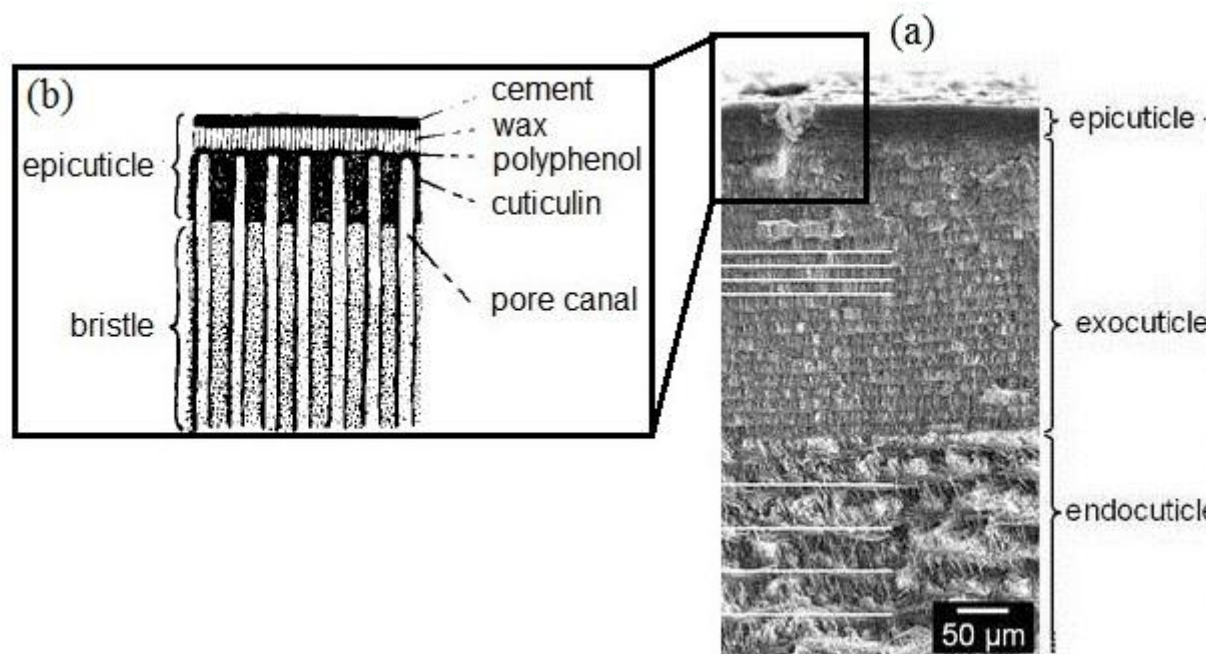


Figure 1.3 Microstructure of arthropod cuticle. (a) SEM micrograph of American lobster *Homarus americanus*, cross-section of integument showing cuticle layers composed of non-chitinous epicuticle and chitinous exo- and endocuticle, (b) schematic illustration of layers constituting the epicuticle. *Image courtesy: Fabritius, et al., 2011(a); Wigglesworth, 1948 (b).*

1.3 Integrated Pest Management

Integrated pest management (IPM) is a balanced, tactical approach used to provide effective and long-term solution to pest problems.(Bianchi et al., 2006, Manlay et al., 2007). IPM is a collective term that refers to the incorporation of various controls which are often grouped into a range of measures including: cultural, biological, mechanical and or physical; chemical and or green chemical (Kogan, 1998). These principles are combined to create a pest management program that maintains the pest population below economically injurious levels (EIL), that is “the lowest population density of pest species that will cause economic damage; or the amount of pest injury which will justify the cost of control” (Stern et al., 1959). In the case of stored grains, these standards dictate nil tolerance of live pests or evidence of pest-induced grain damage. This is particularly challenging for the design of

pesticides with excellent suppression effectiveness yet low in toxicity. These management schemes are based on a cost / profit analysis, which takes into account the interests of, and the impacts on, the producers, society and the environment. Identified control practices that pose least hazard to people, property and the environment has summarised below in **Table 1.2** (Altieri, 2002). IPM programs are site-specific where environmental factors that affect the pest and its ability to thrive are highlighted and deployed against a pest.

Using the information derived from the identification of pests, accurate measurement of pest populations, assessment of damage levels and knowledge of available tactics to create conditions that are unfavourable to the pest (Flint, 2012). It has been shown that combining a range of approaches produces much more effective overall control of the pest than using just on approach alone (Colette et al., 2001, Hassan and Bakshi, 2005). The advantages of employing IPM are:

- maintaining a balanced ecosystem with introduction and application of proper controls that protects its living things and the non-living environment
- promote a healthy environment by reducing the usage of synthetic pesticides that remain in the soil as long-lasting pollutants and as potential threat to organisms
- cost effective by avoiding unnecessary pesticide expenses
- create a good public image as demand for clean, healthy food supply without the use of synthetic pesticides are gaining much more attentions

Table 1.2 Stored grain pest control strategies used in IPM

<i>Category</i>	<i>Description</i>	<i>Advantage</i>	<i>Disadvantage</i>
Cultural	Agronomic practices designed to optimise growing and storing conditions to increase crop tolerance to pests	Preventative rather than curative , cheapest of all control measures	Highly knowledge- and skill-dependent, difficult to evaluate their effectiveness, labor and time consuming
Biological	Introduction of pests' natural enemies or through genetic modification approaches to elevate tolerance of plants under pest attack	Target specific, relatively permanent measure	Highly knowledge- and skill-dependent, great effort needed to understand ecological impacts which can also be costly
Mechanical	Manipulating storage atmosphere and temperature and/or building physical barriers like row covers or trenches	Broad-spectrum	Requires capital investment
Physical	Biophysical approaches functioned either at micro or sub-micron scale	Broad-spectrum and target specific in similar size scale	Slower in action
Bio-derived chemicals	Bio-degradable or bio-compatible chemicals	Leave no toxic residuals on the products	Larger doses required, production costs may be high
Synthetic chemicals	Artificially synthesised compound	Provide rapid and broad spectrum eradication of insect pests	Non-specific toxic effect which applies to most living organisms

1.3.1 Australian strategies and perspective for pest control of stored grain

In Australia, the most common ways for grain storage are sealed silos, unsealed silos and bunkers. Aeration cooling to control temperature and grain moisture, along with good hygiene by keeping silos and farm equipment clean and free of old grain are IPM tactics emphasized in the country (Bullen, 2007). Nevertheless, chemical fumigants including phosphine and methyl bromide are often applied to meet the strict trading policy that no live insect are to be detected at any stage of the receival process (ATSE, 2002, GTA, 2014). Despite the significant input into Australian agricultural growth and its value over the past few decades since 1940s, there has been continuing public concerns over the impact of these chemicals on human health and the environment (Davies et al., 1994, Emerson et al., 1999). An evolving market that demands products grown without use of synthetic and harmful pesticides has grown rapidly over the years. Australia has good history combating these issues by taking various tactics, one of which is the study of using inert dust, diatomaceous earth (DE) as crop protection agents (Armitage et al., 1999). Absorbacide[®] and Dryacide[®] are DE dust based product, sold as non-toxic pesticides (Entosol, 2015, MountSylviaDiatomite, 2015). Due to concerns to the negative impact it brought on grain grading quality and wearing of processing machinery, DE has been used mainly as a capping treatment and applied only the top 30 cm layer or dusted onto the surface of the grain mass (Jackson and Webley, 1994, Fields, 1998).

1.4 Chemical Insecticides

Of the available control methods for insect pests in stored grain, chemical insecticides are a major strategy for controlling insect pests, and are a key component of current IPM practices. These are a group of compounds that are formulated to kill, harm, repel or mitigate one or multiple species of insects. In addition, there are also ovicides and larvicides, which are used to target insects at specific developmental stages. Applications of these chemicals are monitored under assessment process namely maximum residual limit (MRL) to ensure the food is safe for consumption.

MRL refers to the maximum residue limit value of pesticides that are legally permitted on food which also indicate if pesticide have been used properly (Juraske et al., 2012). Classification of these substances is achieved in several ways; by their chemical structure, source, target species or toxicological action. Toxicological action (or modes of action) is a term that describes where the biochemical interactions between the pest organism and insecticides are taking place. Knowledge of the chemical's target site and their actions has practical importance to help with product selection (IRAC, 2010). **Table 1.3** lists groups insecticides, where modes of action are also indicated. A copy of the complete classification based on Mode of Action (MoA) produced by the Insecticide Resistance Action Committee's (IRAC) is attached in **Appendix 1**.

Table 1.3 Classification of major group of chemical insecticides and their modes of action (IRAC, 2010).

Chemical (organic)		
Type	Examples	Modes of Action
<i>Natural/botanical</i>	Azadirachtin	Insect growth regulator (IGR), inhibit biosynthesis of juvenile molting hormone, reduce feeding and disrupt molting
	Nicotine	Nicotinic acetylcholine receptors agonist (central nervous system ganglia), cause twitching, convulsions, death
	Petroleum oil	Desiccant, disrupts epicuticle wax layer
	Pyrethroids (Pyrethrum)	Nerve cell membrane destabiliser, cause tremor, hyperactivity, paralysis
	Rotenone	Respiratory enzyme inhibitor

<i>Synthetic</i>	Benzoylphenyl ureas (BPUs)	IGR, chitin synthesis inhibitor
	(1) Carbamate (Methomyl) (2) Organophosphate (Malathion)	Nervous system enzyme (acetylcholinesterase) inhibitor, cause paralysis
	Diacylhydrazines	IGR, ecdysone hormone mimic
	Formamidines	Enzyme (monoamine oxidase) inhibitor on neural transmitter
	Organochlorine	Acts on neurons (sodium/potassium channel and GABA receptor, γ -aminobutyric acid) to cause tremors and convulsions
	Organosulfur	Similar to pyrethrum with added ovicidal activity
	Pyriproxyfen	IGR, juvenile hormone mimic
	Chemical (inorganic)	
Types	Examples	Modes of Action
	Fumigants (phosphine and sulfuryl fluoride)	Nonspecific (multi-site) inhibitors
	Sulphur	Inhibit enzymes involved in energy production
	Arsenic	Poison, uncouple oxidative phosphorylation
Bio-rational		
Types	Examples	Modes of Action

<i>Biochemical</i>	Pheromone (Methoprene)	(1) insect repellants (2) feeding deterrents
<i>Microbiological</i>	Spinosad	Nicotinic acetylcholine receptors allosteric activators
	<i>Bacillus thuringiensis</i> (Bt) toxins	Disrupt of insect midgut membrane (Bravo et al., 2007)
	Entomopathogenic fungi, virus	Spores on the insect germinate, and penetrate through cuticle

Organosynthetic pesticides are the most widespread form of insecticide, and various types were introduced from the 1930s to 1990s; starting with chlorinated hydrocarbons, then organophosphates, methylcarbamates and most recently pyrethroids (Casida and Quistad, 1998). These chemicals share a common mode of action, and mainly attack the nervous systems of insects via one of two metabolic processes: depolarisation of cell membrane ion channels or inhibition of neurotransmitter acetylcholinesterase that interrupts homeostasis and results in death in a short period of time (Lalonde and Brown, 1954, Narahashi, 1981, Casida et al., 1983, Fukuto, 1990, Casida, 1993). The use of these broad-spectrum synthetic chemicals contributed significantly to growth in global food production in recent decades (Carvalho, 2006). However, this heavy reliance has led to detrimental impacts on the greater eco-system and human health, due to the persistent accumulation of these substances in the environment and human body, where they act as irritants, systemic poisons and potential carcinogens (Pimentel et al., 1992). Furthermore, the overuse of chemical insecticides has led to the emergence of pesticide resistant insect strains due to selection pressure (illustrated in **Figure 1.4**) (Dyte, 1974, Champ and Dyte, 1977, Dyte and Halliday, 1985), which necessitates the use of even higher quantities of pesticide to control insect infestations. Genes harbouring mutations that causes pesticide resistance have been identified, although the underlying mechanisms are yet to be fully understood (Schlipalius et al., 2002, Hardstone and Scott, 2010, Nath et al., 2011, Kaur et al., 2012, Ridley et al., 2012,

Schlupalius et al., 2012, Pittendrigh et al., 2014). Research into new synthetic insecticides to overcome the issue of resistance has been constrained due to regulatory restrictions, along with the highly expensive and prolonged development process.

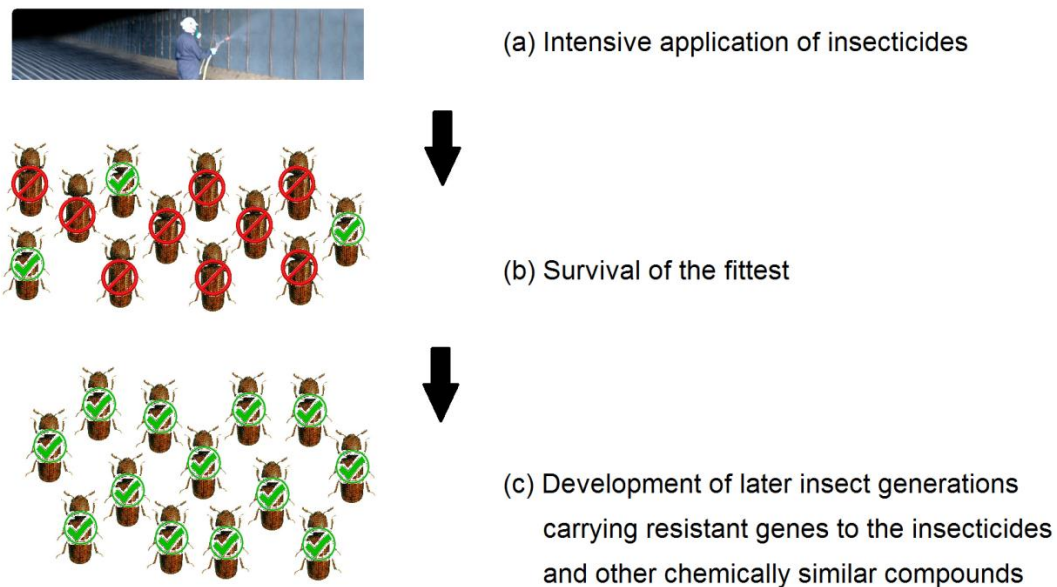


Figure 1.4 Development of insecticide resistance.

In spite of the high costs involved, chemical insecticides are still widely used in most agricultural production around the world as the main control for insect pests, especially in developing countries (Wilson and Tisdell, 2001). However, the focus of development of new types of pest control agents has shifted towards environmentally compatible, non-toxic approaches that are difficult to develop resistance to (Sunstein, 2003, Ishaaya et al., 2007, Dayan et al., 2009).

1.5 Diatomaceous Earth (DE)

DE is so far recognized as a promising candidate to address the problem of pesticide resistance because of its broad-spectrum activity against wide variety of insect pests and the low mammalian toxicity. DE consists of the fossilized remains of algae (phytoplankton also known as diatoms). It occurs as lightweight, soft and

permeable sedimentary deposits of marine rocks found along coastlines or as fresh water accumulations in lakes or marshes (Ross, 1981). Single diatomaceous earth particles (frustules) range from less than 3 μm to over 1 mm in diameter, but are typically 10 to 200 μm (**Figure 1.5**).

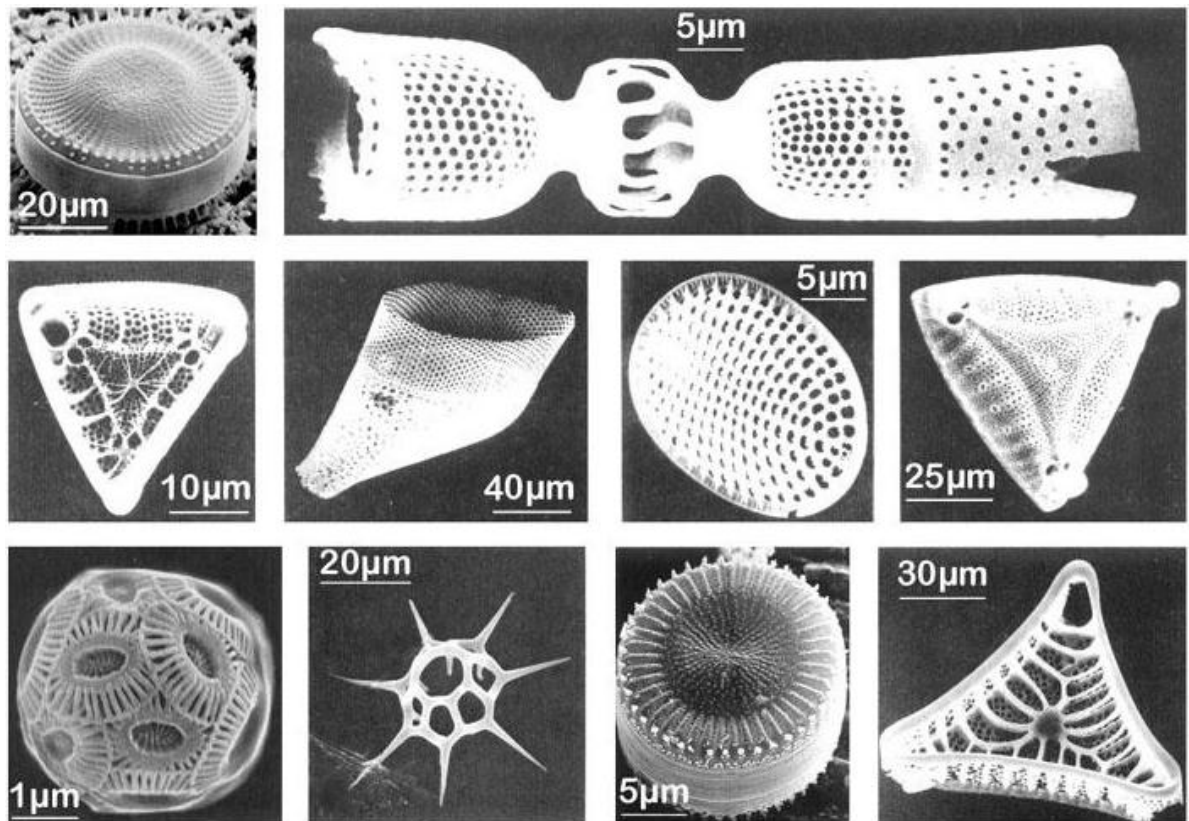


Figure 1.5 Examples of different morphology and microstructures of diatom frustules that comprise diatomaceous earth (Imgarcade.com, 2015).

Diatomaceous earth is typically comprised of 80 to 90% hydrated silica, 2 to 4% alumina and 0.5 to 2% iron oxide. It is most often used as a filtration aid, polishing additive, absorbent, reinforcement in plastics, anti-block in plastic films, porous catalysts support and as an insecticide (Dolley and Moyle, 2003, USGS, 2013)

The earliest available record that shows the control of arthropods using these abundant resources can be tracked back to four millennia ago in China where birds and mammals were observed to bathe in dirt for eliminating mites and other parasites on themselves. Civil practices of applying dusts as crop protectants were found in the Aztec culture of ancient Mexico from 14th century (Golob, 1997). More recent research

has demonstrated the broad spectrum insecticidal properties of DE against a wide range of insect pests, including *R. dominica*, with varying susceptibility (Korunic, 1998). *Tribolium spp.* was found to have the highest tolerance to the dust followed by *Rhyzopertha*. The mode of action has been linked to the sorptive and abrasive properties of DE (Faulde et al., 2006, Saez and Fuentes Mora, 2007). As insects crawl through grains covered in DE particles, their bodies pick up the fine particles, which adsorb the waxy lipid epicuticle, interfering with water balance and causing significant internal water loss (Alexander et al., 1944b, Alexander et al., 1944a). The irregular and sharp edges of DE particles can also scratch and leave open wounds that accelerate internal water evaporation (Wigglesworth, 1944). Some researchers have also postulated that the insecticide effect is due to the blockage of spiracles, the respiratory canal, or the digestive system via ingestion (Carlson and Ball, 1962). However, other experiments conducted excluded mechanisms such as suffocation, as no dust was found around insect spiracles (Imgarcade.com, 2015). Packing of fine dusts in insect's digestive tract has been observed, but no direct evidence was available to support its lethality (Carlson and Ball, 1962, Imgarcade.com, 2015). Death by desiccation remains the most favorable theory as the mode of action of DE insecticide by far (Korunic, 1998).

Factors that determine DE's efficacy as a grain protectant fall into three categories: DE, insect, and the experimental conditions (Fields et al., 2002). These parameters are summarised in **Table 1.4** and further discussed in the following sections.

1.5.1 Effect of experimental conditions

Given that DE appears to work by desiccation, decreases in humidity and grain moisture content can render DE more effective (Arthur, 2000, Fields and Korunic, 2000, Arthur, 2002, Vayias and Athanassiou, 2004, Chanbang et al., 2007). This holds true for application of DE in either wet or dry form, although dry application was found to have greater activity against insects (Korunic et al., 1998).

Table 1.4 Factors determining diatomaceous earth insecticide efficacy

<i>I. Diatomaceous earth</i>	<i>II. Insects</i>	<i>III. Experimental conditions</i>
1. Quantity (dosage)	1. Species	1. Type of crop
2. Particle size and shape	2. Strains	2. Integrity of grain kernels
3. Absorption capacity	3. Life-stage	3. Grain moisture content
4. Source of origin: salt- or fresh-water	4. Population density	4. Relative humidity
		5. Culturing temperature
		6. Storage container material
		7. Application method: wet/dry

Higher temperature is generally believed to enhance insect mortality due to DE as it (1) promotes insect movement therefore increases DE exposure and cuticular damage (Arthur, 2000), and (2) accelerates the rate of water evaporation from insects (Wigglesworth, 1945, Potier et al., 2000). Yet the optimum temperature remains inconclusive as controversial results have also been reported (Chauvin and Plusquellec, 1991, Korunic, 1998, Fitremann et al., 2007).

Higher mortality is also often associated with better adherence of DE to the surface of grains (Korunic et al., 1998). The shape and softness of different grain varieties, as well as the degree of processing, i.e. whole or milled, all influence the adhesion of DE particles (Subramanyam and Roesli, 2000, Athanassiou et al., 2003, Vayias and Athanassiou, 2004, Athanassiou et al., 2005, Kavallieratos et al., 2005, Kavallieratos et al., 2007). Furthermore, rougher grain surfaces have the ability to carry more DE particles, which facilitates contact with insect cuticle, hence causing a greater insecticidal effect (Brown and Kolb, 1955, Sahasrabudhe and Chadha, 1969).

1.5.2 Effect of insects

The type of insect species, strain and life-stage are all important factors affecting the efficacy of DE. Variations in insect behaviour between different species (clustering

or roaming) and different life stages (boring or external feeding) affect the accumulation of DE particles on the insects, and hence efficacy. Differences also exist in insect cuticle composition, both between species and at different life-stages, which may either counteract or facilitate the drying effect of DE (Vayias and Athanassiou, 2004, Germinara et al., 2009). Higher population density affects insect movement results in less contact with DE thus less damage (Roth and Willis, 1951). Morphology of the insect cuticle also plays a significant role in the efficacy of DE. Thicker, more hairy cuticles and cuticles composed with a harder, waxier composition are less susceptible to this type of insecticide (Carlson and Ball, 1962, Ebeling, 1964, Lyon, 1965).

1.5.3 Effect of DE properties

Insect mortality due to the accumulation of DE particles on insects moving through grain is directly related to the concentration of DE applied to the grain (le Patourel and Singh, 1984). The roughness of the individual DE particles is also an important factor, with rougher DE particles tending to accumulate on insects more readily, leading to a greater insecticidal effect (Smith and Goodhue, 1942, Korunic, 1998, Saez and Fuentes Mora, 2007). The absorption capability of the DE particles has a decisive connection to insecticidal efficacy, where DE particles with greater surface area have a higher capacity to adsorb wax molecules, reducing the dose needed to cause insect mortality (Ebeling, 1971, McLaughlin, 1994, Korunic, 1998). Efficacy also depends on the silica content of the DE (McLaughlin, 1994, Saez and Fuentes Mora, 2007), which varies based on where the DE is sourced (freshwater or saltwater deposits). DE collected from marine deposits has been shown to be more effective; requiring only half the effective dosage compared to DE of freshwater origin (Golob, 1997, Schmitz et al., 2007). Interestingly, this dependence on silica content occurs even though it has been shown that the mode of action is not chemically related (Alexander et al., 1944a, imgarcade.com, 2015).

1.5.4 Advantages and disadvantages

Researchers have concluded that the mode of action of DE on insects works mainly via a physical mechanism; with little effect due to its chemical nature (Alexander et al., 1944a, Alexander et al., 1944b). This is a very important advantage of this type of insecticide. Genetic mutation is easily triggered under selection pressure when insects are frequently exposed to pesticides that act via interruption of very specific biochemical processes. In contrast, inert dusts like DE cause insect mortality by a much slower, systematic approach by targeting the overall covering cuticles, which minimises the likelihood of genetic resistance. Nevertheless, a change in behavioral response to the dust and avoidance of contact within arthropod communities is possible (J. Jennings, personal communications, July 15, 2013). Further advantages of DE include its long protection time (does not degrade), and very low toxicity to mammals and other higher animals (de Lisle, 1970, Fernandez et al., 1998), and the low impact on the soundness (baking qualities) of the resulting flour and baked products (Korunic et al., 1996). Despite the prominent and lasting insecticidal effects on insect activity and low mammalian toxicity of DEs, the challenges facing the use of DE in commercial agriculture are still significant (Quarles, 1992). The recommended dosage for DE application ranges from 500 to 3500 parts per million (ppm), which is much greater compared with 0.05 to 20 ppm required on conventional chemical pesticides. At these high levels, DE was reported to alter grain colour to a less pleasant, paler to off-white appearance. Large amount of DE powder can cause handling difficulties and induce respiratory tract irritation on the workers. Bulk density (also known as hectoliter mass) and angle of repose are important indicators for grain handling and milling quality, which are influenced by the percentage of bulk volume occupied by grain, its density, surface characteristic and coefficient of friction (Marshall et al., 1986, Kunert et al., 2007). Higher dosages of DE on grain kernels will raise the friction between kernels, causing reduced flowability (grain handling issues) and decreased bulk densities, which consequently degrades commodity value (Korunic et al., 1998). Reflecting on its abrasive nature, there is a

general concern over the potential for DE treated grain to increase wear on processing machinery.

1.6 Sugar fatty acid esters

Sugar ester is a new type of insecticide that has the advantage of having low mammalian toxicity, so residue limits are much less of a safety concern. SE are 'green chemicals' that have been used as generally regarded as safe (GRAS) ingredient in food, cosmetics and the pharmaceutical industry (Osipow et al., 1956), owing to their antimicrobial activity, biocompatibility and biodegradability, as well as plasticising and antistatic properties (LeHen-Ferrenbach and Hill, 2010). Sugar esters are produced by a variety of plants e.g. *Lycopersicon* (wild tomato), *Nicotiana* (tobacco plants), *Solanum* (wild potato) and *Petunia* of the Solanaceae family (MountSylviaDiatomite, 2015), and in the early 1980's, these compounds were identified as being the primary contributor to the long known insect deterrent properties of these plants (Roush and McKenzie, 1987). Sugar esters are considered as attractive alternative for pest control because of their nontoxicity to mammals, selectivity, abundance and relatively low cost.

1.6.1 Insecticidal activity

Apart from being broad-spectrum (a term referring to the capability of effective control over multiple insect species), the inhibition mechanism of sugar esters is also target specific (Michaud and McKenzie, 2004). Specifically, inhibition involves ovipositional and feeding deterrence upon direct contact (Colette et al., 2001, Flint, 2012, Luz et al., 2012). Studies on the chemical properties of sugar esters have identified two key factors that relate to the insecticidal activity; the degree of substitution on the saccharide (sugar) group and the chain length of the fatty acid (Chortyk et al., 1996). The term monoester refers to the replacement of hydroxyl

esters has been proposed to be either via suffocation (blocking spiracles with condensed water) or desiccation (penetrating into and disrupting the waxy epicuticle layer by emulsification) (Puterka et al., 2003) (Ebeling, 1971) (Stadler and Buteler, 2009). Both actions are well related to its wetting properties, which can be understood in terms of hydrophilic-lipophilic balance (HLB) values. HLB value refers to the balance between hydrophile and lipophile groups of a surfactant; a system proposed by W. C. Griffin in 1949 to help determine the emulsification performance of surfactants. **Table 1.5** shows different HLB values and their correlation with functional properties as described by Griffin's method for calculation (Griffin, 1949).

Table 1.5 HLB values and the related functional properties.

Oil soluble		Water dispersible		Water soluble		
0	3	6	9	12	15	18
<i>HLB value</i>				<i>Functional property</i>		
2-3				Anti-foaming agent		
3-6				Water-in-oil emulsifier		
7-9				Wetting and spreading		
8-16				Oil-in-water emulsifier		
13-15				Detergents		
15-18				Stabiliser		

However, it was found in previous studies that HLB value doesn't fully correlate with insecticidal efficacy, indicating that more mechanisms underlie insecticidal activity,

such as surface chemistry and the structural physiology of both insects and the grain that is treated (Puterka et al., 2003).

In this regard, contact angle is perhaps a more suitable means of quantifying surface wetting properties, which not only takes into account the chemical nature of the liquid phase (water), but also the surface chemistry (hydrophobic or hydrophilic) and the surface texture e.g. water on a smooth wax surface has a contact angle greater than 90° (100-110°) whereas a rough surface can further increase this to extreme hydrofuge (Noble-Nesbitt, 1970). In this instance, the capillary force (F_c) (**Equation 1.1**), or the wetting force, based on the contact angle between the rim of the liquid and the capillary tube wall (**Figure 1.7**), determines whether liquid gets drawn in ($\theta < 90^\circ$) or out ($\theta > 90^\circ$) from tracheoles (although the threshold value may vary due to irregular surfaces and wetting hysteresis) (Wasserthal, 1996).

$$F_c = 2\pi r \sigma \cos\theta$$

Equation 1.1 Wilhelmy balance method, where “ F_c ” is the wetting force, “ r ” is the radius of the tube, “ σ ” is the surface tension (N.m⁻¹) and “ θ ” is the contact angle (Yuan and Lee, 2013).

Importantly, contact angle data has been previously correlated with the killing effects of essential oils (Gillilan, 2012) and herbicides (Singh et al., 1984). Contact angle of less than 40° was found to improve pesticide efficacy and cause rapid knockdown of arthropods (Hollis et al., 2015). Therefore, an understanding of the contact angle of wet applied sugar esters that come into contact with insects may give some clues as to the mode of action (proposed suffocation and desiccation theories) (Wigglesworth, 1957, Lockey, 1988).

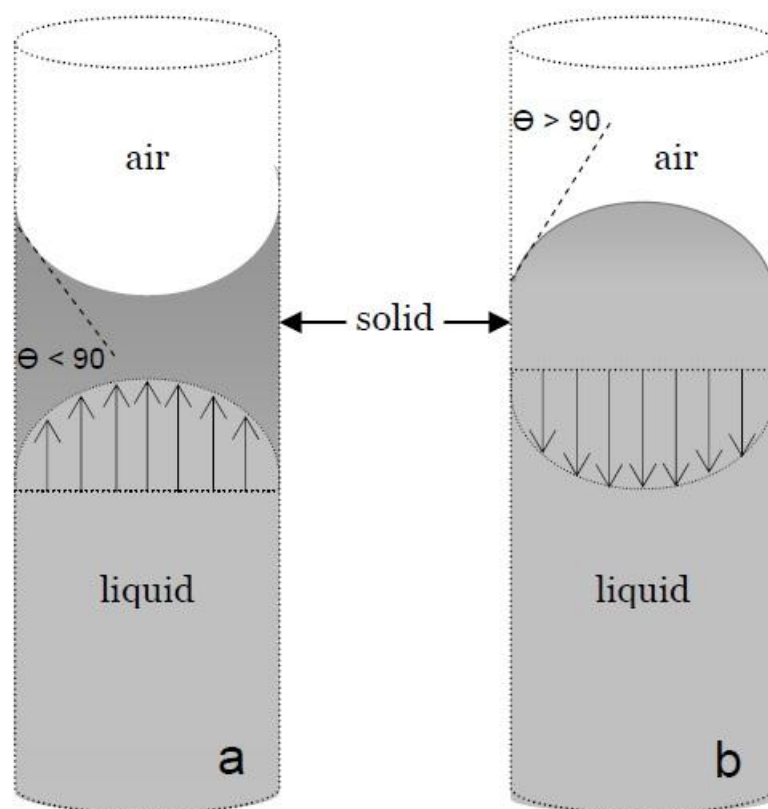


Figure 1.7 Schematic representation of a cross section of tracheoles with liquid inside. *Adopted from Stadler & Buteler, 2009.*

1.6.2 Extraction from natural sources

Extraction of sugar esters from natural sources (e.g. tobacco/tomato/potato leaves) can be accomplished by the whole-leaf wash method. Specifically, whole tobacco leaves are immersed in organic solvent (e.g. dichloromethane, CH_2Cl_2) to remove the wax from the leaf surfaces. Afterward, sugar ester is extracted by soaking in an mixed solution of hexane and methanol (Severson et al., 1985b). A mixture of many different sugar esters is often obtained (**Figure 1.8**) which are identified using analytical techniques such as FTIR, NMR and GC/MC (Severson et al., 1985b, Farone et al., 2002).

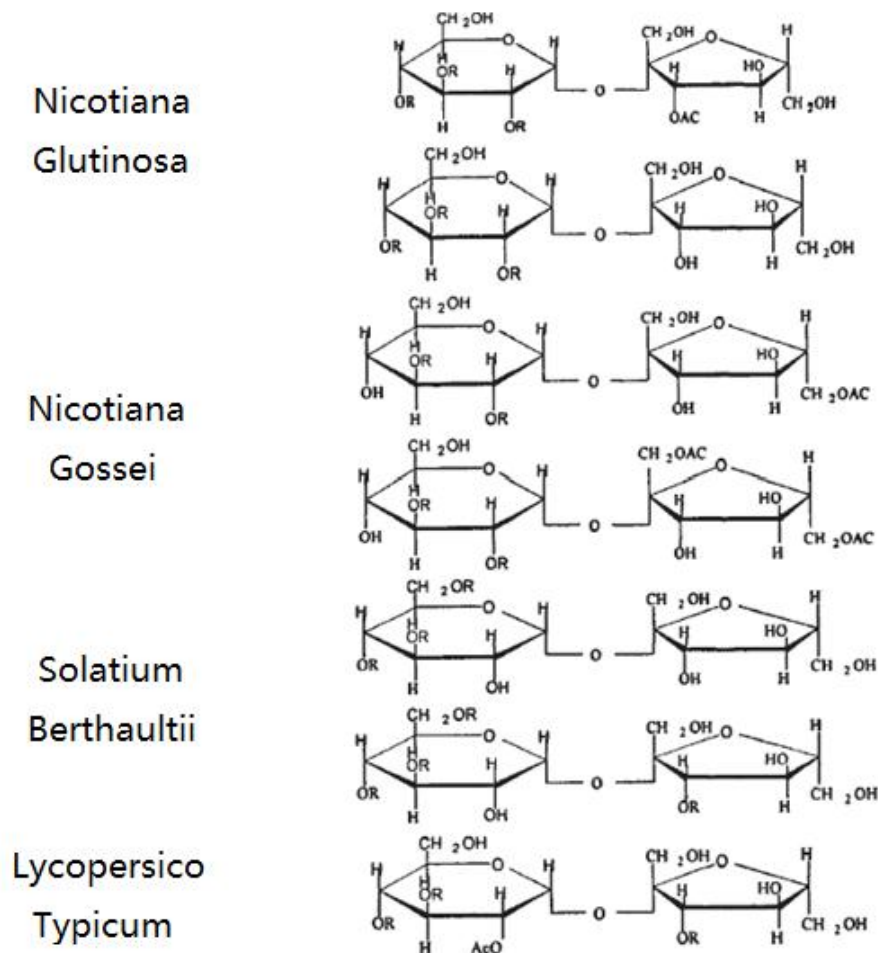


Figure 1.8 Molecular structures of naturally produced sugar esters (Severson et al., 1985a, Li et al., 2008, Wang, 2008).

However, extraction of sugar ester from natural sources for industrial application is impractical, owing to the small quantities that can be extracted. The highest production of sugar esters was found in the leaf of wild tomato (*Lycopersicon pennellii*), which contains about 400 μg per square centimetre of leaf (Denholm and Rowland, 1992). The average quantity reported for *Nicotiana* spp. is generally below 100 $\mu\text{g}/\text{cm}^2$, and the highest yield found in *Nicotiana trigonophylla* (158 $\mu\text{g}/\text{cm}^2$) still works out to produce only 2.8 g of SE for every 1 kg of plant leaf (Koiwai et al., 1983). Given the utilisation rate is lower than 1 %, along with the potential labour and other associated costs, commercialisation via this method is not viable.

1.6.3 Synthetic methods

Alternatively, carbohydrate technology (the study of chemical synthesis using polyfunctional carbohydrates as raw material) can provide a solution. Artificial synthesis started in 1960's, where transesterification methods was established as the first viable commercial route (Polat et al., 1997b). Since then, commercial methods to produce SEs through esterification, acylation or ester exchange reactions between sugar and fatty acids or their derivatives have been developed (Nicolaou and Mitchell, 2001). In most cases, a mixture of monoester, diester or polyester would be obtained via these methods due to the similar reactivities of the primary hydroxyl groups (Chortyk et al., 1996). Based on the type of reaction, sugar ester can be synthesised by several methods, including: transesterification method, acyl chloride esterification method, regioselective chemical synthesis method, regioselective enzymatic synthesis.

1.6.3.1 Transesterification method

In organic chemistry, transesterification is the process that the organic group R'' in an ester exchanges with the organic group R' in an alcohol. This transesterification reaction is often catalysed by acidic or basic catalysts (**Figure 1.9**).

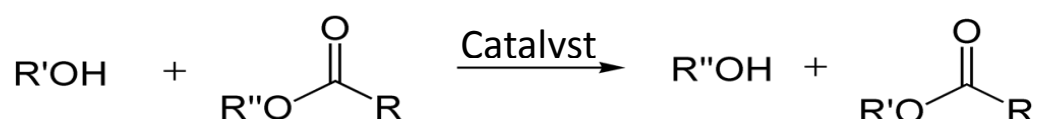


Figure 1.9 Transesterification reaction.

In case of fabricating sugar ester, the reaction can be further modified (**Figure 1.10**), where R represents the functional group in the corresponding fatty acid. For

example, when fatty acid methyl ester starts the transesterification reaction with sugar, methanol is formed and subsequently removed by evaporation at elevated temperatures. When the transesterification reaction is carried out without catalyst or at low temperature, the reaction is unpractically slow. However, too high temperature will cause the loss of solvent or decomposition of reactants. Thus, the use of catalyst is inevitable to accelerate the transesterification reaction (Plat and Linhardt, 2001).

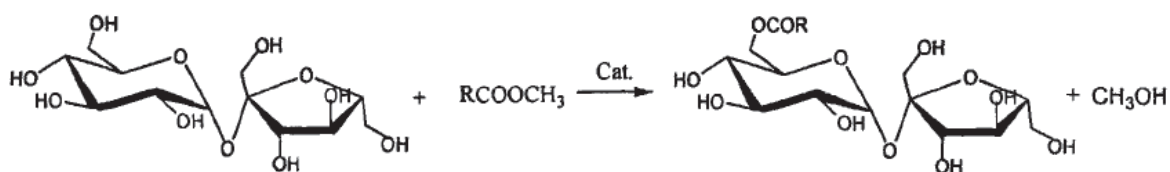


Figure 1.10 Transesterification reaction of sucrose forming sugar ester.

According to the reaction processes, transesterification methods include; organic solvent method, aqueous method, solvent-less method and micro-emulsion method.

(a) Organic solvent method

In an organic solvent method, dimethylformamide (DMF) is firstly used as the solvent (Cruces et al., 2001). Fatty acid methyl ester and catalyst (e.g. K₂CO₃) are dissolved in DMF at low pressure (12 KPa and 100 °C). Transesterification was carried out for ca. 3 to 5 h; and during this process, the produced methanol was removed by heating. The solvent and unreacted starting materials are subsequently removed through a purification process to obtain the sugar ester. Besides K₂CO₃, KHCO₃, NaOH or NaHCO₃ can also be used as the catalyst for this reaction. Due to the difficulty in removing the toxic DMF solvent from the resultant sugar ester, this method cannot be used to produce sugar esters for use with food products (Xiaojie Liu, 2006).

(b) Microemulsion method

Propylene glycol is another organic solvent that can be used to synthesise sugar ester. This method is also called the micro emulsion method (Osipow and Rosenblatt, 1967). Sugar, fatty acid methyl ester, catalyst and sodium aliphataate (emulsifier) are blended in propylene glycol. In this process, a micro-emulsion with sizes of 10 to 60 nm is formed and transesterification is carried out in this system at ca. 100 °C. The advantage of this method lies in the high yield of monoester. However, large amounts of emulsifier are required, which makes purification of the product difficult.

(c) Aqueous solvent method

Similar to the organic solvent method mentioned above, sugar ester may be formed in aqueous solution (Wang, 2008). The sugar is dissolved in water, and is then heated. Catalyst is added together with fatty acid. Afterward, the pressure is decreased and water is removed to obtain the final sugar ester. The use of toxic organic solvents can thus be avoided by using aqueous solvent instead. However, the reaction condition has to be carefully controlled to avoid the hydrolysis of fatty acid esters by the catalyst, which reduces the yield and complicates purification by leaving more unreacted starting material

(d) Solvent free method

In this route, transesterification between sugar and fatty acid ester is performed without using any solvents (Plat and Linhardt, 2001, Le Coent et al., 2003, Fitremann et al., 2007). At elevated temperatures (170 to 190 °C), fatty acid ester is dissolved in melted sugar and the transesterification reaction occurs between them. At this high temperature, sugar is unstable and is easily degraded, thus the synthesis condition has to be carefully controlled and monitored. However, the absence of solvent simplifies purification of the resulting products

1.6.3.2 Acyl chloride esterification method

In this method, acyl chloride (RCOCl) and sugar are reacted in nitrogen containing solvents (e.g. pyridine, quinolone, etc) (**Figure 1.11**) (Liu, 1999, Wang, 2008). Sugar is then acylated and raw sugar ester can be thus obtained.

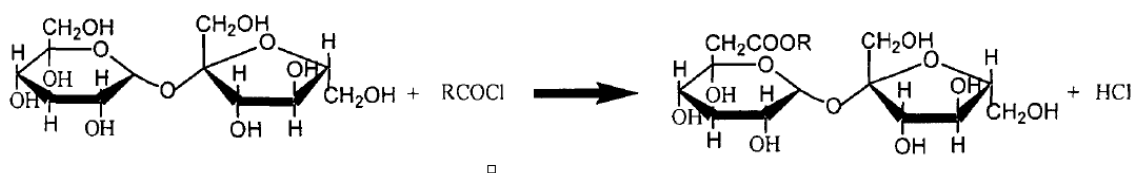


Figure 1.11 Acyl chloride esterification of sucrose forming sugar ester.

The advantage of this method lies in its high yield of monoesters. However, the high cost and toxicity of nitrogen containing solvents greatly limits its practical application. The acyl chloride esterification can also take place by dispersing sugar in excessive amount of acetic acid. Acyl chloride is then added dropwise under nitrogen atmosphere to form sugar ester. Although toxic solvents can be avoided in this process, the residual HCl in the system cannot be removed completely, which results in a product unsuited for use on food.

1.6.3.3 Regioselective chemical synthesis method

In a sugar molecule, both primary and secondary hydroxyl groups co-exists (such as at 6, 6', 1' and 2, 3, 3', 4, 4' positions in sucrose respectively, **Figure 1.12**) (Husband et al., 1998).

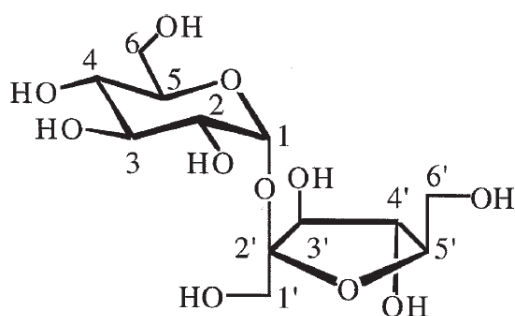


Figure 1.12 Molecular structure of sucrose.

Due to the similar reaction activity among the primary or secondary hydroxyl groups, partially substituted sugar esters with fatty acid groups on different positions will be obtained simultaneously from the above mentioned transesterification or acyl chloride esterification methods. Such sugar derivatives, which are partially substituted, are very hard to separate in the mixed products. To properly assess the effect of different degrees of esterification (mono-ester, di-ester etc) on insecticidal properties, it is desirable to selectively react fatty acids with a specific hydroxyl group on the sugar, thereby forming a single product. Hence, in the preparation of sugar esters, great care and attention should be paid to the regioselective chemical synthesis of sugar fatty acid ester. Regioselective chemical synthesis methods include:

(a) Mitsunobu esterification

Mitsunobu reaction is the reaction that converts an alcohol into different functional groups, such as ester, using triphenylphosphine (Ph_3P or TPP) and an azodicarboxylate such as diisopropyl azodicarboxylate (DEAD) (**Figure 1.13**). (Swamy et al., 2009). The reaction takes place in DMF solvent at room temperature for ca. 20 h.

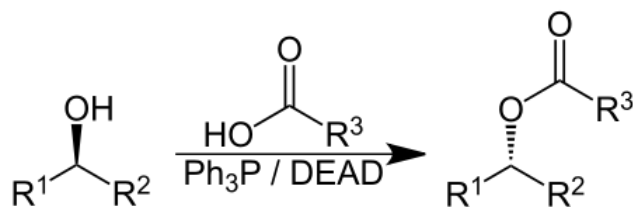


Figure 1.13 Mitsunobu esterification process.

A variety of products are often obtained via this reaction including (in order of relative quantities produced): 6, 6'-di-O-sucrose ester >> 6-O-sucrose ester > 6'-O-sucrose ester.

(b) Direct regioselective synthesis using novel acylating reagents

Catalysing the Mitsunobu reaction by alkaline acylation of sugar results in the products no longer following the priority sequence of; 6-OH = 6'-OH > 1-OH > secondary OH. Instead, the selective acylation on a single hydroxyl site is dependent on the specific acylating reagent and reaction conditions. For example, selective acylation of sucrose using 3-acyl-5-methyl-1,3,4-thiadiazole-2(3H)-thiones in DMF solvent and catalysed by 1,4-diazabicyclo[2.2.2]octane (DABCO) was reported to predominantly produce 6'-acyl sucrose ester, with a small amount of 6-acyl sucrose ester (**Figure 1.14**) (Chauvin and Plusquellec, 1991). Selecting 3-acyl-thiazolidine-2-thione or alkyl isothiocyanate as acylation reagent, and using triethylamine or DABCO as catalyst at room temperature creates 2-O-acyl sucrose at a yield over 40%, which can be further increased up to 70% when NaH is introduced into the reaction system as initiator.

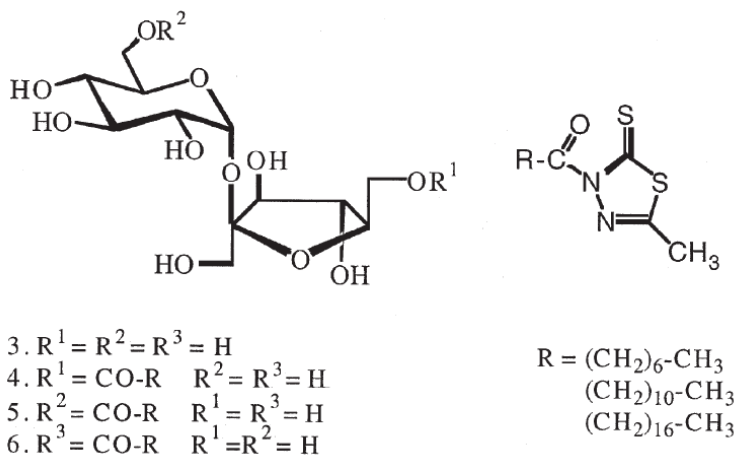


Figure 1.14 Selective acylation of sucrose using 3-acyl-5-methyl-1,3,4-thiadiazole-2(3H)-thiones.

2-O-acyl sucrose has also been shown to undergo in-situ isomerisation with 1,8-diazobicyclo [5.4.0] undec-7-ene (DBU) or trimethylamine (Et_3N) in aqueous solution (**Figure 1.15**). In this method, 6-O-acyl sucrose ester can be formed with a yield of 60% (Baczko et al., 1995).

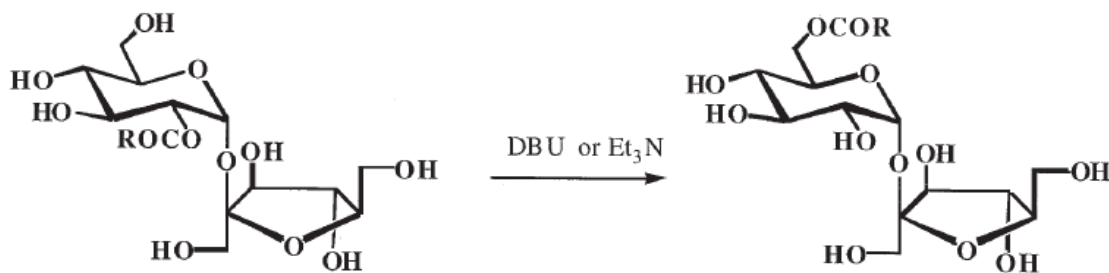


Figure 1.15 In-situ isomerisation of sucrose with DBU or Et_3N .

Another approach lies in the dibutylstannylene-acetal condensation reaction between diol and dibutylstannylene (**Figure 1.16**), which activates one of the hydroxyl groups and passivates the others in the molecule, which is beneficial for the subsequent regioselective synthesis of pure sugar esters. In a typical dibutylstannylene-acetal condensation reaction, diol is refluxed with dibutylstannylene

(Bu_2SnO or $\text{Bu}_2\text{Sn}(\text{OMe})_2$) in methanol or toluene, forming dibutylstannylene-acetal (**Figure 1.17**). Afterward, the dibutylstannylene-acetal intermediate could directly and regioselectively reacted with electrophilic groups (R') catalysed by alkaline in either aqueous or organic solvents. (Vlahov et al., 1997). In the case of synthesising sugar ester by this method, sucrose is firstly reacted with dibutylstannylene by refluxing in methanol to form dibutylstannylene acetal (6-O-acyl sucrose). Then the acetal is separated and reacted with fatty acid anhydrides ($(\text{RCO})_2\text{O}$) or chloride (RCOCl) to form sugar monoester.

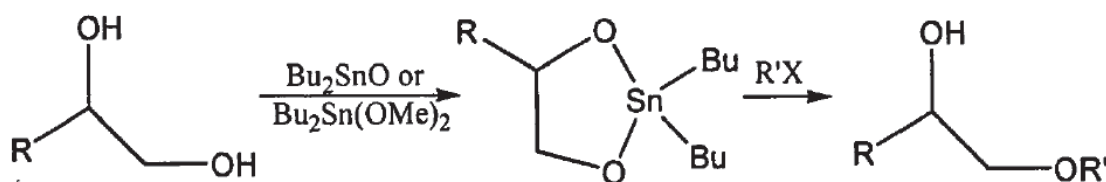


Figure 1.16 Process of dibutylstannylene-acetal condensation forming dibutylstannylene-acetal.

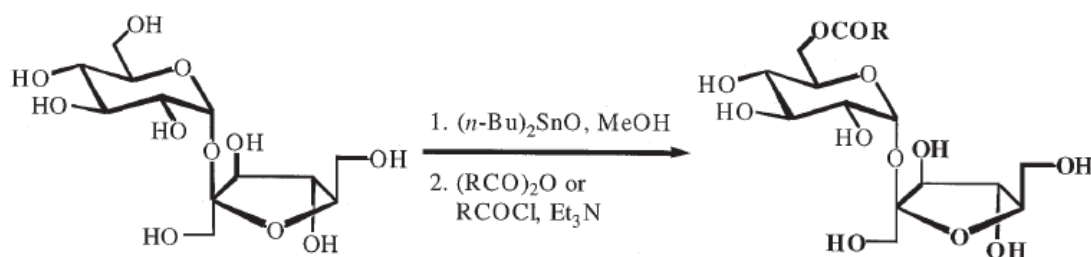


Figure 1.17 Process of sucrose ester formation by dibutylstannylene-acetal condensation.

1.6.3.4 Regioselective enzymatic synthesis

Enzymatic esterification of sugar and fatty acid can take place in organic or aqueous solvents; catalysed by lipase or protease (**Figure 1.18**). Generally, the acylation activity of different hydroxyl groups on a sugar (such as sucrose) catalysed by enzyme ($1'\text{-OH} = 6\text{-OH} > \text{secondary OH} > 6'\text{-OHs}$) is different from the activity sequence in the regioselective chemical method ($6\text{-OH} = 6'\text{-OH} > 1\text{-OH} > \text{secondary OH}$) (Patil et al., 1991). Apart from this, different enzymes also have different catalytic activities on specific hydroxyl sites e.g. lipase of *Candida antarctica* catalyses acylation at $1'\text{-OH}$, while the protease subtilisin catalyses acylation at $6'\text{-OH}$ (Riva et al., 1988).

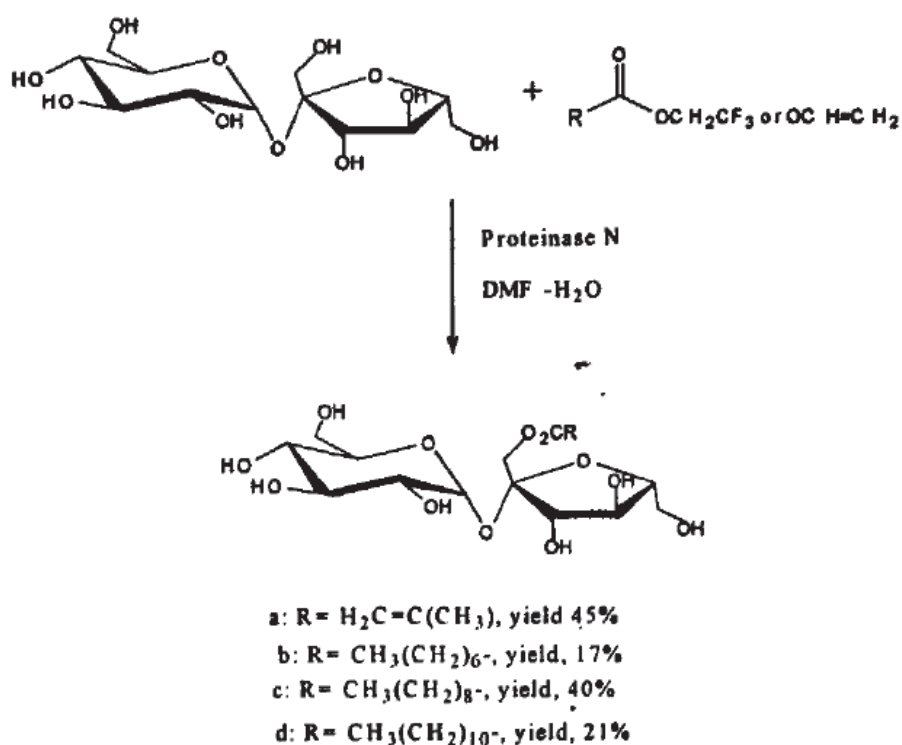


Figure 1.18 Synthesis of sugar esters using enzymes as catalysts.

Usually, regioselective enzymatic synthesis of sugar ester is carried out in organic solvents such as DMF, DMSO or pyridines, which are all toxic and thus make the sugar ester products from this reaction less favorable for application in food. Recently,

other non-toxic solvent (e.g. tert-butanol) or solvent-free methods have been developed. According to the different types of enzyme employed, regioselective enzymatic synthesis can be divided into lipase catalysed and protease catalysed.

(a) *Lipase-catalysed sugar esterifications*

Sugar has been esterified with different fatty acids, catalysed by *Byssoschlamys fulva* NTG9 lipase in tert-butanol solvents, resulting in ca. 40 % esterification (Ku and Hang, 1995). When *Mucor miehei* lipase is applied as catalyst, a 25% yield of 6-O-acyl sucrose was predominantly formed in a solvent-free reaction system (Kim et al., 1998). The yield can be further improved when suitable solid hydrates are introduced into the reaction. Lipase from *Candida antarctica* has also been tested at 40-80 °C, which gave a similar yield of sugar fatty acid ester after a seven-day reaction (Woudenberg-van Oosterom et al., 1996).

Rather than directly using fatty acids, reaction between sugar and vinyl ester can also be catalysed by lipase to achieve with a higher conversion ratio (**Figure 1.19**). The mechanism of this increased conversion ratio lies in the enol tautomerisation, which drives the esterification equilibrium reaction towards product formation (Ferrer et al., 1999).

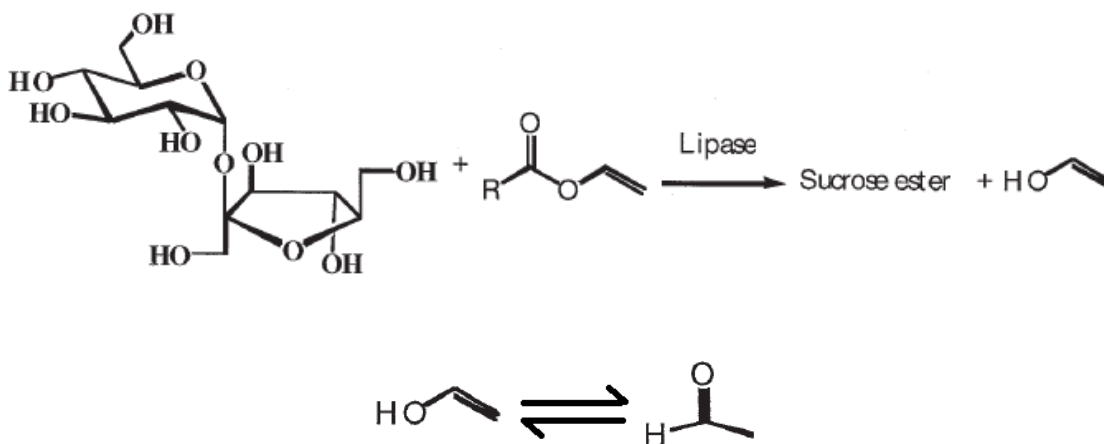


Figure 1.19 Lipase catalysed synthesis of sugar ester using vinyl ester as starting chemicals.

(b) Protease-catalysed sugar esterifications

By using Subtilisin protease in anhydrous DMF, 1'-O-butyl sucrose ester has been synthesised from sugar and trichloroethyl butyrate (**Figure 1.20**).

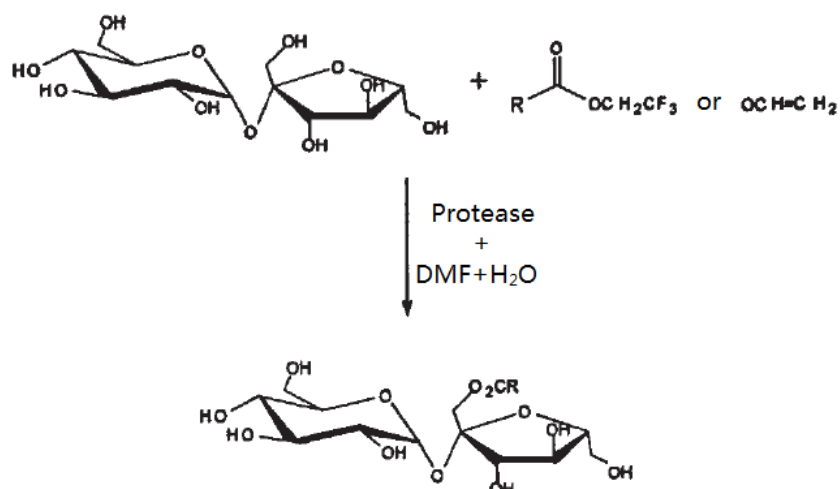


Figure 1.20 Protease catalysed formation of sucrose.

Subtilisin protease also can be applied to the synthesis of 1'-O-methacryl sucrose ester by transesterification of sucrose with trifluoroethyl methacrylate or vinyl methacrylate in anhydrous DMF (Potier et al., 2000). Further study has revealed the effect of the initial ester and water in the synthesis system, which could significantly increase the yield of final sugar esters. Other forms of Subtilisin protease, e.g. commercially available ChiroCLEC-BL, have also been studied as a catalyst, and have been found to promote the transesterification of sucrose with fatty acid vinyl esters; forming sugar esters with yields of up to 60 % (Polat et al., 1997a).

1.6.4 Advantages and disadvantages

Produced from low cost and abundant raw materials with no harmful residues, sugar esters offer an ideal candidate as a new type of control agent. Furthermore, the proposed modes of action of sugar esters are physical, meaning that the development of insect resistance is likely to be restricted (as with DE).

Synthetic compounds prepared using sorbitol, xylitol and sucrose esterified with different fatty acids have been tested at various concentrations (1000-22696 ppm) against a diverse range of insects including; mites (Puterka et al., 2003, McKenzie and Puterka, 2004), aphids (Chortyk et al., 1996, Hassan and Bakshi, 2005), whitefly (Chortyk et al., 1996, GTA, 2014) psyllids (Puterka et al., 2003, McKenzie and Puterka, 2004) and beneficial insects (Michaud and McKenzie, 2004) (**Table 1.6**). At this stage, no data on the efficacy of these compounds against stored grain pests is available. More research is needed to establish lethal effect and the dosage against *R. dominica* and other major stored grain pest insects.

Table 1.6 The effect of synthetic sugar esters against insects of various species ¹⁻⁶

Sugar	Sucrose		Sorbitol		Xylitol	
Fatty Acids	Concentration (ppm)	Mortality %, time & insect sp	Conc.(ppm)	Mortality %, time & insect sp	Conc.(ppm)	Mortality %, time & insect sp
Hexanoate C6:0			1200	0 (15mins) <i>C. pyricola</i>		
(Mono)caprate C6:0	2000	83 (2h) <i>B. tabaci</i>				
Heptanoyl C7:0	1000	95 (24h) <i>M. nicotiana</i>				
monoheptanoyl	1000	17 (24h) <i>M. nicotiana</i>				
diheptanoyl	1000	88 (24h) <i>M. nicotiana</i>				
triheptanoyl	1000	16 (24h) <i>M. nicotiana</i>				
Octanoate C8:0						
	1029	90 (24h) <i>T. citricida</i> †				
	2000	100 (2h) <i>B. tabaci</i>				
	4000	72 (36h) <i>L. dispar</i>				
	8000	36 (5d) <i>A. glycines</i>				
	1200	71 (15mins) <i>C. pyricola</i>	1200	58 (15mins) <i>C. pyricola</i>	1200	15 (15mins) <i>C. pyricola</i>
	2400	41 (15mins) <i>M. sexta</i>	2400	3 (15mins) <i>M. sexta</i>		
	2400	79 (15mins) <i>M. nicotianae</i>	2400	51 (15mins) <i>M. nicotianae</i>		
	1200	97 (15mins) <i>T. urticae</i>	2400	100(15mins) <i>T. urticae</i>		
	1300	95 (24h) <i>L. testaceipes</i>				
	6080	90 (7d) <i>D. citri</i> †				
	8000	0 (24h) <i>C. rufilabris</i>				
	8000	0 (24h) <i>H. axyridis</i>				
	8000	0 (24h) <i>O. insidiosus</i>				
	8000	5.3 (24h) <i>C. coeruleus</i>				
	8000	10.9 (24h) <i>C. sanguinea</i>				
	8000	4.7 (24h) <i>O. v-nigrum</i>				
	22696	90 (4h) <i>T. citricida</i> †				
Octanoyl	1000	85 (24h) <i>M. nicotiana</i>				
Monooctanoyl	1000	11 (24h) <i>M. nicotiana</i>				
dioctanoyl	1000	88 (24h) <i>M. nicotiana</i>				
trioctanoyl	1000	27 (24h) <i>M. nicotiana</i>				
Nonanoyl C9:0	1000	75 (24h) <i>M. nicotiana</i>				
Monononanoyl	1000	16 (24h) <i>M. nicotiana</i>				
dinonanoyl	1000	64 (24h) <i>M. nicotiana</i>				
trionanoyl	1000	13 (24h) <i>M. nicotiana</i>				
Decanoate C10:0			1200	25 (15mins) <i>C. pyricola</i>	1200	73 (15mins) <i>C. pyricola</i>
			2400	0 (15mins) <i>M. sexta</i>	2400	0 (15mins) <i>M. sexta</i>
			2400	71(15mins) <i>M. nicotianae</i>	2400	29 (15mins) <i>M. nicotianae</i>
			1200	100 (15mins) <i>T. urticae</i>	1200	100 (15mins) <i>T. urticae</i>

Dodecanoate C12:0			1200	34 (15mins) <i>C. pyricola</i>
			2400	0 (15mins) <i>M. sexta</i>
			2400	34 (15mins) <i>M. nicotiana</i>
			400	100 (15mins) <i>T. urticae</i>
Dodecanoyl	1000	64 (24h) <i>M. nicotiana</i>		
Monododecanoyl	1000	47 (24h) <i>M. nicotiana</i>		
didodecanoyl	1000	23 (24h) <i>M. nicotiana</i>		
tridodecanoyl	1000	15 (24h) <i>M. nicotiana</i>		
Palmitate C16:0	2000	93 (2h) <i>B. tabaci</i>		
Monopalmitate	2000	88 (2h) <i>B. tabaci</i>		
Oleate C18:1	2000	100 (2h) <i>B. tabaci</i>		

*concentration enlisted is either the highest tested or the lowest required to cause over 90% mortality

† spray petri dish bioassay system with a detached leaf substrate

‡ residual petri dish bioassay system

B. tabaci = *Bemisia tabaci* Biotype B (Sternorrhyncha : Aleyrodidae)

C. coeruleus = *Curinus coeruleus* Mulsant

C. pyricola = Pear Psylla *Cacopsylla pyricola* Foerster

C. rufilabris = lacewing *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae)

C. sanguinea = ladybeetles *Cycloneda sanguinea* L.

D. citri = Asian citrus psylla, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae)

H. axyridis = *Harmonia axyridis* Pallas

M. nicotiana = Tobacco Aphid *Myzus nicotiana* Blackman

M. sexta = Tobacco Hornworm *Manduca sexta* [Johannson]

O. insidiosus = *Orius insidiosus* (Say) (Hemiptera:Anthocoridae)

O. v-nigrum = *Olla v-nigrum* Mulsant (Coleoptera: Coccinellidae)

T. citricida = Brown Citrus Aphid *Toxoptera citricida*

References:

1. Chortyk, Pomonis et al. 1996
2. Puterka, Farone et al. 2003,
3. McKenzie and Puterka 2004
4. McKenzie, Weathersbee III et al. 2004
5. Michaud and McKenzie 2004
6. Alves, Boscolo et al. 2008

1.7 Summary: challenges in the control of stored grain pests

Chemical pesticides have been used in most regions of the world to manage insect infestation on storage grains. Regulatory authorities, however, have been restricting the use of these materials due to unfavourable mammalian toxicity, concerns about worker safety, and their impact on the environment. In addition, most markets are now reluctant to accept grain with chemical residues. Moreover, many target pests have developed resistance to various chemical treatments which considerably limits their use in future. The quick fix of applying a different chemical is no longer acceptable because of associated costs to society including environmental pollution that will take decades to recover, our health and threats to the eco-system. There is a world-wide need for non-toxic natural alternatives such as DE, which has proven itself to be a useful control agent. However, the relatively large doses required for efficacy have limited its application and market acceptance. SE appears to be an ideal new type of insecticide, however there is little known about its effectiveness against stored product pests like LGB.

1.8 Aims and Objectives

The low cost, low mammalian toxicity and demonstrated insecticidal activity of sugar esters makes them good candidates for use in protecting stored grain from insect pests. However, no data on the efficacy of sugar esters against key grain pests is currently available. Thus, the purpose of this project is to study the insecticidal properties of sugar esters against *R. dominica*, a major stored grain pest in Australia. A variety of sugar esters with different fatty acid chain lengths and sugar head groups will be synthesised using a solvent free approach (as this gives good yield, control over the degree of esterification and avoids toxic solvent residues) and then characterised. Their efficacy against *R. dominica* will be assessed by bioassay (mortality and progeny production). If found to be effective, combinations of the most effective sugar ester with DE will also be investigated, in an effort to further reduce the required dose of sugar ester while maintaining low concentrations of DE, thereby avoiding the problems associated with high doses of each material.

The aims and objectives of this project are:

Aim 1: To synthesise several sugar esters with different sugars and lipid acid to test as potential new control agent for stored grain pest management

Objective 1: To synthesise sorbitol and xylitol esters of octanoic acid (C8), decanoic acid (C10) and lauric acid (C12) using a solvent free approach.

Objective 2: To characterise the physical and chemical properties of synthesised products

Objective 3: To purify the resulting sugar ester products from unreacted materials by a phase separation method.

Aim 2: To evaluate the efficacy of sugar esters against *R. dominica*.

Objective 1: To investigate the effect of fatty acid chain length and different sugars on the efficacy of sugar esters against *R. dominica*.

Objective 2: To examine the effect of the purification process on sugar ester insecticidal activity.

Objective 3: To study the efficacy of combinations of sugar esters and diatomaceous earth against *R. dominica*.

Objective 4: to provide possible explanations of the mode of action of these formulations

1.9 Thesis Structure

The thesis is structured with 6 chapters (and 4 appendices to cover supplemental information) included in this thesis. This section provides a short summary of all the chapters involved.

Chapter 1 introduces the background of pest control and management strategies, the issue of chemical resistance, and describes promising alternative methods for overcoming pesticide resistance. The purpose of this work is then explained, and the aims and objectives are outlined.

Chapter 2 provides experimental details, including materials and methods that are followed in chapter 3, 4 and 5.

Chapter 3 describes the successful synthesis of sugar esters using different sugars and fatty acids with various carbon chain lengths. Results from purification of the crude product is then discussed, followed by analysis of the products using FTIR and NMR (for their molecular structure) as well as tensiometer (to determine the surface wettability).

Chapter 4 presents the results of bioassays of the sugar esters against LGB. Trends in efficacy are discussed and the modes of action are also investigated by both optical and electron microscopic methods.

Chapter 5 demonstrates the effect of combining sorbitol octanoate with DE dust in comparison with each individual component. Lethal doses are determined, to understand if any synergy between the DE and sugar ester are present in the mixture. The impact of the formulation on grain quality was also studied via the changes in bulk density and angle of repose.

Chapter 6 summarises the research results for this thesis and provides a perspective for future applications of sugar esters combined with DE as pest control agents in stored grain.

Supplementary information is provided in the appendices, where appendix 1 presents the IRAC MoA Classification Scheme, and appendix 2, 3, 4 and 5 included complete NMR spectra, supplementing insect mortality data, contact angle and SEM images, respectively.

References

Alexander, P., J. A. Kitchener and H. V. A. Briscoe (1944a). Inert dust insecticides: Part II. The nature of effective dusts. Annals of Applied Biology **31**(2): 150-156.

Alexander, P., J. A. Kitchener and H. V. A. Briscoe (1944b). Inert dust insecticides: Part III. The effect of dusts on stored products pests other than *Calandra granaria*. Annals of Applied Biology **31**(2): 156-159.

Altieri, M. A. (2002). Agroecology: the science of natural resource management for poor farmers in marginal environments. Agriculture, Ecosystems & Environment **93**(1-3): 1-24.

Armitage, D. M., D. A. Collins, D. A. Cook and J. Bell (1999). The efficacy of siliceous dust alternatives to organophosphorus compounds for the control of storage mites. Proceedmqe of the 7th International Working Conference on Stored-product Protection Beijing, P. R. China, Sichuan Publishing House of Science and Technology.

Arthur, F. H. (2000). Toxicity of Diatomaceous Earth to Red Flour Beetles and Confused Flour Beetles (Coleoptera: Tenebrionidae): Effects of Temperature and Relative Humidity. Journal of Economic Entomology **93**(2): 526-532.

Arthur, F. H. (2002). Survival of *Sitophilus oryzae* (L.) on wheat treated with diatomaceous earth: impact of biological and environmental parameters on product efficacy. Journal of Stored Products Research **38**(3): 305–313.

Athanassiou, C. G., N. G. Kavallieratos, L. P. Economou, C. B. Dimizas, B. J. Vayias, S. Tomanović and M. Milutinović (2005). Persistence and Efficacy of Three Diatomaceous Earth Formulations Against *Sitophilus oryzae* (Coleoptera:Curculionidae) on Wheat and Barley. Journal of Economic Entomology **98**(4): 1404-1412.

Athanassiou, C. G., N. G. Kavallieratos, F. C. Tsaganou, B. J. Vayias, C. B. Dimizas and C. T. Buchelos (2003). Effect of grain type on the insecticidal efficacy of SilicoSec against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Crop Protection **22**(10): 1141–1147.

ATSE (2002). Pesticide Use in Australia. Victoria, Australia, Australian Academy of Technology Science and Engineering.

Baczko, K., C. Nugier-Chauvin, J. Banoub, P. Thibault and D. Plusquellec (1995). A new synthesis of 6-O-acylsucroses and of mixed 6,6' -di-O-acylsucroses. Carbohydrate Research **269**(1): 79 – 88.

Beament, J. W. L. (1964). The Active Transport and Passive Movement of Water in Insects. Advances in Insect Physiology **2**: 67 -129.

Bianchi, F. J. J. A., C. J. H. Booij and T. Tscharrntke (2006). Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. Proceedings of the Royal Society B: Biological Sciences **273**(1595): 1715-1727.

Bravo, A., S. S. Gill and M. Soberón (2007). Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. Toxicon **49**(4): 423–435.

Brown, J. B. and D. K. Kolb (1955). Applications of low temperature crystallization in the separation of the fatty acids and their compounds. Progress in the Chemistry of Fats and other Lipids **3**: 57-72.

Bullen, K. (2007). Insect Control in Stored Grain. Q. Department of Primary Industries and Fisheries, DPI&F, QLD.

Carlson, S. D. and H. J. Ball (1962). Mode of Action and Insecticidal Value of a Diatomaceous Earth as a Grain Protectant. Journal of Economic Entomology **55**(6): 964-970.

Carvalho, F. P. (2006). Agriculture, pesticides, food security and food safety. Environmental Science & Policy **9**(7-8): 685–692.

Casida, J. E. (1993). Insecticide action at the GABA-gated chloride channel: Recognition, progress, and prospects. Archives of Insect Biochemistry and Physiology **22**(1-2): 13–23.

Casida, J. E., D. W. Gammon, A. H. Glickman and L. J. Lawrence (1983). Mechanisms of Selective Action of Pyrethroid Insecticides. Annual Review of Pharmacology and Toxicology **23**: 413-438.

Casida, J. E. and G. B. Quistad (1998). Golden Age of Insecticide Research: Past, Present, or Future? Annual Review of Entomology **43**: 1-16.

CGC (2013). Official Grain Grading Guide. C. I. Services. Canada, Canadian Grain Commission.

Champ, B. R. and C. E. Dyte (1977). FAO Global Survey of Pesticide Susceptibility of Stored Grain Pests. FAO Plant Protection Bulletin **25**(2): 49-67.

Chanbang, Y., F. H. Arthur, G. E. Wilde and J. E. Throne (2007). Efficacy of diatomaceous earth to control *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) in rough rice: Impacts of temperature and relative humidity. Crop Protection **26**(7): 923–929.

Chapman, R. F. (2013). Insects : structure and function. New York, Cambridge University Press.

Chauvin, C. and D. Plusquellec (1991). A new chemoenzymatic synthesis of 6'-O-acylsucroses. Tetrahedron Letters **32**(29): 3495 - 3498.

Chortyk, O. T., G. J. Pomonis and A. W. Johnson (1996). Syntheses and Characterizations of Insecticidal Sucrose Esters. Journal of Agricultural and Food Chemistry **44**(6): 1551-1557.

Colette, W. A., L. K. Almas and G. L. Schuster (2001). Evaluating the Impact of Integrated Pest Management on Agriculture and the Environment in the Texas Panhandle. Western Agricultural Economics Association Annual Meetings. Logan Utah.

Cruces, M. A., F. J. Plou, M. Ferrer, M. Bernabé and A. Ballesteros (2001). Improved synthesis of sucrose fatty acid monoesters. Journal of the American Oil Chemists' Society **78**(5): 541-546.

Davies, P. E., L. S. J. Cook and D. Goenarso (1994). Sublethal responses to pesticides of several species of Australian freshwater fish and crustaceans and rainbow trout. Environmental Toxicology and Chemistry **13**(8): 1341–1354.

Dayan, F. E., C. L. Cantrell and S. O. Duke (2009). Natural products in crop protection. Bioorganic & Medicinal Chemistry **17**(12): 4022–4034.

de Lisle, A. L. (1970). Animal feed supplement and method of eliminating worms from animals. U. S. Patent. United States of America, Phoenix Gems, Inc.,.

Denholm, I. and M. W. Rowland (1992). Tactics for Managing Pesticide Resistance in Arthropods: Theory and Practice. Annual Review of Entomology **37**: 91-112.

Dolley, T. P. and P. R. Moyle (2003). History and Overview of the U.S. Diatomite Mining Industry, with Emphasis on the Western United States. U. S. G. S. U.S. Department of the Interior. **Bulletin 2209–E**.

Dyte, C. E. (1974). Problems Arising from Insecticide Resistance in Storage Pests. EPPO Bulletin **4**(3): 275–286.

Dyte, C. E. and D. Halliday (1985). Problems of development of resistance to phosphine by insect pests of stored grains. EPPO Bulletin **15**(1): 51–57.

Ebeling, W. (1964). Permeability of Insect Cuticle. he Physiology of Insecta M. Rockstein. New York, Academic Press Inc. **3**: 508-523.

Ebeling, W. (1971). Sorptive dusts for pest control. Annual Review of Entomology **16**: 123-158.

Edde, P. A. (2012). A review of the biology and control of *Rhyzopertha dominica* (F.) the lesser grain borer. Journal of Stored Products Research **48**: 1-18.

Emerson, G. M., N. McL Gray, G. A. Jelinek, D. Mountain and H. J. Mead (1999). Organophosphate poisoning in perth, western australia, 1987–1996. The Journal of Emergency Medicine **17**(2): 273–277.

Entosol (2015). Dryacide.

Fabritius, H., C. Sachs, D. Raabe, S. Nikolov, M. Friák and J. Neugebauer (2011). Chitin in the Exoskeletons of Arthropoda: From Ancient Design to Novel Materials Science. Chitin Formation and Diagenesis. N. S. Gupta. London New York, Springer Dordrecht Heidelberg 35-60.

FAO (2009). How to Feed the World in 2050. High Level Expert Forum. Rome, Agricultural Development Economics Division, Economic and Social Development Department.

FAOSTAT (2013a). Total World's Cereals (excluding beer) Supply 1961 - 2009. <http://faostat3.fao.org/home/index.html#DOWNLOAD>, FAO.

FAOSTAT (2013b). World Crops Production, Cereals Total 1961 - 2012. <http://faostat3.fao.org/home/index.html#DOWNLOAD>, FAO.

Farone, W. A., T. Palmer and G. J. Puterka (2002). Polyol ester insecticides and method of synthesis. . U. S. Patent. United State of America, Ava Chemical Ventures L.L.C.

Faulde, M. K., M. Tisch and J. J. Scharninghausen (2006). Efficacy of modified diatomaceous earth on different cockroach species (Orthoptera, Blattellidae) and silverfish (Thysanura, Lepismatidae). **79**(3): 155-161.

Fernandez, M. I., B. W. Woodward and B. E. Stromberg (1998). Effect of diatomaceous earth as an anthelmintic treatment on internal parasites and feedlot performance of beef steers. Animal Science **66**(03): 635-641.

Ferrer, M., M. A. Cruces, M. Bernabé, A. Ballesteros and F. J. Plou (1999). Lipase-catalyzed regioselective acylation of sucrose in two-solvent mixtures. Biotechnology and Bioengineering **65**(1): 10-16.

Fields, P. and Z. Korunic (2000). The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored-product beetles. Journal of Stored Products Research **36**(1): 1–13.

Fields, P. G. (1998). Diatomaceous earth: advantages and limitations. Proceedings of the 7th International Working Conference on Stored-Product Protection, Beijing, China, Sichuan Publishing House of Science and Technology.

Fields, P. G., S. Allen, Z. Korunic, A. McLaughlin and T. E. Stathers (2002). Standardized testing for diatomaceous earth. Proceedings of the 8th International Working Conference on Stored-Product Protection, Wallingford, United Kingdom, CAB International.

Fitremann, J., Y. Queneau, J.-P. Maître and A. Bouchu (2007). Co-melting of solid sucrose and multivalent cation soaps for solvent-free synthesis of sucrose esters. Tetrahedron Letters **48**(23): 4111–4114.

Flinn, P. W., D. W. Hagstrum, C. Reed and T. W. Phillips (2010). Insect population dynamics in commercial grain elevators. Journal of Stored Products Research **46**(1): 43 - 47.

Flint, M. L. (2012). IPM in practice : principles and methods of integrated pest management Oakland, University of California Division of Agriculture and Natural Resources.

Fukuto, T. R. (1990). Mechanism of action of organophosphorus and carbamate insecticides. Environmental Health Perspectives **87**: 245–254.

Germinara, G. S., A. D. Cristofaro and G. Rotundo (2009). Antennal olfactory responses to individual cereal volatiles in *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae). Journal of Stored Products Research **45**(3): 195–200.

Gillilan, J. (2012). Improvement of U.S. EPA Minimum Risk Essential Oils' Pesticide Activity Through Surfactant Enhancement and Synergy, The Ohio State University. **Doctorate of Philosophy.**

Godfray, H. C. J., J. R. Beddington, I. R. Crute, L. Haddad, D. Lawrence, J. F. Muir, J. Pretty, S. Robinson, S. M. Thomas and C. Toulmin (2010). Food Security: The Challenge of Feeding 9 Billion People. Science **327**(5967): 812-818.

Golob, P. (1997). Current status and future perspectives for inert dusts for control of stored product insects. Journal of Stored Products Research **33**(1): 69-79.

Griffin, W. C. (1949). Classification of Surface-Active Agents by 'HLB'. Journal of the Society of Cosmetic Chemists **1**(5): 311-326.

GTA (2014). GTA Wheat Standards 2014/2015. Australia, Grain Trade Australia.

Hackman, R. H. (1964). Chemistry of the insect cuticle. The Physiology of Insecta. M. Rockstein. New York, Academic Press. **3**: 471-506.

Hassan, A. R. and K. Bakshi (2005). Pest Management, Productivity and Environment: A Comparative Study of IPM and Conventional Farmers of Northern Districts of Bangladesh. Pakistan Journal of Social Sciences **3**(8): 1007-1014.

Hill, D. S. (2008). Pests of Crops in Warmer Climates and Their Control, Springer: 59-78.

Hodges, R. J., J. C. Buzby and B. Bennett (2011). Foresight Project on Global Food and Farming Futures. Journal of Agricultural Science **149**(S1): 37–45.

Hollis, S., C. McDonald and J. Rader (2015). Methods for Treating Arthropods. U. S. Patent. United States OMS Investments, Inc. **20150017116**.

Husband, F. A., D. B. Sarney, M. J. Barnard and P. J. Wilde (1998). Comparison of foaming and interfacial properties of pure sucrose monolaurates, dilaurate and commercial preparations. Food Hydrocolloids **12**(2): 237-244.

Imgarcade.com (2015). Images For (Diatomaceous Earth Microscopic View). Retrieved June 13th 2015.

IRAC (2010). Mode of Action Classification. I. R. A. Committee, Insecticide Resistance Action Committee.

Ishaaya, I., A. R. Horowitz and R. Nauen (2007). Insecticides Design Using Advanced Technologies. Netherlands, Springer Berlin Heidelberg.

Jackson, K. and D. Webley (1994). Effects of Dryacide® on the physical properties of grains, pulses and oilseeds. Proceedings of the 6th International Working Conference on Stored-product Protection - Volume 2, Canberra Australia, CABI, London.

Juraske, R., P. Fantke, A. C. R. Ramírez and A. González (2012). Pesticide residue dynamics in passion fruits: Comparing field trial and modelling results. Chemosphere **89**(7): 850-855.

Kaur, R., D. I. Schlipalius, P. J. Collins, A. J. Swain and P. R. Ebert (2012). Inheritance and relative dominance, expressed as toxicity response and delayed development, of phosphine resistance in immature stages of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). Journal of Stored Products Research **51**: 74–80.

Kavallieratos, N. G., C. G. Athanassiou, F. D. Mpakou and A. E. Mpassoukou (2007). Factors Affecting Laboratory Bioassays with Diatomaceous Earth on Stored Wheat: Effect of Insect Density, Grain Quantity, and Cracked Kernel Containment. Journal of Economic Entomology **100**(5): 1724-1731.

Kavallieratos, N. G., C. G. Athanassiou, F. G. Pashalidou, N. S. Andris and Ž. Tomanović (2005). Influence of grain type on the insecticidal efficacy of two diatomaceous earth formulations against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Pest Management Science **61**(7): 660–666.

Kim, J. E., J. J. Han, J. H. Yoon and J. S. Rhee (1998). Effect of salt hydrate pair on lipase-catalyzed regioselective monoacylation of sucrose. Biotechnology and Bioengineering **57**(1): 121-125.

Kogan, M. (1998). Integrated Pest Management: Historical Perspectives and Contemporary Developments. Annual Review of Entomology **43**: 243-270.

Koiwai, A., F. Suzuki, T. Matsuzaki and N. Kawashima (1983). The fatty acid composition of seeds and leaves of *Nicotiana* species. Phytochemistry **22**(6): 1409-1412.

Korunic, Z. (1998). Diatomaceous earths, a group of natural insecticides. Journal of Stored Products Research **34**(2-3): 87-97.

Korunic, Z., S. Cenkowski and P. Fields (1998). Grain bulk density as affected by diatomaceous earth and application method. Postharvest Biology and Technology **13**(1): 81-89.

Korunic, Z., P. G. Fields, M. I. P. Kovacs, J. S. Noll, O. M. Lukow, C. J. Demianyk and K. J. Shibley (1996). The effect of diatomaceous earth on grain quality. Postharvest Biology and Technology **9**: 373-387.

Ku, M. A. and Y. D. Hang (1995). Enzymatic synthesis of esters in organic medium with lipase from *Byssoschlamys fulva*. Biotechnology Letters **17**(10): 1081-1084.

Kunert, A., A. A. Naz, O. Dedek, K. Pillen and J. Le´on (2007). AB-QTL analysis in winter wheat: I. Synthetic hexaploid wheat (*T. turgidum* ssp. *dicoccoides* × *T. tauschii*) as a source of favourable alleles for milling and baking quality traits. Theoretical and Applied Genetics **115**(5): 683-695.

Lalonde, D. I. V. and A. W. A. Brown (1954). The effect of insecticides on the action potentials of insect nerve. Canadian Journal of Zoology **32**(2): 74-81.

Le Coent, A. L., M. Tayakout-Fayolle, F. Couenne, S. Briancon, J. Lieto, J. Fitremann-Gagnaire, Y. Queneau and A. Bouchu (2003). Kinetic parameter estimation and modelling of sucrose esters synthesis without solvent. Chemical Engineering Science **58**(2): 367-376.

le Patourel, G. N. J. and J. Singh (1984). Toxicity of amorphous silicas and silica-pyrethroid mixtures to *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Journal of Stored Products Research **20**(4): 183–190.

LeHen-Ferrenbach, C. and K. Hill (2010). Sugar-Based Surfactants. Fundamentals and Applications. C. C. Ruiz, CRC Press.

Li, S., Z. Song, Z. Liu and S. Bai (2008). Characterization and insecticidal activity of sucrose octanoates. *Agronomy for Sustainable Development* **28**(2): 239-245.

Liu, Z. (1999). Purification processes of crude sucrose ester. *Journal of Wuhan Polytechnic University*(3): 11-14.

Lockey, K. H. (1988). Lipids of the insect cuticle: origin, composition and function. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* **89**(4): 595–645.

Lorini, I. and H. Beckel (2006). Efficacy of “diatomaceous earth” to control the main stored grain pests. Proceedings of the 9th International Working Conference on Stored Product Protection, Sao Paulo, Brazil, Research Information Ltd.

Luz, C., J. Rodrigues and L. F. N. Rocha (2012). Diatomaceous earth and oil enhance effectiveness of *Metarhizium anisopliae* against *Triatoma infestans*. *Acta Tropica* **122** 29–35.

Lyon, R. L. (1965). Structure and toxicity of insecticide deposits for control of bark beetles. U. S. D. o. Agriculture. Washington, USA, USDA. **1343**.

Manlay, R. J., C. Feller and M. J. Swift (2007). Historical evolution of soil organic matter concepts and their relationships with the fertility and sustainability of cropping systems. *Agriculture, Ecosystems & Environment* **119**(3–4): 217–233.

Marshall, D. R., D. J. Mares, H. J. Moss and F. W. Ellison (1986). Effects of Grain Shape and Size on Milling Yields in Wheat. II*. Experimental Studies. *Australian Journal of Agricultural Research* **37**(4): 331-342.

McKenzie, C. L. and G. J. Puterka (2004). Effect of sucrose octanoate on survival of nymphal and adult *Diaphorina citri* (Homoptera: Psyllidae). *Journal of Economic Entomology* **97**(3): 970-975.

McLaughlin, A. (1994). Laboratory trials on desiccant dust insecticides. Proceedings of the 6th International Working Conference on Stored Product Protection, Canberra, Australia, CAB International, Wallingford, United Kingdom.

Michaud, J. P. and C. L. McKenzie (2004). Safety of a novel insecticide, sucrose octanoate, to beneficial insects in Florida citrus. *Florida Entomologist* **87**(1): 6-9.

MountSylviaDiatomite (2015). Absorbacide. 2015.

Narahashi, T. (1981). Mode of action of chlorinated hydrocarbon pesticides on the nervous system. Toxicology of Halogenated Hydrocarbons Toxicology of Halogenated Hydrocarbons M. A. Q. Khan and R. H. Stanton, Pergamon Press Inc. : 222–242.

Nath, N. S., I. Bhattacharya, A. G. Tuck, D. I. Schlipalius and P. R. Ebert (2011). Mechanisms of Phosphine Toxicity. Journal of Toxicology **2011**: 494168.

Nicolaou, K. C. and H. J. Mitchell (2001). Adventures in Carbohydrate Chemistry: New Synthetic Technologies, Chemical Synthesis, Molecular Design, and Chemical Biology. Angewandte Chemie International Edition **40**(9): 1576-1624.

Noble-Nesbitt, J. (1970). Structural aspects of penetration through insect cuticles. Pesticide Science **1**(5): 204–208.

Oerke, E.-C., H.-W. Dehne, F. Schönbeck and A. Weber (1999). Crop Production and Crop Protection: Estimated Losses in Major Food and Cash Crops. Amsterdam, Netherlands, Elsevier.

Oerke, E. C. (2006). Crop losses to pests. Journal of Agricultural Science **144**: 31-43.

Osipow, L. and W. Rosenblatt (1967). Micro-emulsion process for the preparation of sucrose esters. Journal of the American Oil Chemists Society **44**(5): 307-309.

Osipow, L., F. D. Snell, W. C. York and A. Finchler (1956). Methods of Preparation Fatty Acid Esters of Sucrose. Industrial & Engineering Chemistry **48**(9): 1459-1462.

Parks, C. G., K. Conrad and G. S. Cooper (1999). Occupational exposure to crystalline silica and autoimmune disease. Environmental Health Perspectives **107**(suppl 5): 793-802.

Patil, D. R., D. G. Rethwisch and J. S. Dordick (1991). Enzymatic synthesis of a sucrose-containing linear polyester in nearly anhydrous organic media. Biotechnology and Bioengineering **37**(7): 639-646.

Pimentel, D., H. Acquay, M. Biltonen, P. Rice, M. Silva, J. Nelson, V. Lipner, S. Giordano, A. Horowitz and M. D'Amore (1992). Environmental and economic costs of pesticide use. BioScience **42**(10): 750-760.

Plat, T. and R. Linhardt (2001). Syntheses and applications of sucrose-based esters. Journal of Surfactants and Detergents **4**(4): 415-421.

- Polat, T., H. G. Bazin and R. J. Linhardt (1997a). Enzyme Catalyzed Regioselective Synthesis of Sucrose Fatty Acid Ester Surfactants. Journal of Carbohydrate Chemistry **16**(9): 1319-1325.
- Polat, T., H. G. Bazin and R. J. Linhardt (1997b). Enzyme Catalyzed Regioselective Synthesis of Sucrose Fatty Acid Ester Surfactants. Journal of Carbohydrate Chemistry **16**(9): 1319-1325.
- Potier, P., A. Bouchu, G. Descotes and Y. Queneau (2000). Proteinase N-catalysed transesterifications in DMSO–water and DMF–water: preparation of sucrose monomethacrylate. Tetrahedron Letters **41**(19): 3597–3600.
- Potter, C. (1935). The biology and distribution of *Rhizopertha dominica* (Fab.). Transactions of the Royal Entomological Society of London **83**(4): 449-482.
- Prusky, D. (2011). Reduction of the incidence of postharvest quality losses, and future prospects Food Security **3**(4): 463–474.
- PulseAus (2013) Australian Pulse Standards 2013/2014. 1-98
- Puterka, G. J., W. Farone, T. Palmer and A. Barrington (2003). Structure-Function Relationships Affecting the Insecticidal and Miticidal Activity of Sugar Esters. Journal of Economic Entomology **96**(3): 636-644.
- Quarles, W. (1992). Diatomaceous earth for pest control. The IPM Practitioner **14**(5/6): 1-16.
- Ridley, A. W., D. I. Schlipalius and G. J. Daghish (2012). Reproduction of phosphine resistant *Rhizopertha dominica* (F.) following sublethal exposure to phosphine. Journal of Stored Products Research **48**: 106–110.
- Riva, S., J. Chopineau, A. P. G. Kieboom and A. M. Klibanov (1988). Protease-catalyzed regioselective esterification of sugars and related compounds in anhydrous dimethylformamide. Journal of the American Chemical Society **110**(2): 584-589.
- Ross, T. E. (1981). Diatomaceous earth as a possible alternative to chemical insecticides. Agriculture and Environment **6**(1): 43–51.
- Roth, L. M. and E. R. Willis (1951). Hygroreceptors in Coleoptera. Journal of Experimental Zoology **117**(3): 451–487.

- Roush, R. T. and J. A. McKenzie (1987). Ecological Genetics of Insecticide and Acaricide Resistance. Annual Review of Entomology **32**: 361-380.
- Saez, A. and V. H. Fuentes Mora (2007). Comparison of the desiccation effects of marine and freshwater diatomaceous earths on insects. Journal of Stored Products Research **43**(4): 404-409.
- Sahasrabudhe, M. R. and R. K. Chadha (1969). Chromatographic analysis of sorbitan fatty acid esters. Journal of the American Oil Chemists Society **46**(1): 8-12.
- Schlupalius, D. I., Q. Cheng, P. E. B. Reilly, P. J. Collins and P. R. Ebert (2002). Genetic linkage analysis of the lesser grain borer *Rhyzopertha dominica* identifies two loci that confer high-level resistance to the fumigant phosphine. Genetics **161**(2): 773-782.
- Schlupalius, D. I., N. Valmas, A. G. Tuck, R. Jagadeesan, L. Ma, R. Kaur, A. Goldinger, C. Anderson, J. Kuang, S. Zuryn, Y. S. Mau, Q. Cheng, P. J. Collins, M. K. Nayak, H. J. Schirra, M. A. Hilliard and P. R. Ebert (2012). A core metabolic enzyme mediates resistance to phosphine gas. Science **338**(6108): 807-810.
- Schmitz, A., A. Sehrbrock and H. Schmitz (2007). The analysis of the mechanosensory origin of the infrared sensilla in *Melanophila acuminata* (Coleoptera; Buprestidae) adduces new insight into the transduction mechanism. Arthropod Struct Dev. **36**(3): 291-303.
- Severson, R. F., R. F. Arrendale, O. T. Chortyk, C. R. Green, F. A. Thome, J. L. Stewart and A. W. Johnson (1985a). Isolation and characterization of the sucrose esters of the cuticular waxes of green tobacco leaf. Journal of Agricultural and Food Chemistry **33**(5): 870-875.
- Severson, R. F., R. F. Arrendale, O. T. Chortyk, R. Green, F. A. Thome, J. L. Stewart and A. W. Johnson (1985b). Isolation and characterization of the sucrose esters of the cuticular waxes of green tobacco leaf. Journal of Agricultural and Food Chemistry **33**(5): 870-875.
- Singh, M., J. R. Orsenigo and D. O. Shah (1984). Surface tension and contact angle of herbicide solutions affected by surfactants. Journal of the American Oil Chemists' Society **61**(3): 596-600.
- Smith, C. M. and L. D. Goodhue (1942). Particle Size in Relation to Insecticide Efficiency. Industrial & Engineering Chemistry **34**(4): 490-493.

- Stadler, T. and M. Buteler (2009). Modes of entry of petroleum distilled spray-oils into insects: a review. Bulletin of Insectology **62**(2): 169-177.
- Stern, V. M., R. F. Smith, R. van den Bosch and K. S. Hagen (1959). The integrated control concept. Hilgardia **29**: 81-101.
- Subramanyam, B. and R. Roesli (2000). Inert Dusts. Alternatives to Pesticides in Stored-Product IPM. B. Subramanyam and D. W. Hagstrum: 321-380.
- Sunstein, C. R. (2003). The Cost-Benefit State: The Future of Regulatory Protection. American Bar Association.
- Swamy, K. C. K., N. N. B. Kumar, E. Balaraman and K. V. P. P. Kumar (2009). Mitsunobu and Related Reactions: Advances and Applications. Chemical Reviews **109**(6): 2551-2651.
- Teena, M., A. Manickavasagan, A. Mothershaw, S. El Hadi and D. S. Jayas (2013). Potential of Machine Vision Techniques for Detecting Fecal and Microbial Contamination of Food Products: A Review. Food and Bioprocess Technology **6**(7): 1621-1634.
- UN (2009). World Population To Exceed 9 Billion by 2050: Developing Countries to Add 2.3 Billion Inhabitants with 1.1 Billion Aged Over 60 and 1.2 Billion of Working Age. D. o. E. a. S. Affairs. New York, United Nations.
- Upadhyay, R. K. and S. Ahmad (2011). Management Strategies for Control of Stored Grain Insect Pests in Farmer Stores and Public Ware Houses. World Journal of Agricultural Sciences **7**(5): 527-549.
- USGS (2013). Mineral Commodity Summaries 2013. U. S. G. S. U.S. Department of the Interior. Virginia: 52-53.
- Vayias, B. J. and C. G. Athanassiou (2004). Factors affecting the insecticidal efficacy of the diatomaceous earth formulation SilicoSec against adults and larvae of the confused flour beetle, *Tribolium confusum* DuVal (Coleoptera: Tenebrionidae). Crop Protection **23**(7): 565–573.
- Vlahov, I. R., P. I. Vlahova and R. J. Linhardt (1997). Regioselective Synthesis of Sucrose Monoesters as Surfactants. Journal of Carbohydrate Chemistry **16**(1): 1-10.

Wang, Q. (2008). Regioselective synthesis, characterization and physicochemical properties of sucrose esters. Faculty of Chemical, Environmental and Biological Science and Technology. Dalian, Dalian University of Technology

Doctor of Philosophy 134.

Warheit, D. B., C. M. Sayes, K. L. Reed and K. A. Swain (2008). Health effects related to nanoparticle exposures: Environmental, health and safety considerations for assessing hazards and risks. Pharmacology & Therapeutics **120**(1): 35-42.

Wasserthal, L. T. (1996). Interaction of Circulation and Tracheal Ventilation in Holometabolous Insects. Advances Insect Physiology **26**: 297-351.

WFP (2013, 2013). Types of Insect Pests. Retrieved 22 September 2013

Wigglesworth, V. B. (1944). Action of inert dusts on insects. Nature **153**(3886): 493-494.

Wigglesworth, V. B. (1945). Transpiration Through the Cuticle of Insects. The Journal of Experimental Biology **21**: 97-114.

Wigglesworth, V. B. (1957). The Physiology of Insect Cuticle. Annual Review of Entomology **2**: 37-54.

Wilson, C. and C. Tisdell (2001). Why farmers continue to use pesticides despite environmental, health and sustainability costs. Ecological Economics **() 39**: 449–462.

Woudenberg-van Oosterom, M., F. van Rantwijk and R. A. Sheldon (1996). Regioselective acylation of disaccharides in tert-butyl alcohol catalyzed by *Candida antarctica* lipase. Biotechnology and Bioengineering **49**(3): 328-333.

Xiaojie Liu, G. H., Changgui Yuan (2006). Advance in Research, Production and Application of Sucrose Ester. Food and Fermentation Industries **27**(11): 64.

Yuan, Y. and T. R. Lee (2013). Contact Angle and Wetting Properties. Surface Science Techniques. G. Bracco and B. Holst. Berlin Heidelberg, Springer-Verlag: 3-34.

Chapter 2

Experimental Design and Methodology

2.1 Chemical and Materials

Octanoic acid, decanoic acid, lauric acid, phosphoric acid, sorbitol and xylitol were obtained from Sigma-Aldrich. Calcium hydroxide and ethanol (denatured, 98%) were obtained from Chem-supply. All chemicals were used as purchased from the supplier without any further purification. Dryacide was supplied by Entosol Australia Pty Ltd. Conductive gel, Carbon Dag 154, was supplied by Acheson Chemicals for insect sample preparation for electron microscopic study.

2.1.1 Diatomaceous earth

Diatomite rock (Mt Sylvia Pty Ltd, Australia) is first broken down into small chunks (~1 cm diameter) using a hammer. The pieces of rock are then crushed into a coarse powder with a vibrating ring mill (2 sec, chrome steel grinding bowl). This powder is then suspended in deionized water (10 g / 100 ml). The suspension is sonicated for 30 min, and then left to stand for 5 min to allow coarse sediments to settle. The supernatant solution is collected by siphoning, transferred to a plastic tub, and left to stand for a further 30 min. This supernatant is again collected by siphoning, and left to stand overnight. The resulting sediment is dried in an oven at 90°C overnight. Once dry, the resulting solids are crushed in a vibrating ring mill (3 min, chrome steel grinding bowl) to form diatomaceous earth powder (DE), particle size average 224 ± 87.4 nm which was measured using a NanoSight NS300 Nanoparticle tracking analyser.

2.2 Equipment

<i>Equipment</i>	<i>Model/Specification</i>	<i>Manufacturer</i>
Buchner funnel, porcelain		Wheel, China
Filter flask, glass		Schott Duran, Germany
Filter paper	No.1, 6 μ m	Advantec, Japan
Guko gasket rubber		Deutsch & Neumann, Germany
Hot plate with magnetic stirrer	MR Hei-Standard	Heidolph, Germany
Water purifier (18.2 M Ω)	Purelab Option Q7	Elga, UK

2.3 Synthesis of Sugar Esters

To prepare the sugar esters, sugars were directly esterified with respective fatty acids at elevated temperature catalyzed by phosphoric acid according to the procedure detailed in (Farone et al., 2002). The synthesis of sorbitol octanoate ester is used as an example:

Sorbitol (60.01g, 0.329 mol) is first mixed with octanoic acid (43.85g, 0.304 mol, molar ratio of 1.1:1) in a round bottom flask with stirring. The round bottom flask is then connected to a condenser, and the mixture was refluxed with stirring using an oil bath. Once the temperature reached ca. 150 °C, phosphoric acid (3.21g, 0.032 mol) was added into the flask. The mixture was then continuously stirred and refluxed at 150 °C for 28 h (13.5 h in the case of xylitol decanoate ester). The round bottom flask was then allowed to cool to room temperature, except for decanoate ester and laurate ester, which need to be kept at 40 and 60 °C respectively in order to maintain in liquid form. Once cooled, calcium hydroxide (27.01g, 0.364 mol) was added to neutralize the phosphoric acid. The products, termed crude sugar esters, were purified using an established method with slight modification (Wagner et al., 1991). Specifically, 100 g products were firstly diluted with 500 ml of ethanol (5 times w/v). The precipitated calcium phosphate salt was removed by vacuum filtration, resulting in a clear amber colored solution. 900 ml of water (9 times v/v) was then added into the filtrate with

stirring, and the resulting liquid was refrigerated at 4 °C overnight to facilitate phase separation of sugar ester. The separated sugar ester precipitate was collected using a pipette or by decanting the water phase. Lastly, the collected phase was then further washed with 1 part of acetone (w/v) to remove any unreacted fatty acids. Recover the solvent washed precipitate to obtain final purified sugar esters which are thick transparent amber color liquids. Purified and unpurified products were characterized by Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance spectroscopy (NMR).

2.4 Characterisation of Sugar Esters

2.4.1 FTIR

Functional groups in the synthesized sugar esters were identified through Nicolet 6700 FTIR spectrophotometer (Thermo Scientific, US) equipped with the attenuated total reflection (ATR) platform (**Figure 2.1**), a mercuric cadmium telluride (MCT-A) detector and a potassium bromide (KBr) beam splitter. Prior to the experiment, the detector was cooled to 77 K using liquid nitrogen. A droplet of the liquid sugar ester sample was pipetted onto the diamond crystal window of the FTIR instrument, which is located at the center of the ATR platform. Then, the swivel tower was screwed down to clamp the sample to the crystal surface and applies pressure via a pointed tip. Spectra were collected at 60 scans with resolution of 4 cm⁻¹ in the frequency region of 4,000-650 cm⁻¹. Spectral data was analysed using OMNIC Peak Resolve software (Thermo Electron Corporation, US).

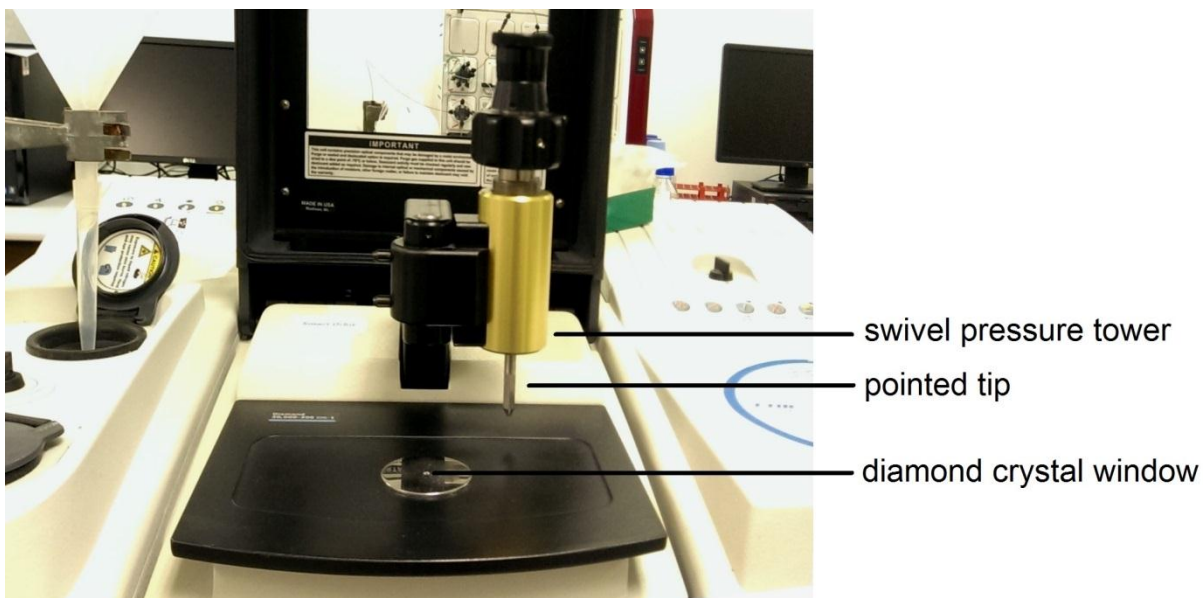


Figure 2.1 FTIR spectrophotometer equipment with attenuated total reflection system

2.4.2 NMR

The molecular structure of the sugar esters was also characterized by ^1H -NMR, using an Agilent 600 MHz DD2 NMR system with a cryoprobe using standard proton (^1H) parameters (**Figure 2.2**).

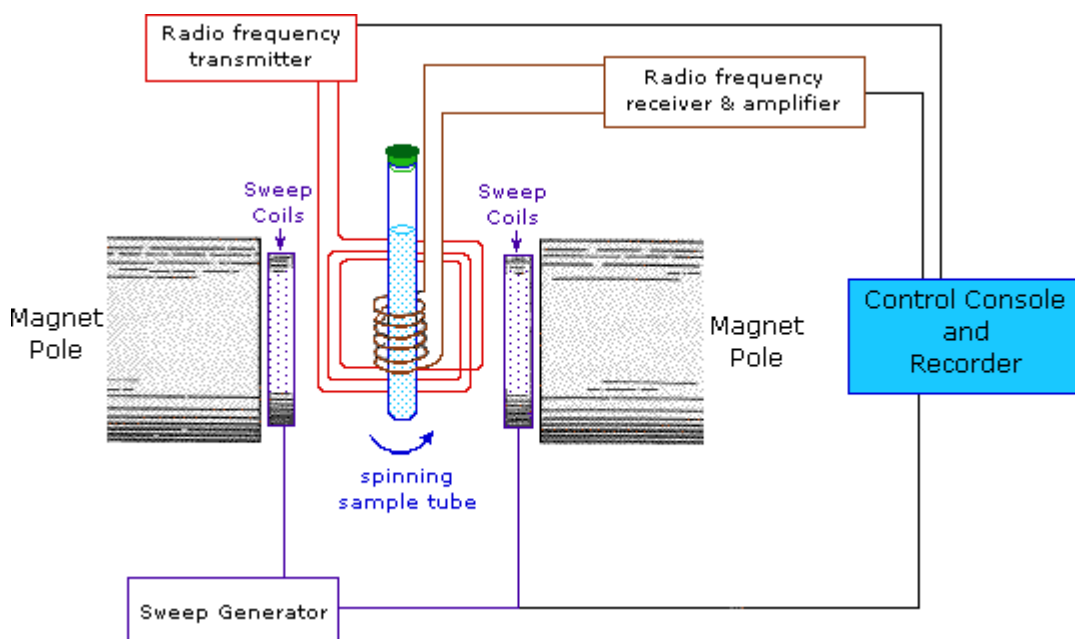


Figure 2.2 Schematic setup of NMR (Taken with permission from pharमतutor.org)

Samples were prepared in deuterated acetone. NMR spectra were collected and analyzed using VNMRJ 4.2 software. Spectral data was automatically phase corrected, and the peak areas (integrals) were normalized relative to the integral for the triplet CH₃ peak for the fatty acid component (~1.3 ppm). Hydrophilic lipophilic balance (HLB) values of each product were calculated using the equation reported by Berguerio et al. (1978) as shown in formula (1), based on calculation of the so-called H ratio (Rabaron et al., 1993). H ratio describes the relative effect of the hydrophilic part of the molecule, which is determined in this case from the sum of all peak integrals for protons in the hydrophilic sugar group, I_{gph} , (the 3.5-5ppm region), and the sum of all peak integrals for the entire molecule, I_{tot} , using formula (2):

$$HLB = \frac{60H}{(H+2)} \quad (1)$$

$$H = I_{gph}/I_{tot} \quad (2)$$

2.4.3 Contact Angle

The surface wetting properties of the sugar esters are studied by examining the contact angle between a water droplet and treated grain. Measurement of static contact angles was performed on the Theta Optical Tensiometer (Attension, Biolin Scientific). Using the sessile-drop method, the water droplet profile on sugar ester coated grains was imaged three times. Correlation of contact angles, surface wettability and insecticidal activity is discussed in Chapter 4.

2.5 Insecticide Bioassays

2.5.1 Preparation of grain

Katana hard white winter wheat was used in these experiments, and is obtained from Flinders Ranges Premium Grain. Grain is stored at -20 °C in a freezer prior to use. Grain for these experiments is first cleaned using a stainless steel insect screen (2

mm mesh, Graintec Scientific, Australia) to remove dust, husks and other debris. The grain is then placed into cylindrical glass jars (117 mm height x 115 mm diameter, 860 ml total volume) which are fitted with metal lids containing a hole (~20 mm diameter) that is covered with glued on tissue paper (Kimwipe) (**Figure 2.3**). These lids prevent insect escape, while allowing for ventilation. These jars of grain are then conditioned in a controlled environment chamber (Thermoline Scientific, Australia) at 30 °C and 55% relative humidity (r.h.) for 2 weeks. Moisture content (m.c.) of conditioned grain is monitored following the conditioning period using a moisture meter (HE-50, Pfeuffer, Germany) to ensure that a consistent grain moisture content equal to recommended maximum storage conditions (11.5 - 12.5%) was reached (Fields et al., 2002).



Figure 2.3 Photo of the jar for the bioassay

2.5.2 Selection of insects

Bioassays are carried out against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) (Strain ID: QRD 14), which were initially provided by Dr. Patrick Collins from the Postharvest Grain Protection Unit, Department of Agriculture and Fisheries

Queensland. These insects were reared on whole wheat (cv. Katana) by transferring ~ 100 adult insects to a jar of clean conditioned grain (300 g per jar), storing in a controlled environment chamber at 30 °C, 55 % r.h. for 2 weeks to allow for egg laying, then removing the adults and returning the jars to the chamber for a further 6 weeks to allow progeny to develop. Newly emerged adult insects from these jars are then collected for use in bioassay studies within a 2 week window (0-2 weeks old).

2.5.3 Comparative bioassays

Comparative bioassays are carried out on grain treated with various doses of sugar ester (100, 400, 1000 and 4000 ppm) and/or diatomaceous earth (100, 400, 700 and 1000 ppm), in an effort to compare the efficacy of these formulations and identify the best performing combinations. Samples for each treatment are prepared in triplicate, with untreated grain serving as the negative control in each bioassay (to quantify non-treatment related insect mortality).

To prepare treated grain samples, clean conditioned grain is first divided up into portions (100 g per replicate). In the case of sugar esters, the grain is spread out on a flat dish to form a monolayer, and then a solution of the sugar ester dissolved in deionized water (1 g / 10 ml) is sprayed onto the grain. The treated grain is then transferred to a labelled 860 ml glass jar closed with a ventilated lid, and stored in a controlled environment chamber at 30 °C and 55 % r.h. for 3 days to allow the treated grains to dry. DE is applied to grain by transferring the grain into an 860 ml glass jar, adding the required quantity of DE dust, then shaking the closed jar for 1 min to disperse the dust over the grain. The lid is then replaced with a ventilated lid. When testing combinations of DE and sugar ester, DE dust was applied 24 hours after sugar ester pre-treatment of the grain.

To start the bioassay, thirty randomly chosen adult insects were carefully counted out and placed into the jars of treated grain. The bioassay jars were kept in a controlled environment chamber at 30 °C and 55 % r.h. for a total of 60 days. Samples were examined after 7 and 14 days to determine the number of dead insects, and also after 60 days to determine progeny production. In order to count the insects, bioassay jars were sieved using a stainless steel insect screen (2 mm mesh, Graitec Scientific,

Australia). Dead insects were collected and live insects were placed back into the jars after each count. Upon completion of each bioassay, jars of grain and insects were sterilized by heating in an oven at 60°C overnight.

Mortality data was first corrected for control mortality using Abbotts' correction, then arcsine transformed to normalize the variance of each set of replicates. Ryan-Einot-Gabriel-Welsch Range (R-E-G-W-Q) test was then performed (using IBM SPSS) to divide means into significantly different groups ($p < 0.05$). The mean number of dead adult insects was the response variable, while dose rates (inclusive of control) were the main effects. Calculations were carried out on the mortality results following 14 days of exposure (so the full lethal effect of the formulation against the insects could be assessed).

2.5.4 Lethal dose bioassays

Based on the results of comparative bioassays, an optimal formulation is identified (Sorbitol octanoate + DE). Lethal dose values (LD_{50} and LD_{90}) and confidence intervals, relative potency and the combination index for this formulation are then determined by carrying out a bioassay on the formulation, its separate components (DE / sugar ester) and a positive control (dryacide) as described in section 2.5.3, at doses that give insect mortality between 10 and 90%. The required doses of each component and their mixture were decided based on the effective dose observed from comparative bioassay studies. For the sugar ester, the doses selected were 3000, 4000, 5000, 6000 and 7000 ppm. For DE, the doses selected were 80, 160, 240, 320 and 400 ppm. For the combined formulation, the doses selected were 20/800, 40/1600, 60/2400, 80/3200 and 100/4000 ppm (DE/sugar ester). Mortality data was subjected to probit analysis (SPSS, IBM) to determine lethal dose values, with relative potency and the combination index calculated according to equations (1) and (2) respectively (Attique et al., 2006):

$$(1) RP = \frac{LD_{50dryacide}}{LD_{50formulation}}$$

$$(2) CI = \frac{LD_{50DE\ combo}}{LD_{50DE\ only}} + \frac{LD_{50SO\ combo}}{LD_{50SO\ only}} + \left(\frac{LD_{50DE\ combo}}{LD_{50DE\ only}} \times \frac{LD_{50SO\ combo}}{LD_{50SO\ only}} \right)$$

2.5.5 Surface bioassay

Sugar esters that displayed significant inhibition profiles were further studied to investigate the underlying mechanism of insecticidal action. Surface bioassay provides a convenient approach to address this research question. Active ingredients, the sugar esters, at the highest concentrations tested in grain bioassays were applied in two ways, either on crushed grain (in-feed method) or on petri-dish (plate method). These methods helped to test the hypothesized modes of action either via ingestion or by contact. Inner plate surface of all petri-dishes were roughened by sand-paper (P1200, 3M) to assist insect movements. For in-feed method, Katana grains, cleaned and conditioned, were crushed into granule by the crushing unit (HE-50, Pfeuffer, Germany) and then mixed with sugar ester solutions prepared in deionized water (1mL at concentration of 4000ppm) by manual shaking for 1 minute. For plate method, same application amounts of sugar ester solution were pipetted onto petri-dish (Techno-Plas, Australia). Allow both preparation to dry overnight at 30 °C and 55 % r.h. in the culturing chamber. About 2 g of treated and crushed grains was added to clean petri-dishes and 2 g of untreated crushed grains was added to the treated petri-dishes as comparison. Twenty randomly selected insects, aged 2 month or younger, were carefully placed into each petri dish. Experiments were performed in one triplicate.

2.6 Grain bulk density

Grain bulk density, also known as hectolitre mass (HLM) or test weight, is measured for both untreated and treated grain at various doses using a Chondrometer (Graintec Scientific, Australia), which consists of an upper and lower cylindrical container (500mL), which are separated by a metal blade. In these experiments, 1 kg of clean, conditioned grain is first treated with formulation (as described in section 2.5.1) and placed into a large bowl. The chondrometer is then assembled, and test grain from the bowl is added to the top cylinder until it is full. The dividing blade is then quickly withdrawn to allow grain to fall into the lower cylinder. The blade is then reinserted, and remaining grain in the top cylinder is returned to the bowl. The weight of grain in

the lower cylinder is measured, and the grain is returned to the bowl. This process is repeated to give a total of 10 measurements, which are then averaged.

2.7 Angle of Repose

The angle of repose (AR) is measured for both untreated and treated grain at various doses using the pilling or fixed funnel method. In these experiments, 1 kg of clean, conditioned grain is first treated with formulation (as described in section 2.5.1) and placed into a large bowl. A plastic funnel (80 mm diameter with an outlet of 20 mm diameter) is then positioned 50 mm above the centre of a circular plastic plate (90 mm diameter, Techno-Plas, Australia) using a retort stand. 80 g of grain is then collected from the sample bowl, and added to the funnel (using a finger to block the outlet). Once filled, the grain is allowed to fall onto the plastic plate until the funnel is empty, forming a conical heap. Tapping of the funnel is necessary if the grain becomes jammed in the funnel neck. The height of the cone, denoted by the letter H, and radius of the circular plate, denoted by the letter R were used to calculate respective AR using equation 3 (Kaleemullah and Gunasekar, 2002).

$$\tan^{-1} \left(\frac{2H}{R} \right) \quad (3)$$

All experiments were repeated 10 times and an average of the results was used in the report.

2.8 Optical Microscopy

External morphology of treated grain and insects from bioassays were first examined using a BK Plus Imaging System (Visionary Digital, USA) (**Figure 2.4**), which is comprised of a camera (Canon EOS 7D, Japan), macro lens, dual port microscope and objective lenses, motorized camera control (P-51 CamLift), flash (FX2 Lighting System) and transilluminator (SolMate).

While optical microscopy is typically restricted by the inverse relationship between magnification and depth of field, this system allows high magnification, in-focus images of specimens to be collected by taking pictures over a range of focal depths, spanning from where the top-most features are in focus, to where the bottom-most features are in focus. These images are then combined using a focus stacking algorithm in the software package, Zerene Stacker (Zerene Systems LLC, USA) (Visionary-Digital™, 2013).

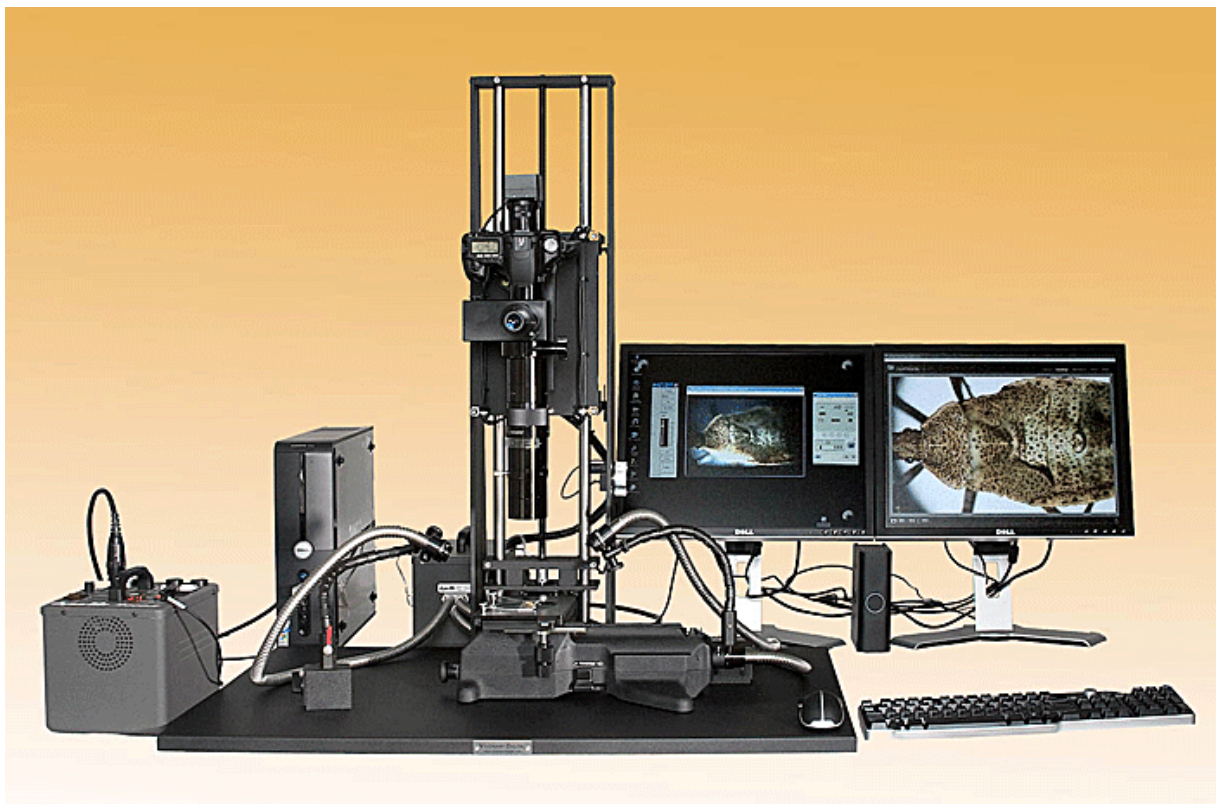


Figure 2.4 Setup of BK Plus Imaging System (Taken with permission from VisionaryDigital.com)

2.9 Scanning electron microscopy (SEM)

Insect and treated grain specimens are prepared for electron microscopy by first mounting them on a metal SEM stub using conductive gel (Carbon Dag 154, Acheson Chemicals). The specimen is then sputter coated with platinum (10 nm thick) using a Cressington 208 HR Sputter Coater (Cressington Scientific Instruments Ltd, England).

SEM studies on prepared specimens were performed using a Quanta 450 FEG microscope (FEI, USA) under high vacuum conditions, operated at 20 kV. Images were taken using the secondary electron detector at 1,000 and 5,000 times magnifications. In the case of insect specimens, images of selected body parts were taken as illustrated in **Figure 2.5**.

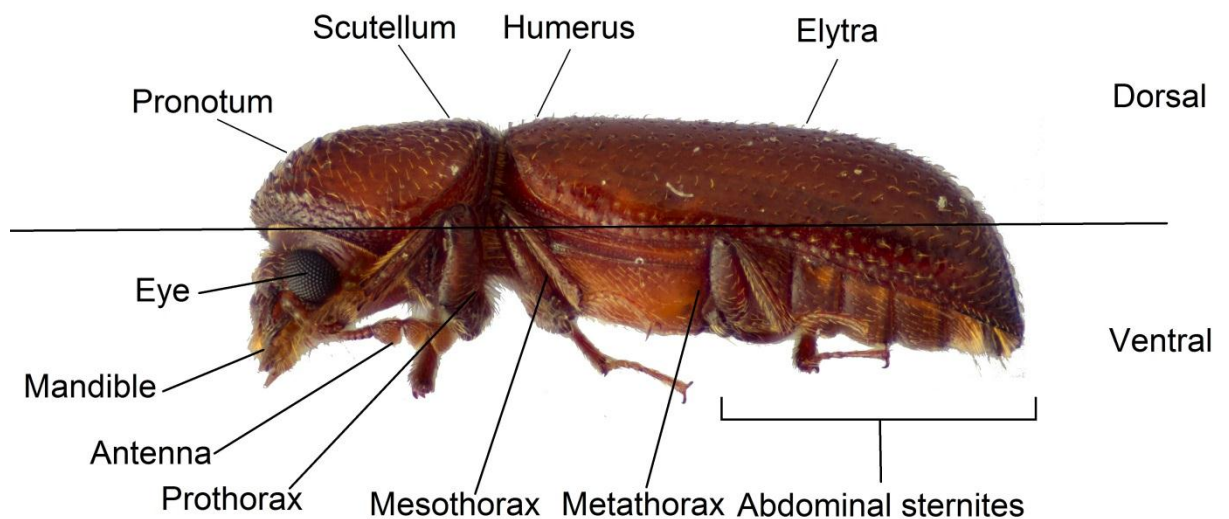


Figure 2.5 Schematic illustration of major insect body parts that are prompted to attacks by surface active insecticides such as DE

References

- Attique, M. N. R., et al. (2006). "Could resistance to insecticides in *Plutella xylostella* (Lep., Plutellidae) be overcome by insecticide mixtures?" J. Appl. Entomol. **130**(2): 122–127.
- Farone, W. A., et al. (2002). Polyol ester insecticides and method of synthesis. . U. S. Patent. United State of America, Ava Chemical Ventures L.L.C.
- Fields, P. G., et al. (2002). Standardized testing for diatomaceous earth. Proceedings of the 8th International Working Conference on Stored-Product Protection, Wallingford, United Kingdom, CAB International.
- Kaleemullah, S. and J. J. Gunasekar (2002). "Moisture-dependent Physical Properties of Arecanut Kernels." Biosystems Engineering **82**(3): 331–338.
- Rabaron, A., et al. (1993). "Physical methods for measurement of the HLB of ether and ester non-ionic surface-active agents: H-NMR and dielectric constant." International Journal of Pharmaceutics **99**: 29-36.
- Visionary-Digital™ (2013). "BK Plus Lab System." Retrieved 09/12/2014, from <http://www.duninc.com/IntegratedSystems1.html>.
- Wagner, F. W., et al. (1991). Separation and purification of sugar esters. U. S. Patent. United State of America, Nebraska Department Of Economic Development. **4,983,731**.

Chapter 3

Synthesis and Characterisation of Sugar Esters

3.1. Introduction

In this chapter, the synthesis of several sugar esters for use as potential new green agents for control of stored grain insect pests is described. Two sugars varied in carbon number (sorbitol and xylitol) and fatty acids varied in chain length (octanoic acid, decanoic acid, and lauric acid) are selected, as previous studies on the chemical properties of sugar esters have identified that the type of sugar group and the chain length of the fatty acid are two key factors that determine their insecticidal activity. Sorbitol is a type of sugar has 6 carbons in the structure where xylitol has 5 carbons. Octanoic acid is a lipid that has a chain length with 8 carbons where decanoic acid has 10 and lauric acid has 12.

As discussed in the literature review, direct esterification between sugar and fatty acid using the solvent-less method is the most time effective and facile way to prepare sucrose fatty acid esters. Synthesis was carried out following the method disclosed in the US Pat. No. 6419941B1, "Polyol ester insecticides and method of synthesis". Monoesters, having one hydroxyl group being replaced with one acyl group on sugar molecule was targeted, as it is believed to an active type of sugar ester molecule that has good insect inhibition property (Puterka et al., 2003). Synthesised sugar esters contain unreacted starting materials (the product is referred to as crude) and so were purified using a phase separation method as described in US Pat. 4983731, which utilises the differential solubility of each component present in the mixture, with slight modification depending on the relative solubilities of each component present (Wagner et al., 1991). The yields of both purified and unpurified sugar esters were then calculated (section 3.3.1), and the products analysed by FTIR (section 3.3.2) and NMR (section 3.3.3), to characterise the composition, molecular structure and HLB values (section 3.3.4) of the resulting products. The products were also characterised by tensiometer (section 3.3.5), to determine contact angle and hence wetting properties of the sugar esters, which will be useful in interpreting bioassay results and understanding the mode of insecticidal action.

3.2. Materials and methods

3.2.1. Synthesis and purification of sugar esters

Sugar esters prepared are sorbitol octanoate (sC8), sorbitol decanoate (sC10), sorbitol laurate (sC12) and xylitol octanoate (xC8), xylitol decanoate (xC10), xylitol laurate (xC12). All chemicals used in this section are detailed in Chapter 2, Section 2.1 where equipment is listed in Section 2.2. Details for the synthetic procedures using solvent-less approach and purification basing on phase separation principles are recorded in Section 2.3.

3.2.2. Characterisation

Sugar esters are characterised to determine if the syntheses are successful in producing the desired products, and to quantify their purity and wetting properties.

3.2.2.1. FTIR

As described in Chapter 2, Section 2.4.1.

3.2.2.2. NMR

As described in Chapter 2, Section 2.4.2

3.2.2.3. Contact angle

As described in Chapter 2, Section 2.4.3.

3.3. Results and Discussion

3.3.1. Product yield

The yield for the 6 types of synthesized sugar esters is calculated and summarized in **Table 3.1**. As can be seen, only very minor material losses (< 3 wt.%) were observed following completion of the reaction. No sediments were observed in all the experiments, which suggests good control of the reaction process has been obtained. Initial purification (step 1) to remove the acid neutralization salt (calcium phosphate) gives product losses of 20–30 wt.%, while the calculated mass of calcium phosphate for this procedure should only account for a loss of 5-7% of total weight. This additional loss may be explained in terms of some of the product being occluded within the precipitates (indicated by FTIR analysis as shown in **Figure 3.3**), as well as losses during handling (residues on glassware etc.). Further washing of the precipitate with ethanol may be applied to obtain these residues.

Table 3.1. Products retained as to total weight of starting chemicals (w/w) after repeated washing.

	Synthesis*	Washing times		
		Step 1	Step 2	Step 3
sorbitol octanoate	98.26%	74.81%	44.89%	43.05%
xylitol octanoate	98.56%	74.67%	68.00%	65.78%
sorbitol decanoate	96.08%	70.72%	49.34%	48.02%
xylitol decanoate	100.00%	70.00%	53.33%	51.30%
sorbitol laurate	98.52%	73.33%	56.67%	-
xylitol laurate	97.40%	80.00%	60.00%	-

* recovery rates of each product at the end of synthesis

Step 1 refers to the weights of remaining products after dissolving in ethanol and filtering, and

step 2 refers to the weights of remaining products after phase separation by adding water.

step 3 refers to the weights of remaining products after acetone washing

In the next step, unreacted sugars are removed using a biphasic solvent system with ethanol and water (step 2). The yield of sugar esters following this process is between 45- 70% (**Table 3.1**), where losses are largely due to the purification process. The decrease in yield may result from several aspects, including (1) fatty acids with different chain length may have different optimum reaction temperatures (leaving more or less unreacted starting material); and (2) the separation process may not be universally suitable for all products due to variation in solubility due to the degree of substitution in sugar molecule, type of sugar, and fatty acid chain length, which all affects the molecule's solubility (Gupta et al., 1983, Wanasundara et al., 2005). Further refinement (step 3) to remove unreacted fatty acids using a biphasic solvent system with acetone and water was carried out, but there was not much to be washed off. There's also technical difficulties where separation of the sugar ester from the fatty acid could not be achieved (sC12 and xC12). For these reasons, all sugar esters used in subsequent studies are purified only using step 1 and 2.

3.3.2. FTIR

Unpurified products were characterized by FTIR to confirm successful synthesis of the sugar esters. The spectra for these products, as well as the starting materials for comparison, are given in **Figure 3.1**, with key spectral peaks listed in **Table 3.2**. In the spectra of sorbitol and xylitol, characteristic peaks corresponding to alcohol hydroxyl groups (-OH) can be observed in the wave length around 3400 cm^{-1} , which are not present in the spectra of fatty acids. Peaks at $1050\text{-}1260\text{ cm}^{-1}$ in both sugars were attributed to C-O stretch.

Table 3.2. Functional group characterisation using FTIR

<i>Compound</i>	<i>Characteristic Peak</i>
All sugars	3400 cm^{-1} (-OH) $1050\text{-}1260\text{ cm}^{-1}$ (C-O)
Fatty acid	$2850, 2950\text{ cm}^{-1}$ (C-H); 1700 cm^{-1} (C=O)
Ester	1750 cm^{-1} C=O vibration in the ester groups (-COO)

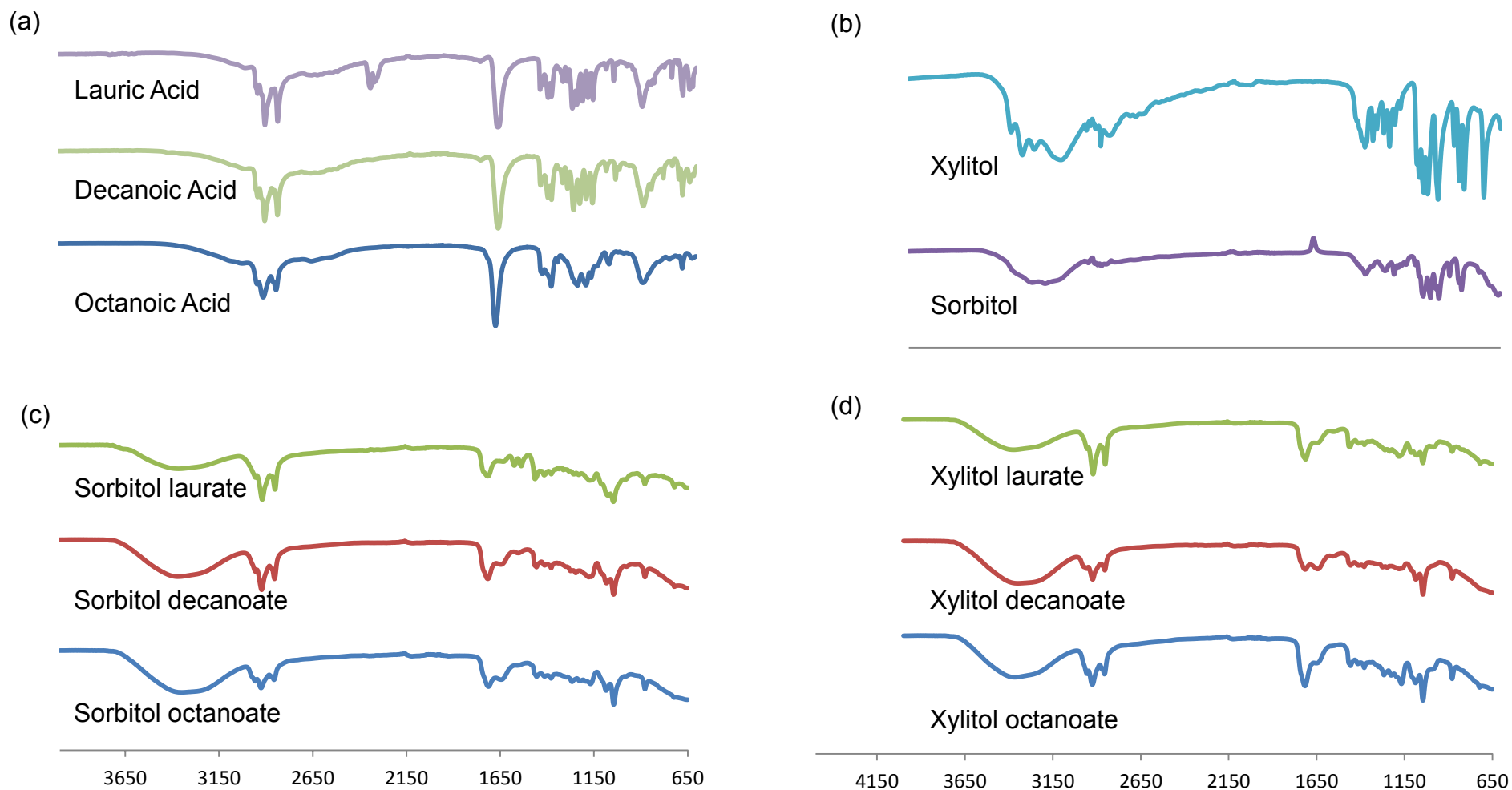


Figure 3.1 FTIR spectra of the raw materials and synthesized esters (a) fatty acids and (b) polyols, and end products (c) sorbitol esters and (d) xylitol esters.

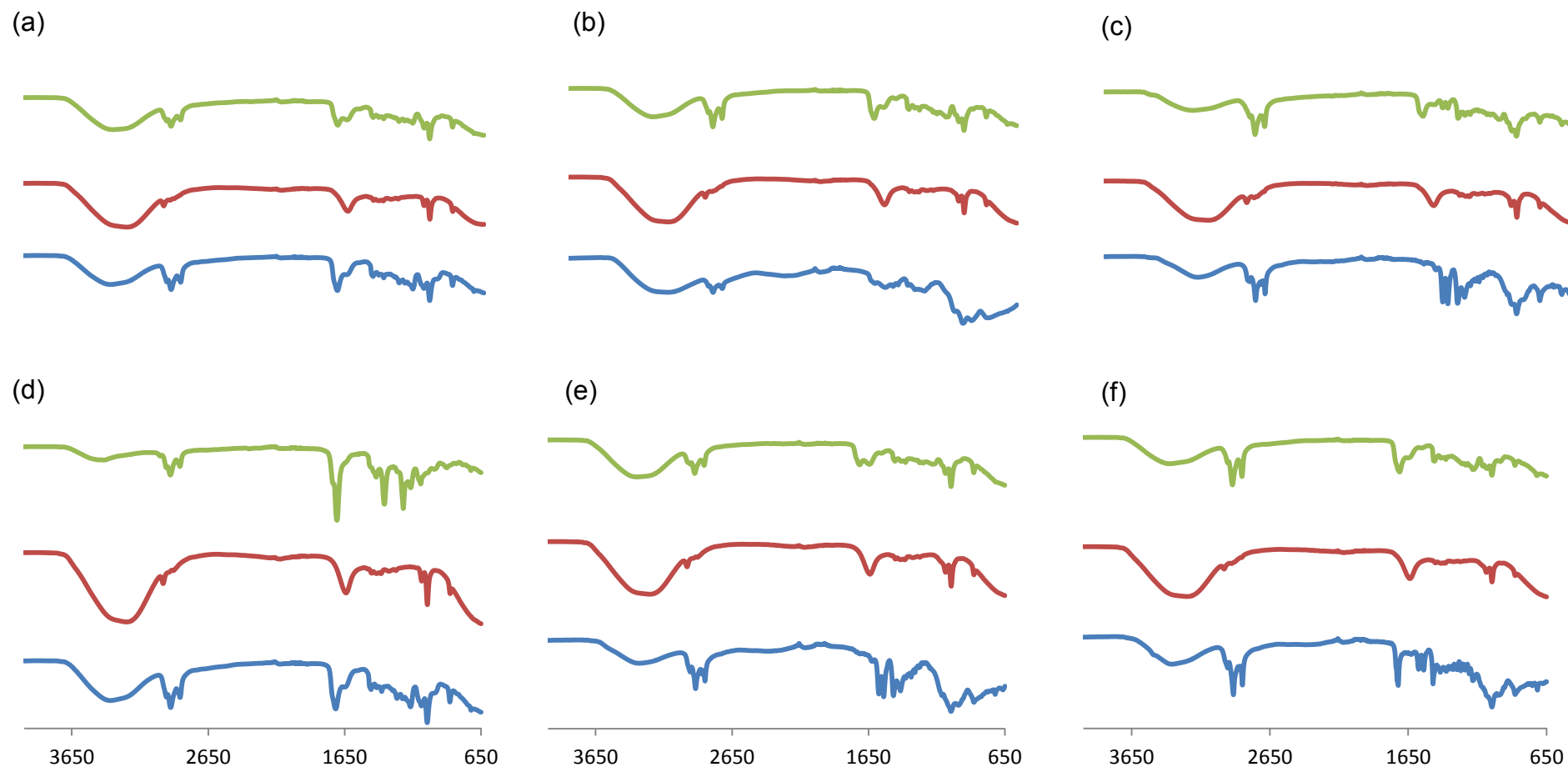


Figure 3.2 FTIR spectra of products obtained from each purification step (a) sorbitol octanoate; (b) sorbitol decanoate; (c) sorbitol laurate; (d) xylitol octanoate; (e) xylitol decanoate; (f) xylitol laurate; where green line refers to the solid salts obtained from step 1, both red and blue line refers to solids obtained from the water phase and the sugar ester phase obtained from step 2, respectively.

For fatty acids, the peaks at 2850 and 2950 cm^{-1} are attributed to the C-H stretch in CH_3 or CH_2 in the long alkyl chains, and the peak at ca. 1700 cm^{-1} is from the C=O groups in the carboxyl groups, which can be observed in all the fatty acids but not in sorbitol or xylitol (Ayala-Bravo et al., 2003).

In the FTIR spectra of all the sugar esters, all the preceding peaks are present, as well as a peak at ca. 1750 cm^{-1} , which results from the C=O vibration in the ester groups (-COO) that is characteristic of sugar ester formation (Deshpande et al., 2013). Thus, from the FTIR results, the existence of sugar esters can be confirmed. FTIR spectra of products from the purification process are given in **Figure 3.2**. This data reveals that the precipitated material is primarily sugar ester, and that a significant quantity of sugar is present in the aqueous phase, as indicated by the presence of noticeable peaks at $\sim 1040 \text{ cm}^{-1}$ (from the sugar), with only comparatively weak peaks present due to fatty acid alkyl chains (2850-2950 cm^{-1}). This implies that the purification process has removed a large amount of the unreacted sugar from the final product.

3.3.3. Proton NMR

Structural analysis of the purified sugar esters using ^1H -NMR spectra was carried out to identify functional groups in the molecule, and to provide compositional information by evaluating the relative quantities of sugar, esterified and free fatty acid molecules (Duus et al., 2000). **Figure 3.3** shows the normalized ^1H NMR spectra of purified sorbitol octanoate (NMR spectra for the remaining sugar esters can be found in **Appendix 2**), with peak areas integrated and normalized relative to the peak for the fatty acid CH_3 group ($\sim 0.87 \text{ ppm}$). The peak observed in all spectra at 2.09 ppm is due to residual acetone in the deuterated acetone solvent, and is used as a reference peak in the spectrum (Gottlieb et al., 1997). Peaks for the acyl chains (0 – 2.5 ppm) and sugars (3.4 – 6.0 ppm) in the products are identified in the spectra and assigned (Sahasrabudhe and Chadha, 1969). The major broad multiplet peaks at δ 1.30 ppm and 1.57 ppm are assigned to the main chain $-\text{CH}_2-$ and $-\text{CH}_2-\text{CH}_2\text{COO}-$ groups of the fatty acids, respectively. Of particular interest are the peaks at $\sim 2.25 \text{ ppm}$ and $\sim 2.3 \text{ ppm}$, which are due to protons adjacent to ester groups and unreacted carboxylic

acid groups respectively. By comparing the integrals for these peaks, the relative molar quantities of reacted and unreacted fatty acids can be determined (**Table 3.3**). From this data, it is clear that a significant quantity of unreacted fatty acid is present in all sugar esters, which will need to be considered when carrying out bioassays. Notably, the laurate esters appear to be more pure than the other esters (less free fatty acid present). Likewise, the molar ratio of sugar to esterified fatty acids can also be determined from $^1\text{H-NMR}$ data, by calculating the integrals for protons associated with the ester group and with the sugar molecules (3.5 – 6.0 ppm), then dividing by the number of associated protons (**Table 3.4**).

Table 3.3. Ratio of reacted to unreacted fatty acids remaining in the purified sugar esters.

	<i>Free Fatty Acid</i>	<i>Esterified Fatty Acid</i>
Sorbitol Octanoate	1	0.89
Sorbitol Decanoate	1	0.62
Sorbitol Laurate	1	1.27
Xylitol Octanoate	1	0.66
Xylitol Decanoate	1	0.59
Xylitol Laurate	1	1.20

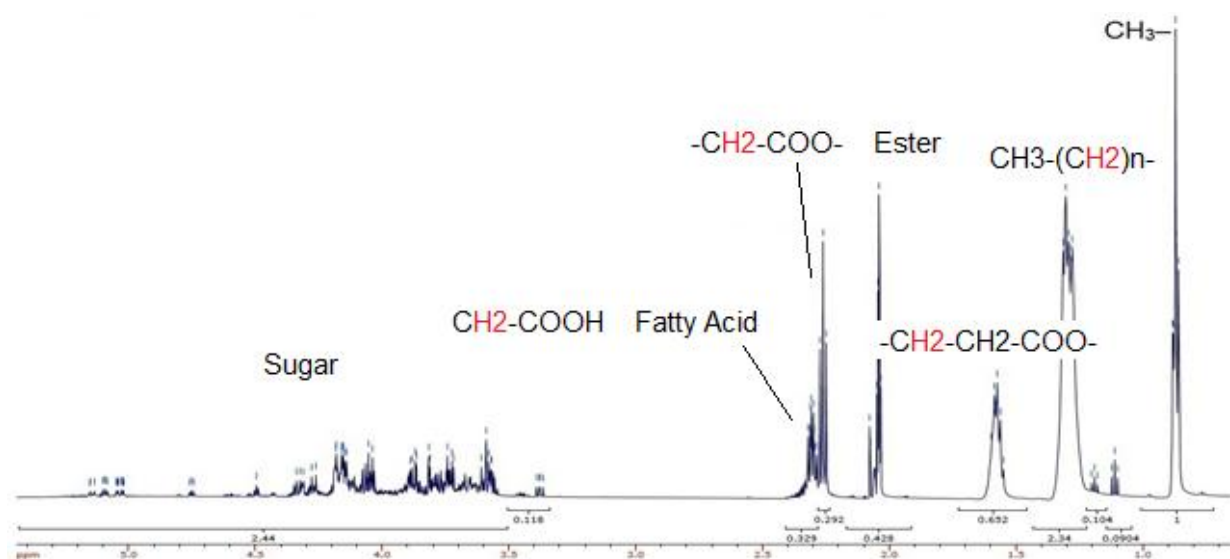
Table 3.4. Fatty acid to sugar ratio for synthesized sugar esters.

	<i>Esterified Fatty Acid</i>	<i>Sugar</i>
Sorbitol Octanoate	1	1.24
Sorbitol Decanoate	1	1.84
Sorbitol Laurate	1	0.73
Xylitol Octanoate	1	2.09
Xylitol Decanoate	1	2.45
Xylitol Laurate	1	0.94

As can be seen, octanoate and decanoate esters of both sugars have ratios larger than 1, indicating that these products are most likely monoesters, with excess

unreacted sugar remaining despite purification. In the case of the laurate esters, a ratio slightly smaller than 1 is calculated, which indicates that they are primarily monoesters, and that very little unreacted sugar remains in the products. This is understood as being reflective of the lower solubility of this sugar ester in water (owing to the longer alkyl chain of the associated fatty acids), which facilitates their separation from water soluble sugars.

Overall, these results indicate that the sugar esters are primarily monoesters, as targeted by our reaction conditions (increased sugar to fatty acid ratio provides more primary hydroxyl groups for the reaction thus promotes higher yield of mono- and di-esters (Akoh and Swanson, 1990).



Peak (δ , ppm)	Assigned functional group
0.87	CH ₃ -
1.30	CH ₃ CH ₂ -
1.57	-CH ₂ -
2.09	((CD ₃) ₂ CO) (solvent)
2.26	-CH ₂ C=O-
3.4–5.2	sorbitol derived ethers

Figure 3.3 Normalized NMR spectra of sorbitol octanoate

3.3.4. Determine the HLB value

After determining the ratio of sugars to fatty acids in the sugar ester, their

respective HLB value can then be calculated. This value enabled the understanding of a surfactant's solubility in a quantifiable way where larger number indicates a higher water-solubility and vice versa. HLB values calculated by Berguerio's equation using NMR data as a function of the total structural protons (polyol core plus alkyl chain) are tabulated for comparison (**Figure 3.4**).

Generally, HLB values of the sugar esters are higher than 8, which means there is good water compatibility according to Griffin's system (**Figure 3.4**) (Griffin, 1949). Xylitol esters, having one less carbon in the polyol core, have lower HLB values compare to sorbitol esters prepared with same fatty acids. Lower HLB indicates a decreased solubility in water thus a higher recovery rate after washing with water.

Sugar esters		HLB value	
sC8.4000		11.3	
xC8.4000		10.5	
sC10.4000		10.1	
xC10.4000		9.3	
sC12.4000		9.2	
xC12.4000		8.4	
Oil soluble		Water dispersible	Water soluble
0	3	6	9
			12
			15
			18

Figure 3.4 HLB values and associated water solubility

3.3.5. Contact Angle

One important aspect of sugar esters that may affect insecticide activity is

wettability, which determines the effectiveness of coating on grains and the potential for blocking insect spiracles (Puterka et al., 2003, Stadler and Buteler, 2009). As shown in **Table 3.5**, pristine grain generally exhibits a hydrophobic behaviour to water, with a contact angle of 127° . This is most likely a result of cuticular wax present on the surface of wheat grain (Troughton and Hall, 1967). However, with increasing quantities of sorbitol octanoate coated on the surface, grains became more wettable, showing smaller contact angles at higher dosage. When the concentration reached 4000 ppm, the wetting property of the grain surface changed to hydrophilic ($\theta = 35^\circ$). This may be because the surface of grain has been fully coated with

Table 3.5. Contact angles of grains with different concentrations of sorbitol octanoate.


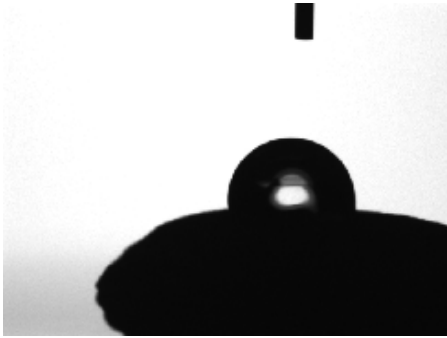
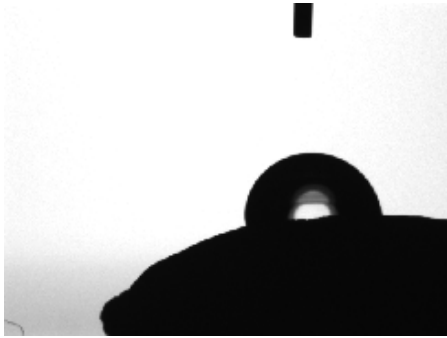
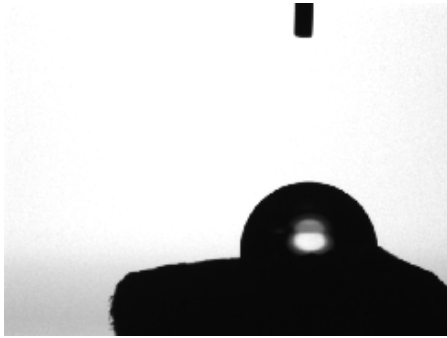
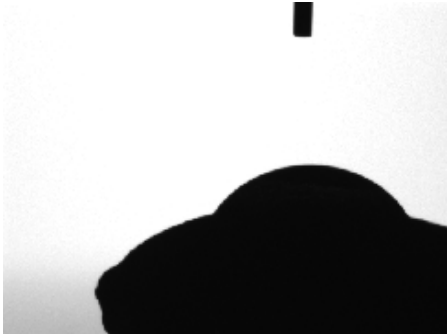
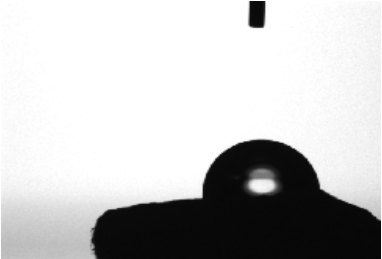
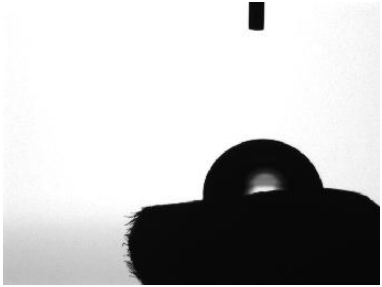

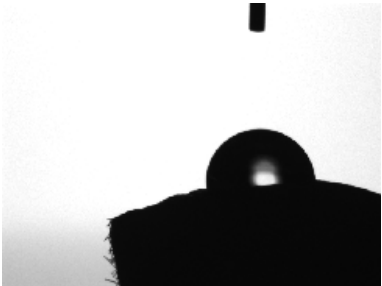


<i>Sample Name</i>	<i>Image</i>	<i>Contact Angle (θ)</i>
Pristine Grain	 A black and white photograph showing a dark, spherical droplet on a dark surface. A small black vertical bar is positioned above the droplet. The droplet is highly rounded, indicating a high contact angle.	127 °
Sorbitol octanoate at 100 ppm	 A black and white photograph showing a dark, spherical droplet on a dark surface. A small black vertical bar is positioned above the droplet. The droplet is slightly less rounded than the pristine grain, indicating a lower contact angle.	100 °
Sorbitol octanoate at 400 ppm	 A black and white photograph showing a dark, spherical droplet on a dark surface. A small black vertical bar is positioned above the droplet. The droplet is significantly less rounded, indicating a lower contact angle.	92 °
Sorbitol octanoate at 1000 ppm	 A black and white photograph showing a dark, spherical droplet on a dark surface. A small black vertical bar is positioned above the droplet. The droplet is very flat, indicating a low contact angle.	92 °
Sorbitol octanoate at 4000 ppm	 A black and white photograph showing a dark, spherical droplet on a dark surface. A small black vertical bar is positioned above the droplet. The droplet is extremely flat, indicating a very low contact angle.	35 °

Table 3.6. Contact angles of grains treated with sugar esters at 1000 ppm concentration.

<i>Sample Name</i>	<i>Image</i>	<i>Contact Angle (θ)</i>
Sorbitol octanoate at 1000 ppm		92 °
Sorbitol decanoate at 1000 ppm		87 °
Sorbitol laurate at 1000 ppm		45 °
xylitol octanoate at 1000 ppm		81 °
xylitol decanoate at 1000 ppm		85 °
xylitol laurate at 1000 ppm		31 °

sugar ester at 4000 ppm. Similar results can also be found with other sugar esters as illustrated in **Appendix 2**, all of which demonstrate how sugar ester coating on the grain surface changed its wetting property from hydrophobic to hydrophilic.

The effect of different sugar esters on the wettability at a fixed concentration is shown in **Table 3.6**. As can be seen, grains demonstrated various wetting behaviour when coated with different sugar esters. Laurate esters have a superior surface wetting activity, by reducing the contact angle of grain from 127° to 45° (sorbitol laurate) and 31° (xylitol laurate). Generally, esters prepared with xylitol yield slightly lower contact angles when coated on the grain surface compared to sorbitol esters. This might result from the shorter carbon chain of the xylitol sugar groups (Soultani et al., 2003).

3.4. Conclusions

In conclusion, sugar esters have been prepared from different sugars (sorbitol and xylitol) and fatty acids (octanic, decanic and lauric acids) using the solvent free method. Yields of 40 to 60% sugar esters are obtained following a two-step refinement method using ethanol and water to remove acid catalyst and unreacted sugars. Free fatty acids were not readily removed from the products. Product recovery may be improved by further washing with appropriate solvents and better designed separation equipment to increase yield. FTIR spectra of products obtained from each purification step indicated that sugar esters were successfully synthesised, and that a significant amount of the product loss occurs due to entrapment within solid precipitates during the first purification step (~20%). Further analysis by proton NMR confirmed that sugar ester synthesis was successful, and that primarily monoesters were formed. This data also showed that unreacted fatty acids and sugars are present in all products. As such, bioassays using these sugar esters should also examine the insecticidal properties of unreacted sugar and fatty acid to account for any possible contribution from these impurities. HLB values for the sugar esters were also calculated, and will be compared with bioassay results. The wetting properties of the sugar esters were also studied via contact angle of water droplets on grain treated with sugar ester. This study demonstrated that the hydrophobic grain surfaces can be wetted at high concentrations, with laurate esters causing wetting at lower doses (1000 ppm) compared to octanoate and decanoate esters (4000ppm).

References:

Akoh, C. C. and B. G. Swanson (1990). Optimized Synthesis of Sucrose Polyesters: Comparison of Physical Properties of Sucrose Polyesters, Raffinose Polyesters and Salad Oils. Journal of Food Science **55**(1): 236-243.

Ayala-Bravo, H. A., D. Quintanar-Guerrero, A. Naik, Y. N. Kalia, J. M. Cornejo-Bravo and A. Ganem-Quintanar (2003). Effects of sucrose oleate and sucrose laureate on in vivo human stratum corneum permeability. Pharmaceutical research **20**(8): 1267-1273.

Deshpande, P. S., T. D. Deshpande, R. D. Kulkarni and P. P. Mahulikar (2013). Synthesis of Sucrose–Coconut Fatty Acids Esters: Reaction Kinetics and Rheological Analysis. Industrial & Engineering Chemistry Research **52**(43): 15024-15033.

Duus, J. Ø., C. H. Gotfredsen and K. Bock (2000). Carbohydrate Structural Determination by NMR Spectroscopy: Modern Methods and Limitations. Chemical Reviews, **100**(12): 4589–4614.

Gottlieb, H. E., V. Kotlyar and A. Nudelman (1997). NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities. J. Org. Chem. **62**: 7512-7515.

Griffin, W. C. (1949). Classification of Surface-Active Agents by 'HLB'. Journal of the Society of Cosmetic Chemists **1**(5): 311-326.

Gupta, R. K., K. James and F. J. Smith (1983). Sucrose esters and sucrose ester/glyceride blends as emulsifiers. Journal of the American Oil Chemists' Society **60**(4): 862-869.

Puterka, G. J., W. Farone, T. Palmer and A. Barrington (2003). Structure-Function Relationships Affecting the Insecticidal and Miticidal Activity of Sugar Esters. Journal of Economic Entomology **96**(3): 636-644.

Sahasrabudhe, M. R. and R. K. Chadha (1969). Chromatographic analysis of sorbitan fatty acid esters. Journal of the American Oil Chemists Society **46**(1): 8-12.

Soultani, S., S. Ognier, J.-M. Engasser and M. Ghoul (2003). Comparative study of some surface active properties of fructose esters and commercial sucrose esters. Colloids and Surfaces A: Physicochem. Eng. Aspects **227**(1): 35–44.

Stadler, T. and M. Buteler (2009). Modes of entry of petroleum distilled spray-oils into insects: a review. Bulletin of Insectology **62**(2): 169-177.

Troughton, J. H. and D. M. Hall (1967). Extracuticular Wax and Contact Angle Measurements on Wheat (*Triticum Vulgare* L.). Australian Journal of Biological

Sciences **20**(3): 509 - 526.

Wagner, F. W., M. A. Dean, R. S. de la Motte and V. H. Stryker (1991). Separation and purification of sugar esters. U. S. Patent. United State of America, Nebraska Department Of Economic Development. **4,983,731**.

Wanasundara, U. N., P. K. J. P. D. Wanasundara and F. Shahidi (2005). Novel Separation Techniques for Isolation and Purification of Fatty Acids and Oil By-Products. Bailey's Industrial Oil and Fat Products. A. E. Bailey and F. Shahidi.

Chapter 4

Insecticidal activity of sugar esters against *Rhyzopertha dominica*

4.1 Introduction

While sugar esters have been shown to have insecticidal properties against a wide range of insect species, there have been no studies carried out to investigate the effect of sugar esters on stored grain pests such as *Rhyzopertha dominica*. This chapter will cover studies on this interaction, and produce beneficial data to help develop an effective, environmentally acceptable formulation to help protect stored grains. The influence of the type of sugar (varied in carbon number) and fatty acid (varied in carbon chain length) on *R. dominica* mortality were investigated (section 4.3.1). As the crude sugar ester products also contain residual starting material, the effect of purification of the sugar ester was also studied by comparing insect mortality due to exposure to purified and unpurified forms of sorbitol octanoate and xylitol octanoate (section 4.3.2). The mode of action of the sugar esters was then investigated (section 4.3.3) by (1) examining for any correlation between mortality and wetting properties, which can be linked to suffocation (blocking spiracles) (section 4.3.3.1), (2) carrying out surface bioassays to examine if mortality is due to ingestion or anti-feedant effects (section 4.3.3.2), and (3) examining insects from bioassay experiments by electron microscopy to determine if any noticeable changes to cuticle structure have occurred (section 4.3.3.3).

4.2 Materials and methods

4.2.1 Sugar esters

Sugar esters used in this study are sorbitol octanoate (sC8), sorbitol decanoate (sC10), sorbitol laurate (sC12), xylitol octanoate (xC8), xylitol decanoate (xC10) and xylitol laurate (xC12) which was prepared by the method described in section 2.3.

4.2.2 Bioassay

Preparation of grain and insects for bioassay studies are described in section 2.5.1 and 2.5.2 respectively. Comparative bioassays to study the insecticidal effect of the different sugar esters were carried out in treated bulk grain against adult *R. dominica* as described in section 2.5.3. Surface bioassays to study the mode of action of the sugar esters were carried out as described in section 2.5.5.

4.2.3 HLB value calculation

HLB values were determined from NMR spectral data, as described in section 2.4.2.

4.2.4 Contact angle

Changes to the surface activeness by the application of sugar esters on grain surface were determined using a tensiometer as described in section 2.4.3.

4.3 Results and Discussion

4.3.1 The influence of size of the sugar molecule and chain length of fatty acid on insecticidal activity of sugar esters

Bioassays on these sugar esters (**Table 4.1**) showed that sugar ester prepared with sorbitol and C8 fatty acid was significantly more effective ($81\% \pm 2\%$) than sorbitol esters with C10 ($16\% \pm 3\%$) and C12 ($3\% \pm 1\%$), and that it is the most effective formulation assayed at 4000ppm. This 4000 ppm dose of sC8 was able to effectively suppress progeny formation (0 ± 0) over the 60 day study period, whereas sC10 and sC12 was less effective. Statistical analysis performed via REGWQ tests ($p < 0.05$) for mean mortality on the 7th and 14th day for all treatments are included in **Appendix 3**.

Table 4.1 Toxicity of sugar ester on LGB prepared with either sorbitol or xylitol and fatty acids (4000ppm) having different chain length in 7, 14 and 60 days.

Sugar esters 4000ppm	Mean mortality (%)		Progeny (60 days)‡	REGWQ test, subset for alpha = 0.05			
	7 days†	14 days†		1	2	3	4
Control	0±0	0±0	432±81	*			
sC8	54±4	81±2	0±0				*
xC8	6±2	30±3	6±7		*	*	
sC10	10±2	16±3	202±56	*	*		
xC10	28±3	50±5	41±16			*	
sC12	2±0	3±1	168±26	*			
xC12	0±0	1±0	131±15	*			

†mean mortality % ± standard deviation

‡progeny ± standard deviation

a). Bioassay carried out on bulk grain, done in triplicate

b). 30 adult (0-2 week old) *R dominica* were used in each replicate

c). Mean mortality on 14 days is accumulative results, these data were subjected to Abbotts' correction and arcsine transformed prior to REGWQ test, SPSS ($p < 0.05$)

In contrast, xylitol esters gave a less clear pattern in insect mortality. Grains treated with xylitol octanoate achieved $30\% \pm 3\%$ mortality; whereas xylitol decanoate caused $54\% \pm 4\%$ mortality and xylitol laurate gave no insect mortality by the 14th day (**Table 4.1**). Note however that the difference in xC8 and xC10 mortality is not statistically significant (Appendix 3ab), so it may be concluded that an overall trend of decreased insect mortality with increasing fatty chain length is present. Less overall mortality compared to sorbitol esters was also found, in agreement with previously reported studies using sugar esters to kill pear psylla, tobacco aphid, tobacco hornworm and mites (Puterka et al., 2003). As with the sorbitol esters, the 4000 ppm dose of xC8 was able to effectively suppress progeny formation (6 ± 2) over the 60 day study period, whereas xC10 and xC12 were less effective. This indicates that the shorter fatty acid chain length (in the sugar ester, residual octanoic acid or both) is critical to inducing progeny suppression in *R. dominica*.

4.3.2 Degree of washing and the impact on insecticidal activity

Sorbitol octanoate (sC8) was selected for this study as the most effective insecticide of the type against other insects. Xylitol octanoate (xC8) was included for comparison. The results of bioassays comparing the efficacy of unwashed and washed sugar esters are presented in **Table 4.2**.

Mortality due to the unpurified (unwashed or crude) sugar ester products is very low, and is indistinguishable from control samples; however progeny appears to be substantially reduced. Purification using the two-step process results in a significant increase in mortality for sorbitol octanoate ($81\% \pm 2\%$) and possible increase for xylitol octanoate ($30\% \pm 3\%$) at 4000ppm. Overall, this result indicates that the presence of unreacted sugars may have diluted the effective dosage of sugar ester, and that the sugars alone do not have any appreciable effect on insect mortality and progeny suppression (See Appendix 3cd for detail analysis).

Interestingly, sugar esters further purified using an acetone wash to remove unreacted fatty acids appears to give lower mortality for sorbitol octanoate ($58\% \pm 7\%$), implying that the octanoic acid impurity also has some insecticidal effect.

Table 4.2 Effect of sugar ester purification (4000ppm) on LGB mortality in 7 and 14 days and progeny formation in 60 days.

	Mean mortality (%)		Progeny 60 Days‡	subset for alpha = 0.05		
	7Days†	14Days†		1	2	3
Control	0±0	0±0	286±52	*		
sC8 crude	0±0	4±1	38±6	*		
sC8 two-steps	54±4	81±2	0±0			*
sC8 three-steps	23±4	58±7	2±1		*	*
xC8 crude	7±2	9±2	15±3	*		
xC8 two-steps	6±2	30±3	6±7	*	*	
xC8 three-steps	13±2	36±4	7±7	*	*	

†mean mortality % ± standard deviation

‡progeny ± standard deviation

a). Bioassay carried out on bulk grain, done in triplicate

b). 30 adult (0-2 week old) *R dominica* were used in each replicate

c). Mean mortality on 14 days is accumulative results, these data were subjected to Abbotts' correction and arcsine transformed prior to REGWQ test, SPSS ($p < 0.05$)

d). sC8 concentration: 4000ppm, xC8 concentration: 4000ppm

e). Two-steps: washed to remove catalyst and unreacted sugars

f). Three-steps: washed to remove catalyst, unreacted sugars and unreacted fatty acids

However, it should be noted that the difference in mortality between two-step and three-step purified sorbitol octanoate is in-significant according to the REGWQ analysis. Furthermore, acetone wash has no significant effect on insect mortality due to xylitol octanoate. For comparison, previous studies involving purification of crude sugar ester to remove any unreacted materials was regarded to cause no difference in its insecticidal effects as with the case of sucrose palmitate (GTA, 2014). Octanoic

acid and sorbitol have both been shown to have insecticidal effects, so the combination as a sugar ester may likewise be having similar effects against *R. dominica* (Legal et al., 1999, Hu et al., 2010). Additionally, it is also possible that the sugar and fatty acid impurities are also having an influence on the observed insecticidal effects.

Regarding progeny production, near complete suppression of progeny after 60 days was observed for xylitol octanoate (15 ± 3 ; 6 ± 7 ; 7 ± 7) and sorbitol octanoate (38 ± 6 ; 0 ± 0 ; 2 ± 1), both in crude and purified forms, which suggests that substantial interference with feeding and growth (Collins and Cook, 2006), or ovipositional deterrence (Jackson et al., 1991) due to the sugar ester is at play. Further study is needed to understand the exact impact of these compounds on LGB's growth, egg laying habit and or the egg.

4.3.3 Investigation of the mode of action of sugar esters

Several possible modes of action have been suggested in the literature for the insecticidal effects of sugar esters, including suffocation, desiccation (disrupting waxy water retaining cuticle layers upon surface contact) or action as a feeding deterrent (through ingestion). Desiccation and suffocation are well related to sugar ester surfactant / wetting properties, which determine the surface activity upon contact with insect surfaces (Puterka et al., 2003). Anti-feedant properties may be determined by isolating the effects of surface contact and ingestion of sugar esters. To further achieve better understanding of mode of action specifically related for interfacial properties of SE and their capability to extract lipids from the cuticle part we explore their hydrophobic/hydrophilic properties.

4.3.3.1 Contact angle

In an effort to see if there is any correlation between insecticidal effect and the surfactant properties of the sugar esters, contact angle measurements (described in

section 3.3.5) are compared with insect mortality from bioassay studies. Contact angle refers to the affinity of a liquid with a surface, where contact angles less than 90° refers to high wettability (hydrophilic surface) and vice versa (Yuan and Lee, 2013). Grain is used as the wetting surface to see the effect of treatment with wet solutions of sugar ester for the bioassays, so this will give some insight as to the state of the sugar ester when the insect comes into contact with the treated grains. A table comparing bioassay results with measured contact angles on grain is given in **Table 4.3**, and images of all contact angle profiles are included in **Appendix 4**.

Table 4.3 Contact angles and their relative mortality and progeny suppression of Katana grains coated with sugar esters at varied concentration (100 to 4000ppm)

	Mean mortality (%)	Progeny 14 Days†	Contact angle (θ°) at different concentration (ppm)			
			60 Days‡	100	400	1000
Plain grain	0±0	432±81		127		
sC8	81±2	0±0	100	92	92	35
xC8	30±3	6±7	108	89	81	31
sC10	16±3	202±56	101	89	87	27
xC10	50±5	41±16	102	96	85	20
sC12	3±1	168±26	102	90	45	29
xC12	1±0	131±15	98	99	31	28

†mean mortality % ± standard deviation

‡progeny ± standard deviation

a). Bioassay carried out on bulk grain, done in triplicate

b). 30 adult (0-2 week old) *R dominica* were used in each replicate

Katana grain without any treatment (serving as a negative control) has a hydrophobic surface ($\theta = 127^\circ$) due to extracuticular wax (Troughton and Hall, 1967). Presence of surface wax is likely to facilitate the uptake of lipophilic organics by reducing the surface energy which helps the sugar ester to spread out and evenly coat the grain (Frost et al., 2007).

Application of sugar esters on grain changed the contact angle extensively, with higher concentration giving a lower contact angle (increased wetting of the surface). Decreased contact angle also occurred at lower concentrations for sugar esters with longer fatty chains. Interestingly, this result suggests that wettability is not a key factor in the insecticidal activity of these sugar esters, with no large differences between esters at doses of 4000 ppm, and low contact angles at 1000 ppm only occurring for sC12 and xC12, which give the lowest insect mortality. This is in contrast with previous studies, which found that high insect mortality is positively correlated with low contact angle (Gillilan, 2012). This implies that the mode of action is not related to high surface wettability (and consequently that suffocation by blocking of insect spiracles is unlikely in this instance).

4.3.3.2 Hydrophilic-lipophilic balance value

Variation in sugar ester's inhibition activity (section 4.3.1) appears to be loosely correlated with a decrease in HLB value for this sugar ester (longer fatty acid chains) (**Table 4.4**).

Table 4.4 HLB value of sugar esters

	HLB value
sC8	11.3
xC8	10.5
sC10	10.1
xC10	9.3
sC12	9.2
xC12	8.4

HLB value was determined by dividing proton number of hydrophilic group by the proton numbers of lipophilic group

Higher HLB (11.3 for sC8) would promote sugar ester suspension (oil in water emulsion) disrupting the insect epicuticle, whereas the lowest HLB values for these sugar esters (8.4 for xC12) correspond well to surfactants that act as wetting or

spreading agents (which is exactly what is observed in the contact angle results – xC12 wets the grain more readily than the other sugar esters. Xylitol esters tend to have lower HLB values which have less overall mortality compared to sorbitol esters.

4.3.3.3 Mode of feeding

The relative contribution of surface contact and ingestion on the insecticidal properties of sugar esters was studied by applying sugar ester suspensions on wheat kernels or directly onto petri-dish surfaces. From these experiments, it was found that both treatments are equally ineffective (**Table 4.5**). In this study, wheat kernels are crushed into small granules to prevent protection from exposure by insects boring into grains, thus the grain surface area is increased. This may dilute the coverage of sugar ester suspension coated on grain surface, hence the reduced toxicity upon contact with insects and with feeding. Therefore, no solid conclusion can be gained from these results.

Table 4.5 Morality of insect feeding on wheat granule with and without sugar ester coating (4000ppm) in 18 days.

		Mean mortality	subset for alpha = 0.05
Negative control		0±0	*
Petri-dish	sC8	0±0	*
	xC8	1±2	*
Grain	sC8	0±0	*
	xC8	0±0	*

- a. Multiple comparison of mean mortality using REGWQ test ($p < 0.05$)
- b. Sugar ester dose: 4000ppm
- c. Grain refers to the application of ester suspension on the grain

4.3.3.4 Scanning Electron microscopic (SEM) study

The effect of exposure to sorbitol octanoate treated grain on the morphology of insect cuticles was examined by scanning electron microscopy (**Figure 4.1**). Antenna,

sternites and elytra were imaged at 1000x and 5000x magnification. No obvious deposition of sugar esters was observed, nor disintegration or other significant structural changes to the insect cuticle. This implies that any changes to cuticle that may be occurring are at a scale smaller than can be observed using this type of instrument (likely at a molecular level), or that insecticidal properties are due to internal chemical effects on the insects. Imaging at higher magnification is difficult to achieve with insect specimens due to severe charging of the insect samples under the electron beam.

Further exploration using other microscopy techniques such as Focused Ion Beam would enable cross-sections of the cuticle and tracheal system to be examined at the submicron scale (Schmitz et al., 2007). Labelling of cuticular compounds using dye- or radio-labelling can also be considered an alternative to assist tracking of any physical changes to the cuticular layers (Collins and Cook, 2006).

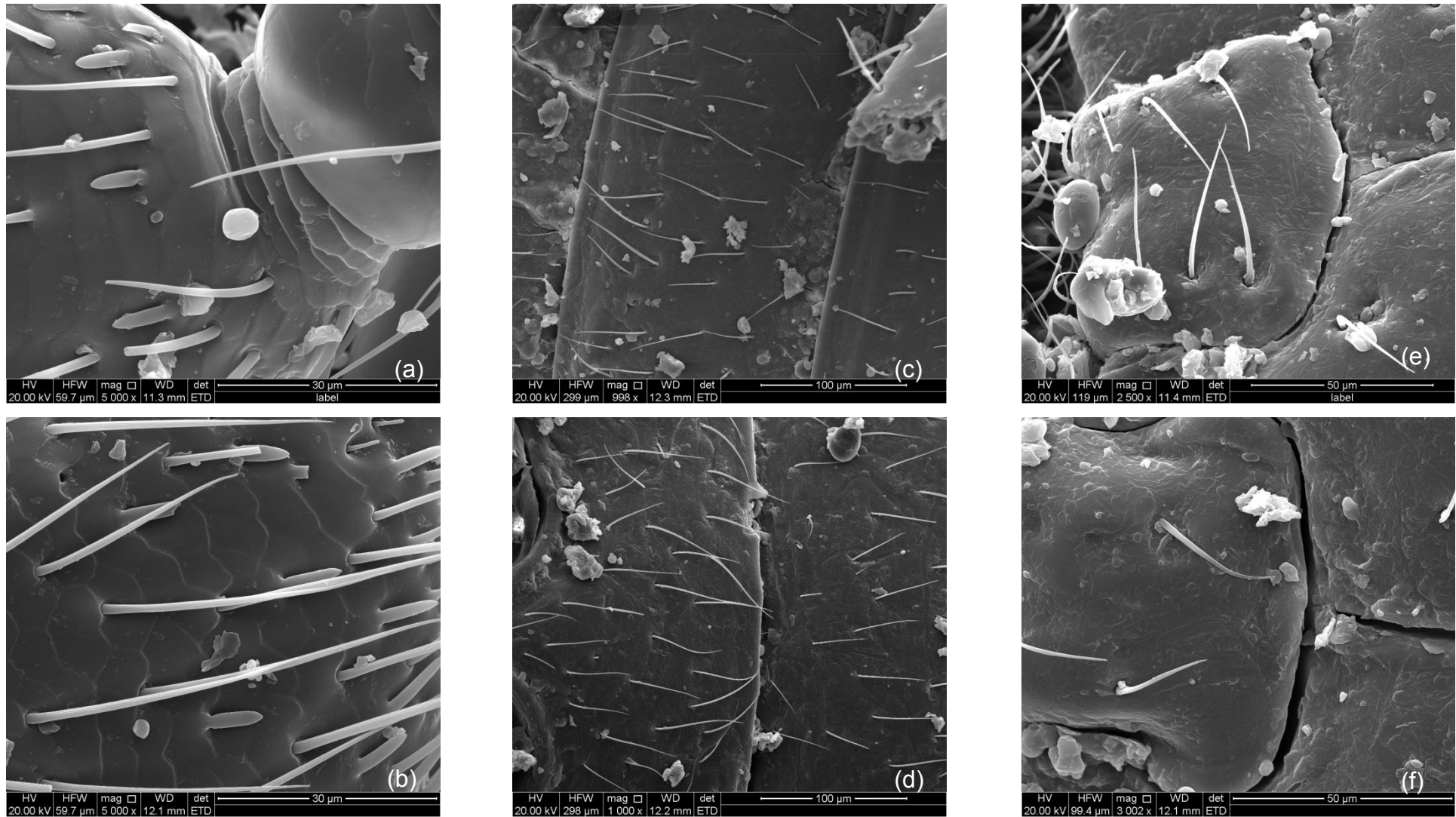


Figure 4.1 SEM images showing the antenna, sternites, and pronotum and elytra of *Rhizopertha dominica*, before (a, c, e) and after (b, d, f) exposure to grains treated with 4000ppm sorbitol octanoate.

4.4 Conclusion

Studies comparing the effect of different fatty acid chain length and choice of sugar were carried out. From these bioassays, Sorbitol octanoate was the most potent sugar ester, with mortality of $81\% \pm 2$ at 4000ppm against LGB after 14 days of exposure. Sorbitol esters were more effective than xylitol esters, while increasing carbon chain length was generally correlated with lower insect mortality.

Removal of unreacted sugars from synthesized crude sugar esters is critical to its insecticidal properties. In studies on sorbitol and xylitol octanoate, removal of unreacted sugars significantly increased insecticidal effectiveness, whereas removal of the unreacted fatty acids had no statistically significant effect for either xylitol or sorbitol esters. Thus, purification of crude sugar esters using ethanol and water to remove extra sugars is the most preferred post-processing method, although sugar esters synthesized without purification can be an economic alternative at much lower costs but reduced insecticidal activity to their refined version.

Subsequent studies to investigate the mode of action of the sugar esters gave some clues as to the mode of action, but were not conclusive. Higher insect mortality was correlated with higher contact angles (less wettability), indicating that modes of action relying on high wettability (such as suffocation) are not likely. Higher HLB is related to better emulsifying properties i.e. disrupting waxy epicuticle layers and therefore giving evidence that desiccation may be a mode of action. Overall, insecticidal activity was positively correlated with calculated HLB values. Studies on the route of exposure gave no information as to if sugar ester acts as a contact poison, kills by ingestion or has anti-feedant properties. No buildup of sugar ester or obvious changes to the cuticle layer were visible to direct observation via electron microscope at up to 5000x magnification.

Further study at higher concentrations may be needed to elucidate the insecticidal mode of action. Other methods such as dye or radio-labelling of sugar ester molecules or dissection of insects to reveal affected areas of the insect body may also be helpful. Further study is also needed to understand how sorbitol octanoate can act to suppress progeny production of LGB (oviposition or progeny development as well

as the underlying mechanism). Increase of dosages may be required to discriminate between the two exposure routes examined in petri-dish studies.

References

Frost, R., O. Carmody, Y. Xi and S. Kokot (2007). Surface characterisation of selected sorbent materials for common hydrocarbon fuels Surface Science **601**(9): 2066-2076.

Gillilan, J. (2012). Improvement of U.S. EPA Minimum Risk Essential Oils' Pesticide Activity Through Surfactant Enhancement and Synergy, The Ohio State University. **Doctorate of Philosophy.**

GTA (2014). GTA Wheat Standards 2014/2015. Australia, Grain Trade Australia.

Hu, J. S., D. B. Gelman, M. E. Salvucci, Y. P. Chen and M. B. Blackburn (2010). Insecticidal activity of some reducing sugars against the sweet potato whitefly, *Bemisia tabaci*, Biotype B. Journal of Insect Science **10**(203).

Jackson, D., R. Severson, V. Sisson and M. Stephenson (1991). Ovipositional response of tobacco budworm moths (Lepidoptera: Noctuidae) to cuticular labdanes and sucrose esters from the green leaves of *Nicotiana glutinosa* L. (Solanaceae). Journal of Chemical Ecology **17**(12): 2489-2506.

Legal, L., B. Moulin and J. M. Jallon (1999). The Relation between Structures and Toxicity of Oxygenated Aliphatic Compounds Homologous to the Insecticide Octanoic Acid and the Chemotaxis of Two Species of *Drosophila*. Pesticide Biochemistry and Physiology **65**(2): 90–101.

Puterka, G. J., W. Farone, T. Palmer and A. Barrington (2003). Structure-Function Relationships Affecting the Insecticidal and Miticidal Activity of Sugar Esters. Journal of Economic Entomology **96**(3): 636-644.

Schmitz, A., A. Sehrbrock and H. Schmitz (2007). The analysis of the mechanosensory origin of the infrared sensilla in *Melanophila acuminata* (Coeloptera; Buprestidae) adduces new insight into the transduction mechanism. Arthropod Struct Dev. **36**(3): 291-303.

Troughton, J. H. and D. M. Hall (1967). Extracuticular Wax and Contact Angle Measurements on Wheat (*Triticum Vulgare* L.). Australian Journal of Biological Sciences **20**(3): 509 - 526.

Yuan, Y. and T. R. Lee (2013). Contact Angle and Wetting Properties. Surface Science Techniques. G. Bracco and B. Holst. Berlin Heidelberg, Springer-Verlag: 3-34.

Chapter 5

Insecticidal activity of sorbitol octanoate combining with diatomaceous earth against *Rhyzopertha dominica*

5.1. Introduction

Based on the results presented in the previous chapter, sugar esters were identified (in particular sorbitol octanoate) that exhibit insecticidal properties. However, the dose esterified required to control *R. dominica* is rather high (> 4000 ppm), so in order to be acceptable by the market, efficacy needs to be increased so lower doses are effective. One way to achieve this, which aligns with the overarching concept of integrated pest management, is to combine sugar esters with other safe insecticides. Diatomaceous earth (DE) is one such material that has been extensively studied for its insecticidal properties.

In this chapter, the insecticidal efficacy of this combination is characterised in a series of two bioassays. First, combinations of sorbitol octanoate (sC8) with DE at various ratios were tested against *R. dominica* to identify the most effective formulation that requires the lowest dose of DE. Sorbitol and octanoic acid (at doses equivalent to that used to prepare this sugar ester at doses of 1000, 2000 and 4000 ppm) were also included in the study to help identify their contribution to insect mortality. Once the optimal formulation is identified, a further bioassay is carried out to determine the lethal dose of the formulation (using probit analysis) compared with a positive control (Dryacide), as well as the lethal dose of the individual DE and sugar ester components. This data is used to determine the relative potency of the formulation, and to determine if any synergistic or antagonistic effects are present in the combined formulation. The possible modes of action of this combined formulation are then investigated by examining insects from the bioassays using optical and electron microscopy. The addition of DE to the formulation can cause handling difficulties, where higher dosages of DE on grain kernels will raise the friction between

kernels, causing reduced flowability and decreased bulk density, which consequently degrades commodity value (Korunic et al., 1998). Therefore, the effect of this formulation on key grain properties (colour, bulk density and angle of repose) is also studied.

5.2. Materials and methods

5.2.1. Sorbitol octanoate

Sorbitol octanoate was produced by following the synthetic methods described in section 2.3, and characterised by FTIR and NMR as described in section 2.4 (results in sections 3.3.2 and 3.3.3 respectively) to confirm successful product formation.

5.2.2. DE and Dryacide

DE dust was prepared and provided by Dr. Lucas Johnson using the procedure detailed in section 2.1.1. Dryacide was purchased from Entosol Australia Pty Ltd.

5.2.3. Comparative bioassay

This is carried out as described in section 2.5.3.

5.2.4. Lethal dose bioassay

This is carried out as described in section 2.5.4.

5.3. Results and discussion

5.3.1. Efficacy of combined formulations of DE and sC8

Data on the efficacy of various doses of DE, sC8 and combinations of these compounds are presented in **Table 5.1**. From this data, it is apparent that DE gives near 100% mortality at doses of 700 ppm, whereas the mortality due to sC8 in this bioassay is comparatively smaller ($31\pm 15\%$ at 4000 ppm). Note that the insect mortality due to sC8 in this bioassay is less than in previous bioassays (Table 4.1), however, this difference is within the range of natural variation in insect response (Robertson et al., 1995). Interestingly, while combinations of 1000 ppm sC8 with DE are less effective than the same dose of DE alone, combination of 2000 and 4000 ppm sC8 with DE results in an overall improvement in insecticidal efficacy. In particular, the optimal combination is 100 ppm DE with 4000 ppm sorbitol octanoate ($96\pm 2\%$ mortality and complete progeny suppression), which is more effective than its component ingredients. The DE dosage in this formulation may potentially have minimal impact on grain properties (Korunic et al., 1996).

Table 5.1 Toxicity of sorbitol octanoate, DE and their combinations against LGB expressed in mortality (7 and 14 day) and progeny suppression (60th day).

	Dose (ppm)	Mean mortality %		Progeny (60 Days)‡	Subset for alpha = 0.05						
		7Days†	14Day†		1	2	3	4	5	6	
Control	0	0±0	0±0	174±39	*						
DE	100	3±1	29±4	188±71		*					
	400	43±5	84±1	36±11					*	*	*
	700	84±2	98±1	4±2							*
	1000	94±1	98±1	0±0							*
sC8	1000	0±0	2±1	169±14	*						
	2000	2±0	2±0	52±13	*						
	4000	12±1	31±4	8±3		*					
sC8/DE	1000/100	0±0	4±2	158±85	*						
	1000/400	9±2	47±4	10±4		*		*			
	1000/700	24±0	68±3	0±0				*	*		
	1000/1000	32±3	74±2	37±22					*	*	
	2000/100	10±3	41±6	1±1		*					
	2000/400	64±1	97±2	0±0							*
	2000/700	64±1	94±1	6±2						*	*
	2000/1000	81±3	100±0	1±1							*
	4000/100	62±3	96±0	0±0						*	*
	4000/400	87±4	100±0	12±8							*
	4000/700	87±2	99±0	0±0							*
	4000/1000	88±2	100±0	0±0							*

†mean mortality % ± standard deviation

‡progeny ± standard deviation

a). Bioassay carried out on bulk grain, done in triplicate

b). 30 adult (0-2 week old) *R dominica* were used in each replicate

c). Mean mortality on 14 days is accumulative results, these data were subjected to Abbotts' correction and arcsine transformed prior to REGWQ test, SPSS ($p < 0.05$)

The second stage of the bioassay study was to further investigate the optimal combination of DE and sC8, to determine the lethal doses that kill 50% (LD₅₀) and 90% (LD₉₀) of the insect population within 14 days. Test concentrations for this bioassay are selected based on results obtained from the first bioassay. Dose-response curves derived from probit analysis of this bioassay data using SPSS are given in **Figures 5.1 - 5.3**. From this data, lethal dose values and the relative potency (based on LD₅₀ values) are determined, which are summarized in **Table 5.2**.

Table 5.2 Lethal doses (LD₅₀ / LD₉₀) and relative potency (vs. Dryacide) of the optimal formulation and its component ingredients in 14 days.

Treatment	LD ₅₀ (ppm)	LD ₉₀ (ppm)	Relative potency (LD ₅₀)
Dryacide	62 (26 - 90)	197 (151 - 307)	-
DE only	49 (17-75)	167 (128 - 243)	1.26
sC8 only	6457 (5737 - 7977)	11668 (9022 - 21501)	0.0097
DE + sC8	2744 (2218 - 3553)	5685 (4170 - 12296)	0.0229
<i>DE</i>	67	139	
<i>sC8</i>	2677	5546	

a) Lethal dose values are reported with the 95% confidence intervals in parenthesis.

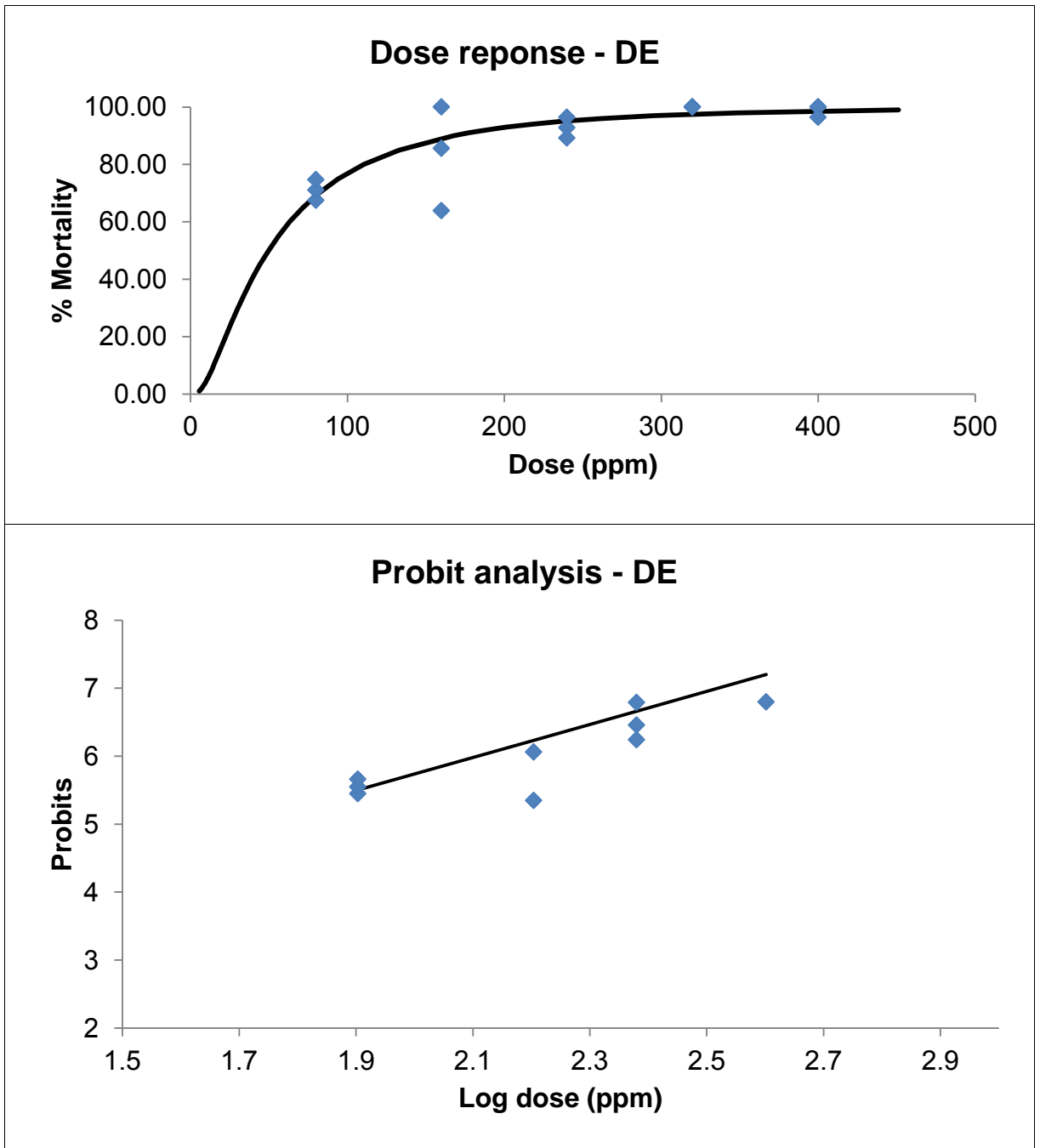


Figure 5.1 Insect mortality of LGB placed in DE treated grains in 14 days.

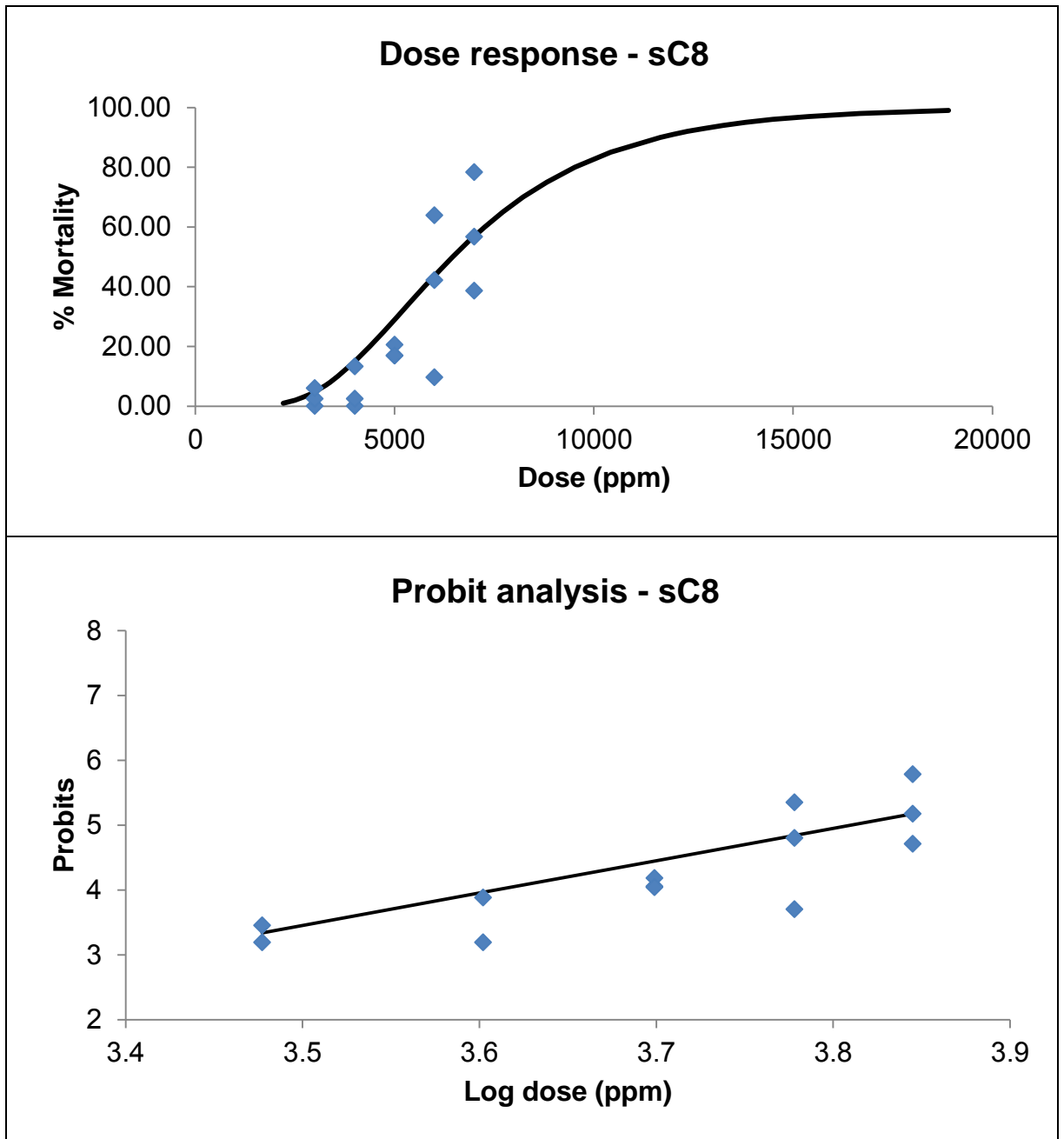


Figure 5.2 Insect mortality of LGB placed in sorbitol octanoate treated grains in 14 days.

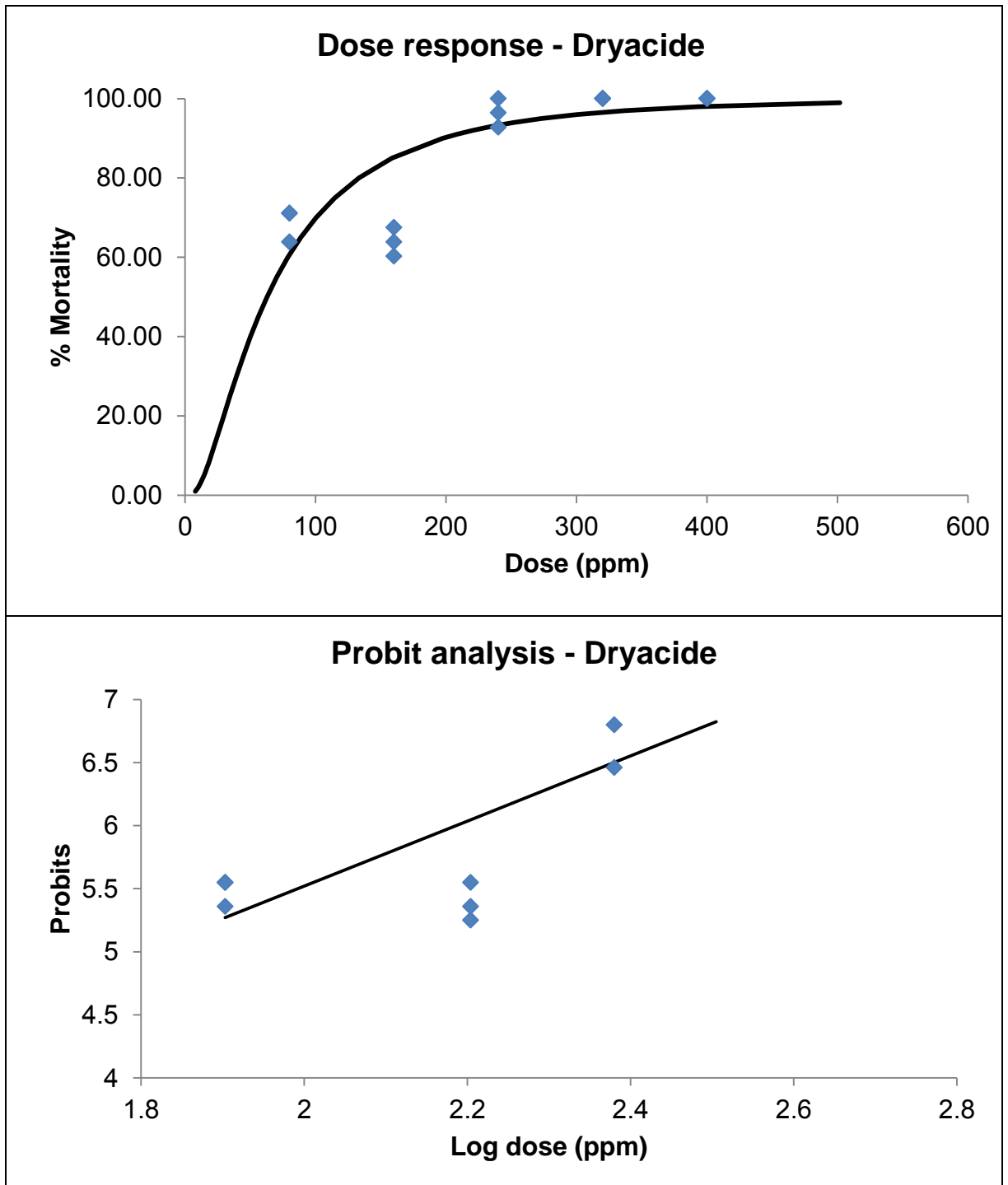


Figure 5.3 Insect mortality of LGB placed in Dryacide® treated grains in 14 days.

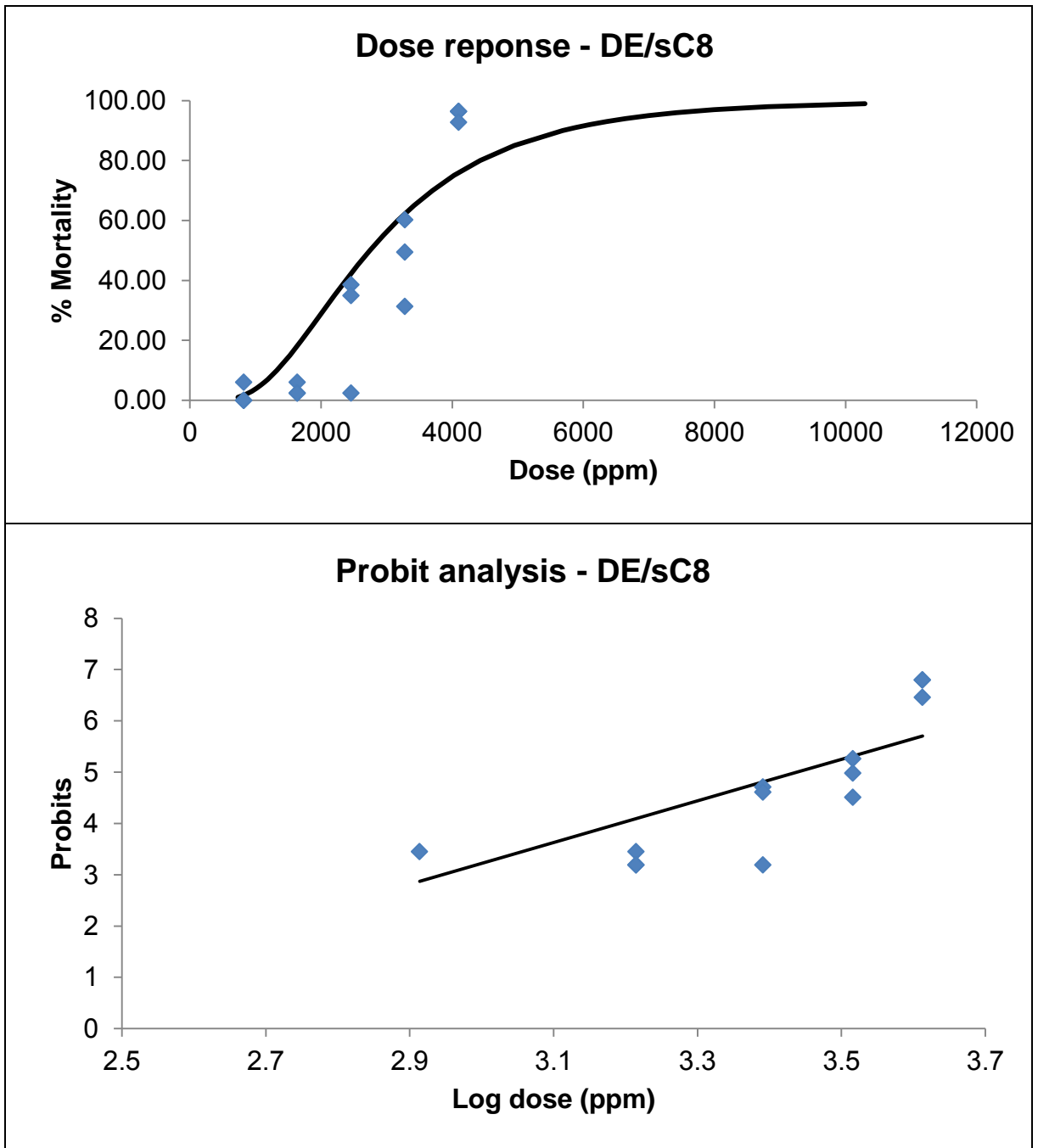


Figure 5.4 Insect mortality of LGB placed in DE / sorbitol octanoate treated grains in 14 days.

In summary, the LD₅₀ for the combined formulation is calculated to be 2745 ppm, or equivalently 67 ppm of DE and 2745 ppm of sC8 (DE: sC8 ratio at 1:40). For sC8, this active concentration was much lower compared to the LD₅₀ for sC8 only (6458 ppm). However for DE by itself, a LD₅₀ value of 50 ppm was determined in this bioassay, which is lower than in the formulation containing sorbitol octanoate. Interestingly, it is noted that the efficacy of the DE in this bioassay (80ppm caused 73%±1% mortality, see Appendix 3g) is much higher compared to most other bioassay studies that were carried out, where a DE dosage of around 400 ppm is needed to cause > 90 % insect mortality. Given the negligible control mortality, and the similarly high efficacy for the positive control (Dryacide, see Appendix 3g), this is ascribed to natural variation in treatment responses between generations (Robertson et al., 1995). Calculation of the combination index for this formulation (Chou, 2010) gives a value of 2.32, which indicates that there is a slight antagonistic effect between the DE and sorbitol octanoate. This may be explained by considering that the wet sorbitol octanoate solution may coat the DE particles when applied to the grain, thereby inhibiting their ability to take up waxes from the insect epicuticle. Alternatively, the sticky sugar ester solution may increase the binding of the DE particles to the wheat grains, inhibiting uptake of the DE particles onto the insects.

5.3.2. Mode of insecticidal action

The mode of action of this combined formulation was investigated by first examining insects exposed to treated grain by optical microscopy. As shown in **Figure 5.5**, insects exposed to grain treated with DE exhibited a positive correlation between DE dose applied to the grain and DE particle accumulation on the insects. In contrast, insects exposed to grain treated with sorbitol octanoate showed no visible accumulation of material on their surfaces (**Figure 5.6**). Interestingly, substantially less attachment of DE onto the insects is noted in the combined formulation (**Figure 5.7**), with DE particles tending to accumulate only around the pronotum and lower part of elytra. Furthermore, an increase in sC8 concentration appears to cause a decrease in DE particle attachment on the insects (**Figure 5.8**). This supports the hypothesis

that antagonistic interactions between DE and sorbitol octanoate in the combined formulation are due to increased adhesion of DE to the grains (and hence less uptake onto the insects). Areas of the insects with higher amounts of DE accumulation were further examined at higher magnification by SEM (**Figure 5.9 and Figure 5.10**). Here, a positive correlation between DE dose and particle attachment (particularly at submicron sizes) is observed, confirming the results gathered from optical microscopy. Hairy sensilla and the dorsal side of insect body tend to attract more DE particles, which tended to accumulate on the tibia, tarsal segments and abdominal sternites (see **Appendix 5**). This distribution of adhered DE is likely related to insect movement through treated grains and container surfaces by crawling, which emphasises contact between the insect and grain surfaces in these areas (Collins and Cook, 2006). Whether the tendency for DE particles to adhere to the antennae alters the sensitivities of peripheral olfactory receptors on the sensillum is unknown (Roth and Willis, 1951, Germinara et al., 2009), but may be worth further investigation. No signs of abrasion were observed on the exocuticle layer at 5000x, indicating that the inhibition mechanism of the DE particles attached on the insect surfaces may be desiccation (Ebeling, 1971).

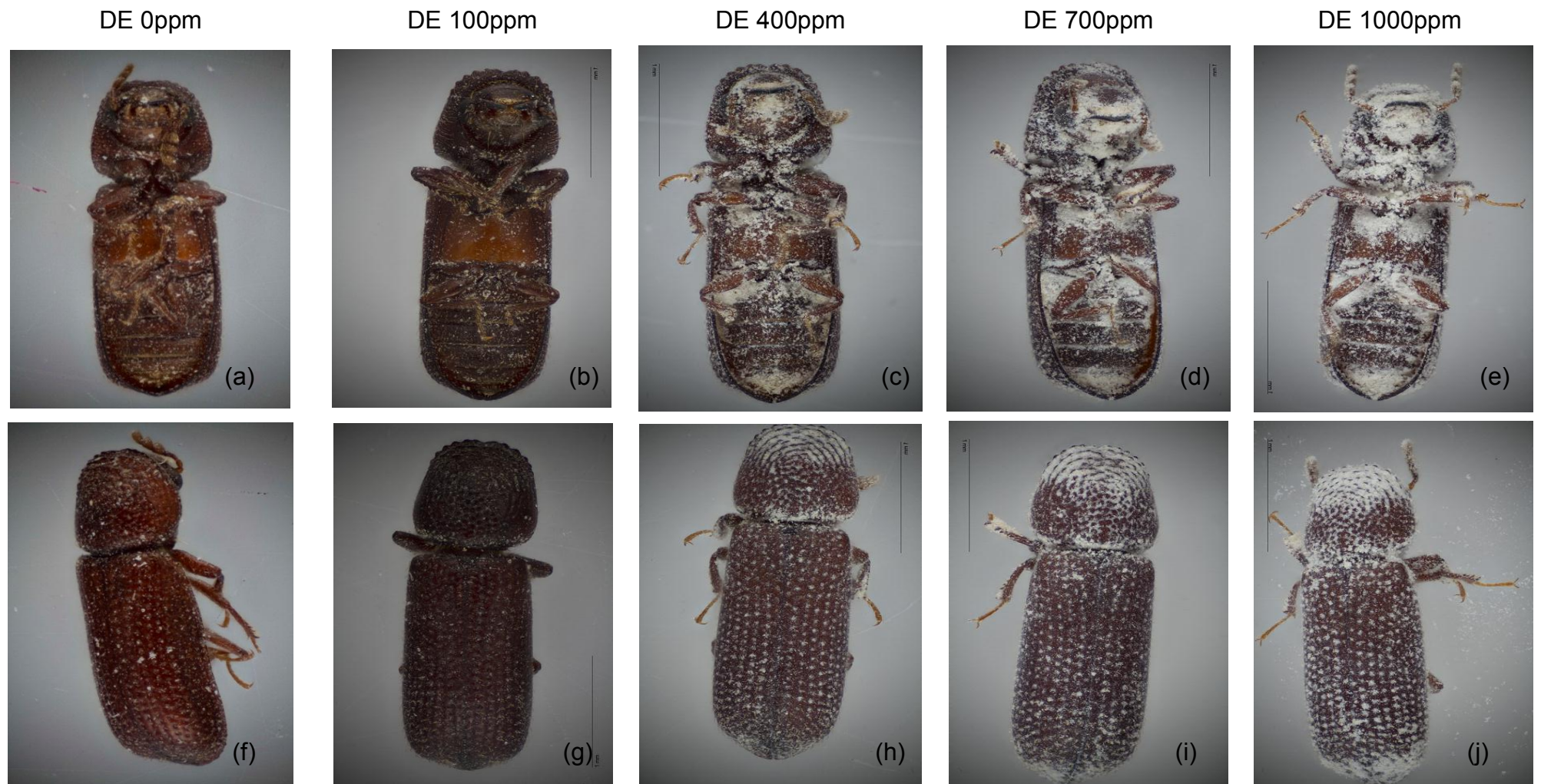


Figure 5.5 Images of insects treated with DE dust at different concentration (scale bar = 1mm). Above: ventral view, below: dorsal view.



Figure 5.6 Images of insects treated with sC8 at different concentration (scale bar = 1mm).

Above: ventral view, below: dorsal view.

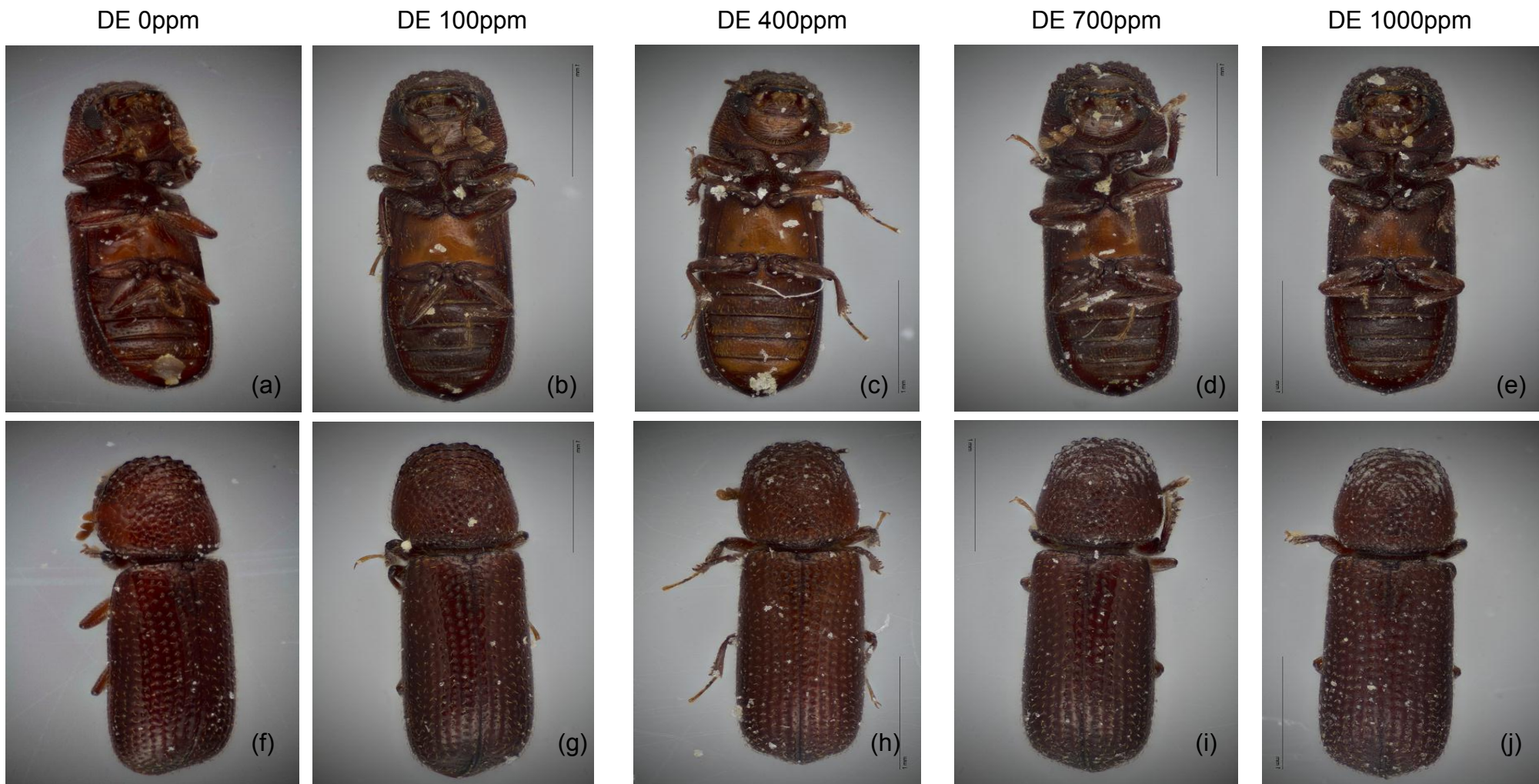


Figure 5.7 Images of insects treated with DE (varied in concentration) combined with 4000ppm sC8 (scale bar = 1mm). Above: ventral view, below: dorsal view.

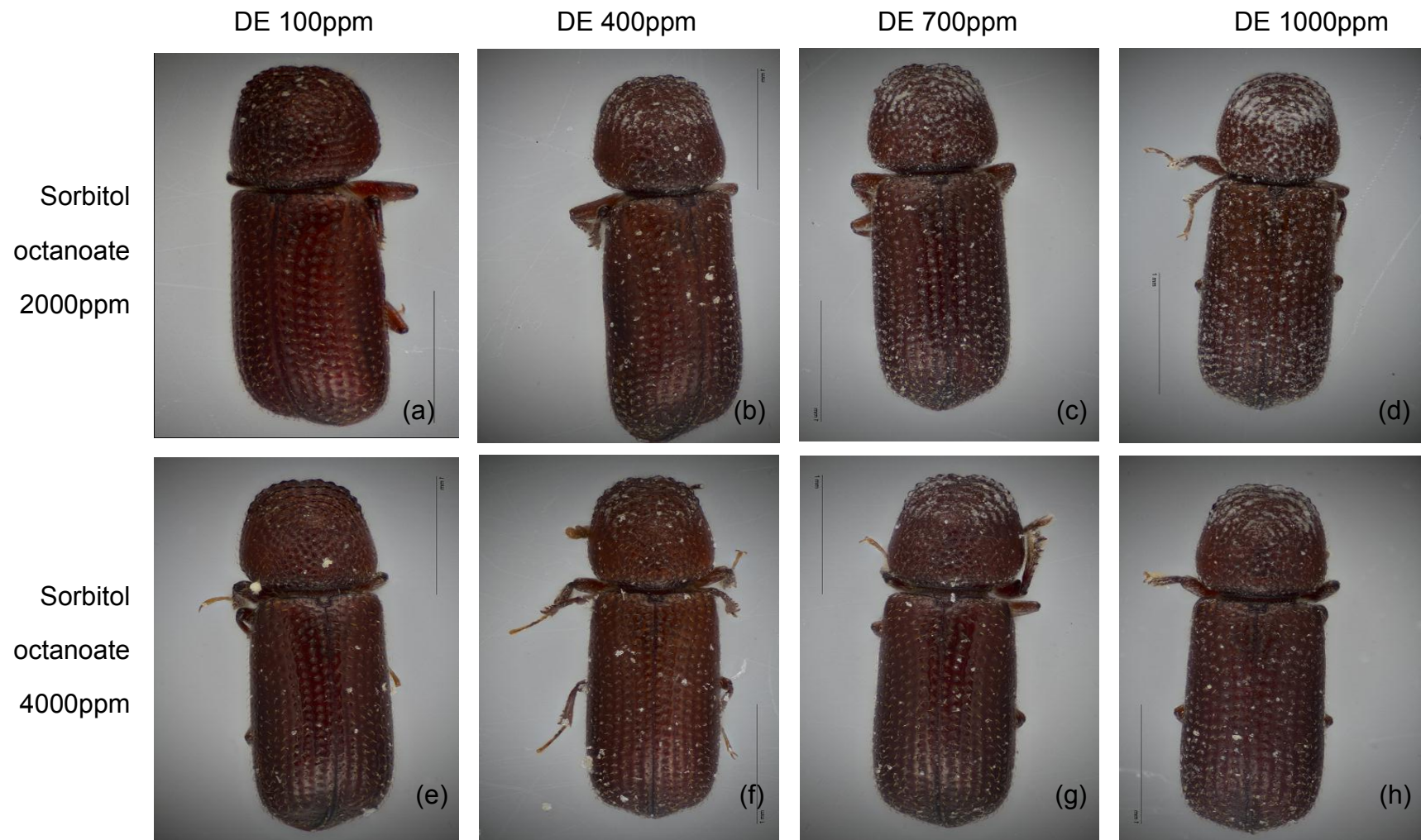


Figure 5.8 Images of insects treated with 2000ppm and 4000ppm sC8 combined with DE at varied levels (scale bar = 1mm). Above: ventral view, below: dorsal view.

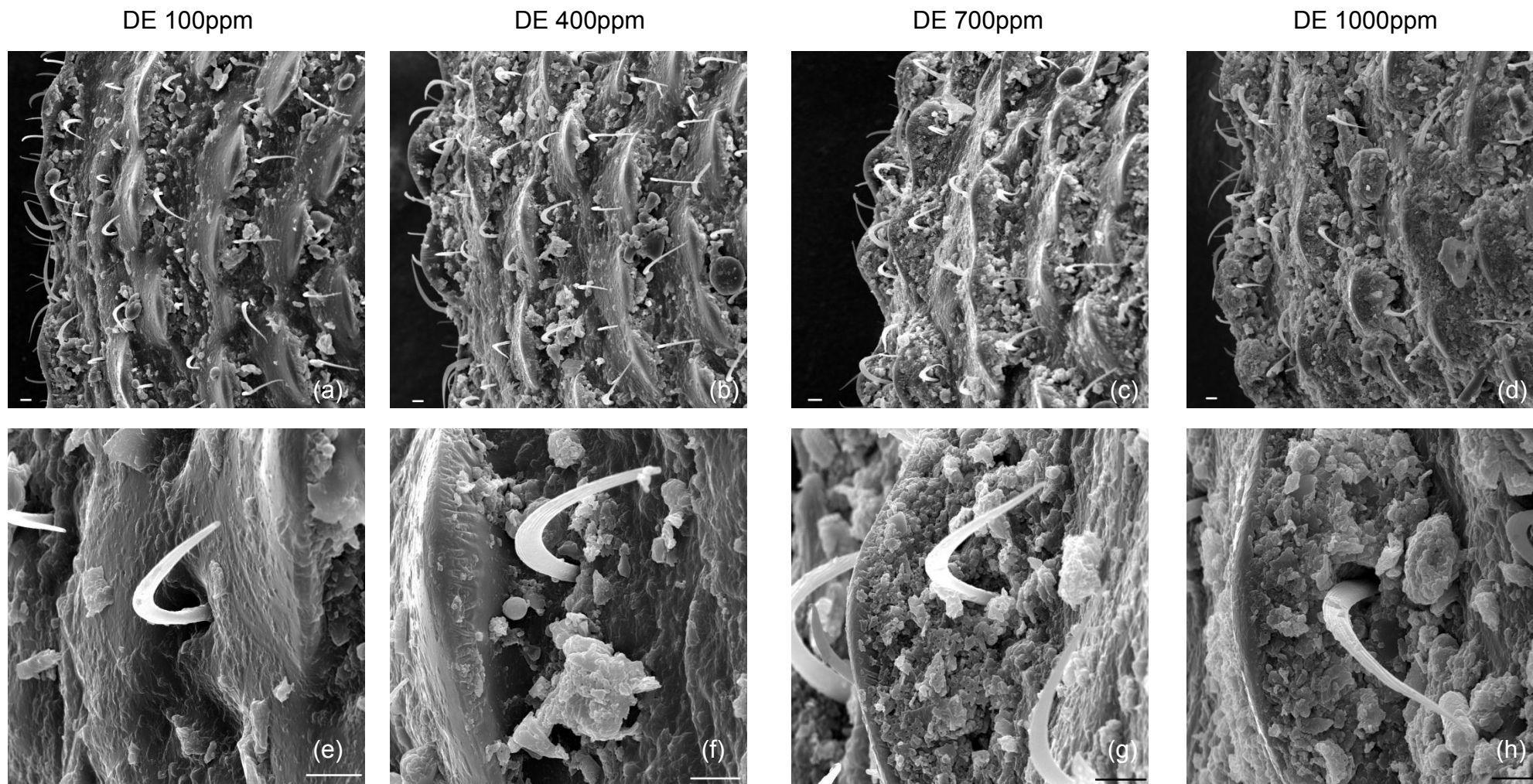


Figure 5.9 Pronotum of *R. dominica* treated by DE-sC8 combined formulation displayed at 1000x and 5000x magnification.

Image magnification, first row = 1000x, second row = 5000x; scale bar = 10µm. DE concentration: 100, 400, 700 and 1000ppm. sC8 concentration 4000ppm

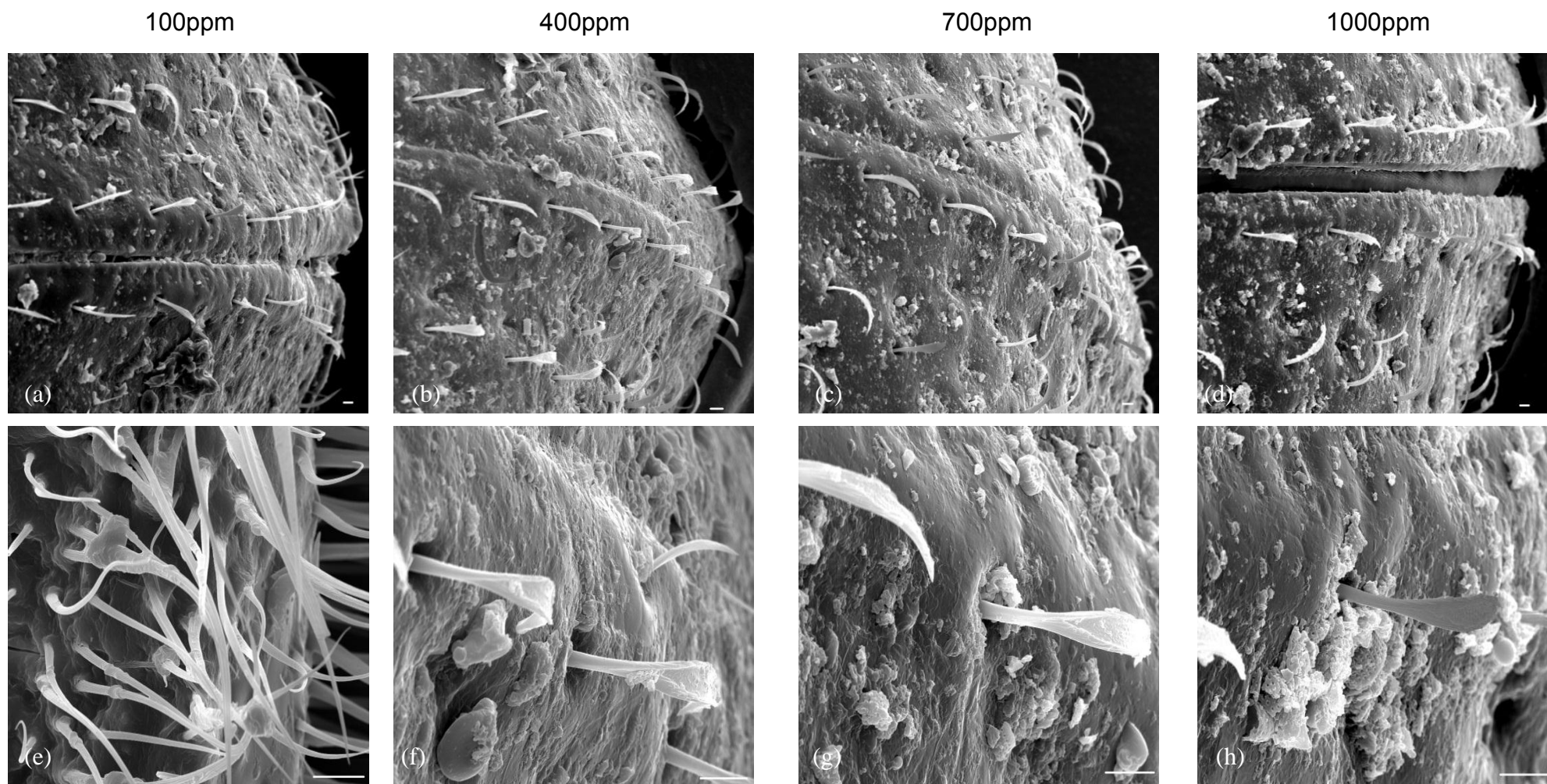


Figure 5.10 Elytra of *R. dominica* treated by DE-sC8 combined formulation displayed at 1000x and 5000x magnification

Image magnification, first row = 1000x, second row = 5000x; scale bar = 10µm. DE concentration: 100, 400, 700 and 1000ppm. sC8 concentration 4000ppm

5.3.3. Effect of formulation treatments on grain properties

5.3.3.1 Grain colour

Optical images of grain treated with DE only and combination of DE with sC8 are shown in **Figure 5.11**. From these images, it is clear that increasing the dose of DE changes the colour of the grain from creamy yellow to pale yellow with white speckles. Treating grain with sugar ester also appears to yield a colour change that is more evenly distributed over the grain surface.

5.3.3.2 Bulk density

The bulk density of grain is an important physical property that is used as a measure of grain quality. The bulk density of grain treated with DE alone and the combined formulation (at different DE doses) was examined using a chondrometer, with the results shown in **Figure 5.12**. Overall, increasing DE concentration leads to a decrease in bulk density, from 82.6 to 75.5 kg/hl. Treatment with the combined formulation has the same effect on bulk density as DE treatment alone, indicating that the sugar ester itself has no impact on the bulk density properties of wheat grain at these doses. Critically, the bulk density for the optimal treatment (100 ppm DE + 4000 ppm sC8) is only marginally smaller than for untreated grain, being well within the limits for market acceptance of the formulation (Posner and Hibbs, 2005).

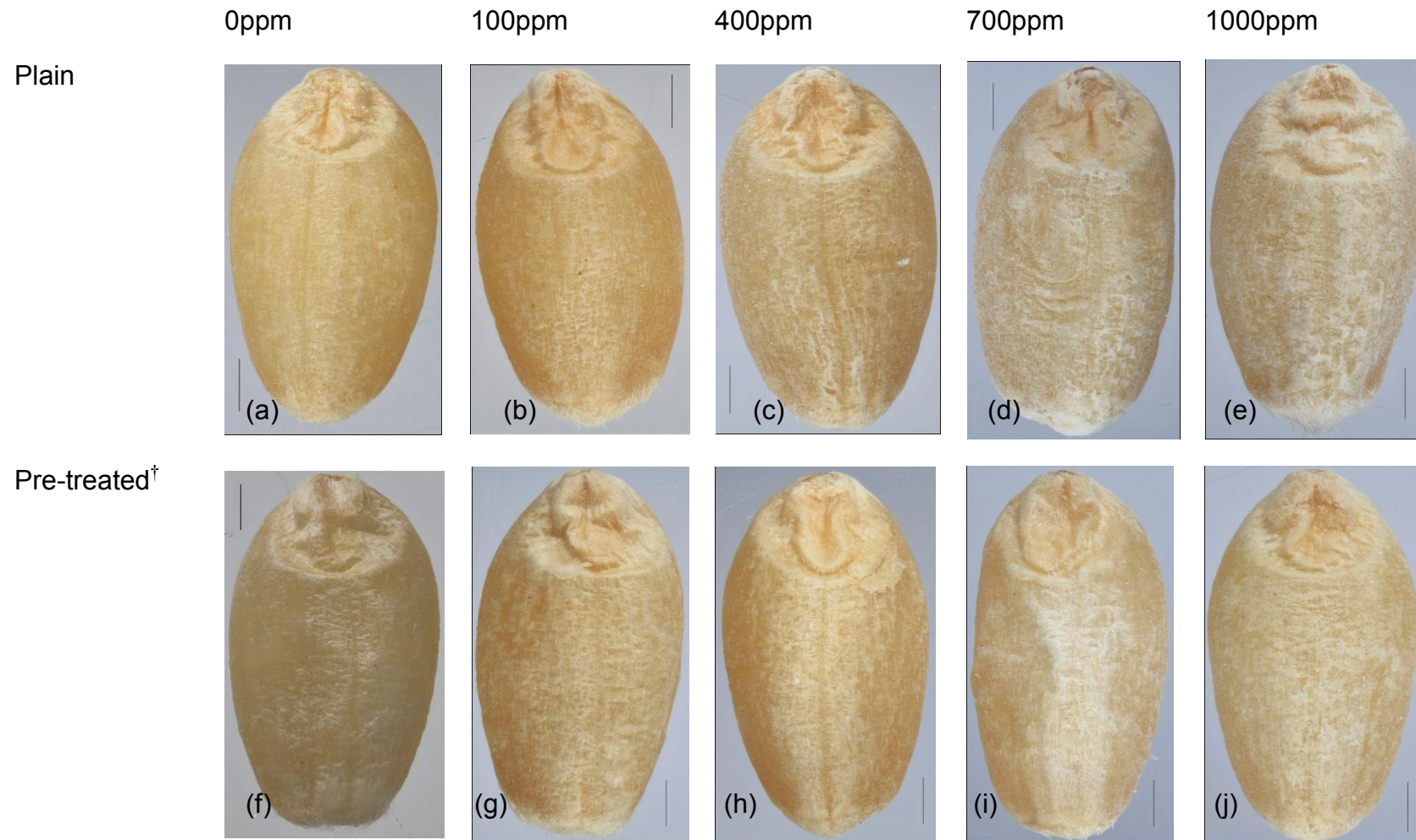


Figure 5.11 Images of Katana grains dusted with DE at varied concentration. [†]Sorbitol octanoate concentration: 4000ppm. Scale bar = 1mm

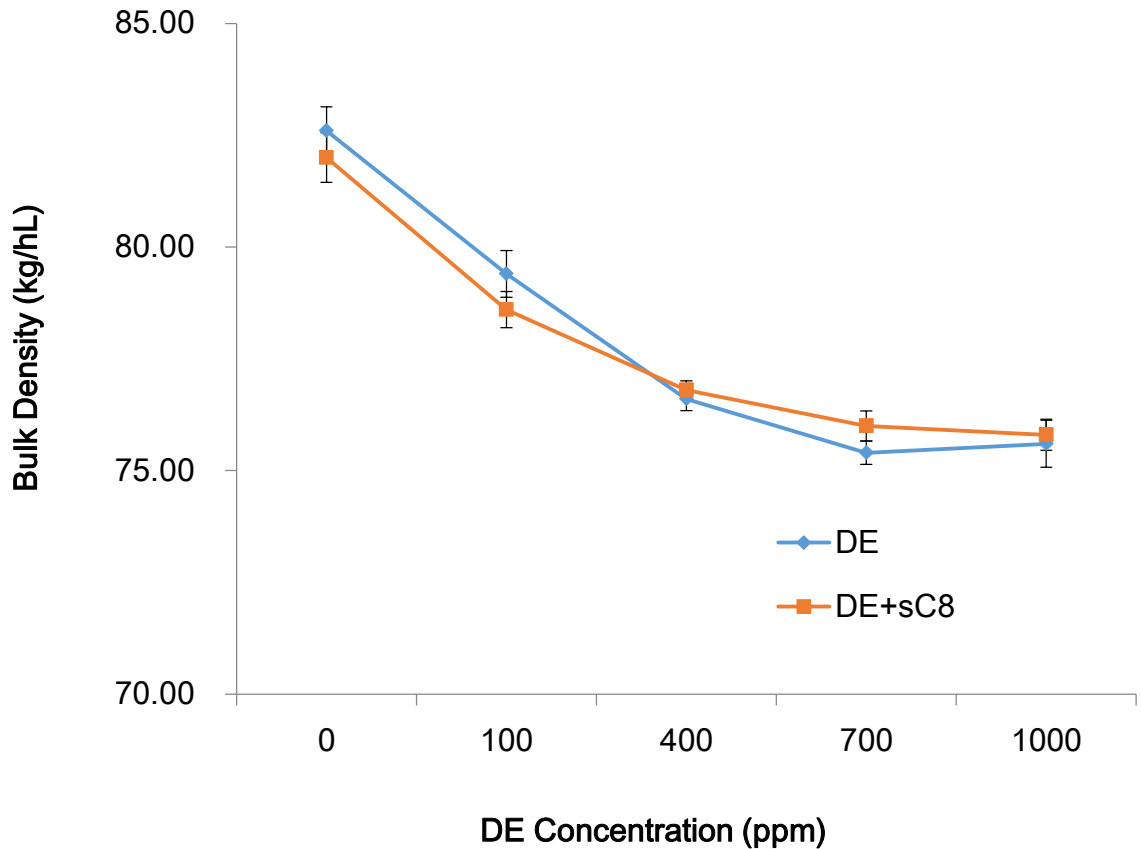


Figure 5.12 Bulk density (kg/hL) of Katana treated with sorbitol octanoate (4000ppm) and DE (100, 400, 700 and 1000ppm).

5.3.3.3 Angle of repose

The flowability of grain during handling and transport is another major concern to the grains industry when considering insecticidal formulations that involve DE dusts. Investment in equipment usually accounts for the highest operating cost, and this equipment is mostly designed to process untreated grains. Modifications to the grain surface that changes the physical properties thus may not be acceptable due to possible slowdowns or even jamming of grain transport equipment (such as augers) during handling. The angle of repose (Φ_R) is an indication of the inter-grain friction (as a function of grain's moisture content), and is the primary measurement used to indicate grain flowability when designing grain handling machinery.

Using the piling method, treatment of grains with DE reveals an initial rapid increase in angle of repose with dose, which plateaus off and remains unchanged at

doses higher than 400 ppm. Treatment of grain with sugar ester (4000 ppm) was found to have a minimal impact on the angle of repose, with only slight differences between untreated and treated grain that are ascribed to small variations in moisture content and surface morphology. Interestingly, combination of sugar ester with DE results in a reduced impact of the DE on the angle of repose by an average ϕ_R of 5° over the dose range tested. This may be explained by the sugar ester forming a lubricating layer between the grain and DE particles (reducing inter-grain frictional forces as represented by its change in ϕ_R (Tabatabaeefar, 2003, Johnson et al., 2011, Kalkan and Kara, 2011)), but which retains some stickiness that gives a small decrease in flowability for grains treated with sorbitol octanoate alone.

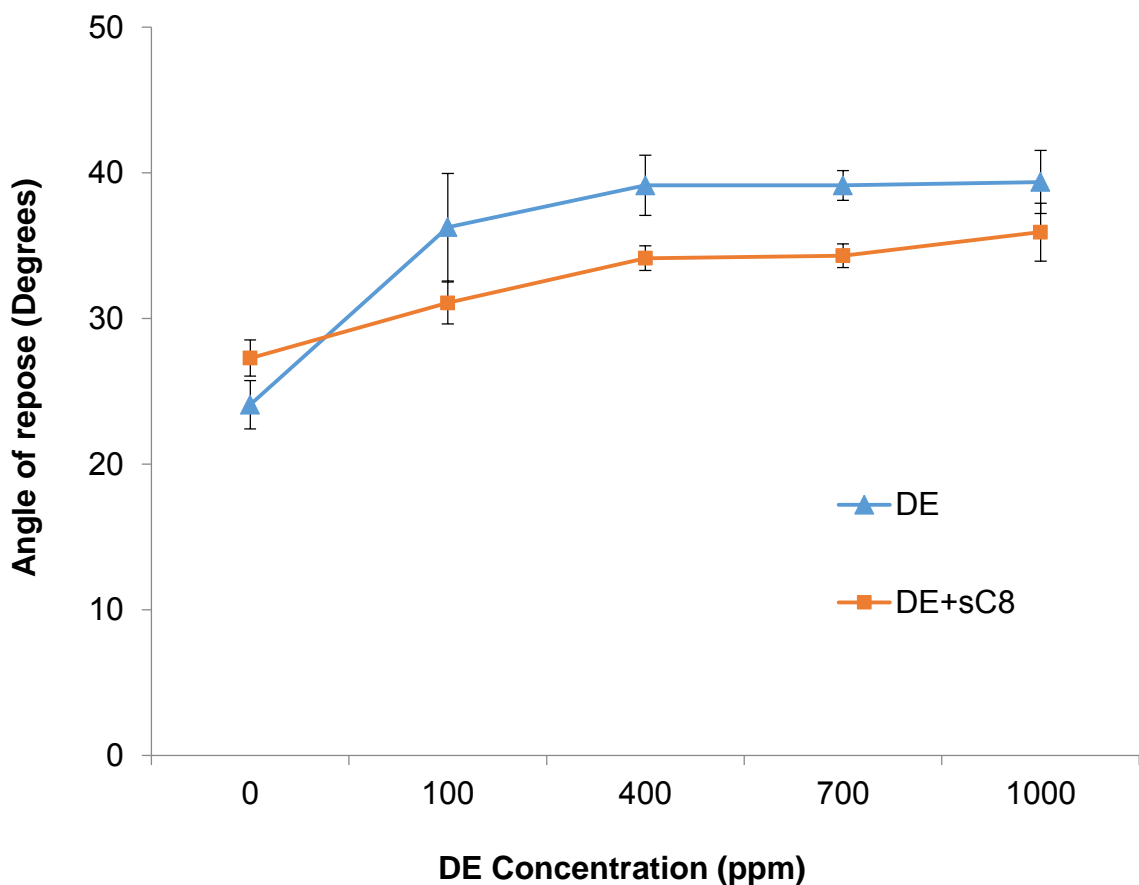


Figure 5.13 Angle of repose of Katana treated with sorbitol octanoate (4000ppm) and DE (100, 400, 700 and 1000ppm).

5.3 Conclusion

In this chapter, the insecticidal activity of sorbitol octanoate (the most potent sugar ester tested in chapter 4) against LGB was found to be further enhanced by combining with DE, with a combination of ester at 4000 ppm and DE at 100 ppm killing almost all of the insect population within 14 days (relative potency of 0.023 vs Dryacide), while also suppressing progeny production. Notably, this formulation performed better than the individual components at their respective doses. However, the combination index for this formulation suggested that there is actually a slight antagonistic interaction between the DE and sorbitol octanoate, which was hypothesised to be due to increased adherence of DE to the grains by the sticky sorbitol octanoate.

The mode of action of these treatments was investigated by examining the cuticle of exposed insects by optical and electron microscope. Accumulation of DE particles on the insects increases with higher DE dose, and tends to gather on the pronotum and the lower part of elytra. This was explained in terms of these parts of LGB coming into contact with treated grain during their crawling action. Substantially less DE accumulated on the insects when combined with sugar ester, supporting the hypothesis of increased adhesion of DE particles to the grains. No signs of abrasion were observed on cuticle surfaces under electron microscope at 5000 times magnification, indicating that of the explanations proposed in the literature, desiccation is the likely mode of action for the DE particles.

Bulk density and angle of repose studies provided an understanding of the formulations impact on the two main marketing concerns; grain weight and flowability. Results indicated that the optimal formulation only minimally reduced bulk density (no change compared to DE only at 100 ppm) and flowability (actually improved the angle of repose by $\sim 5^\circ$ compared with DE only at 100 ppm). This will add value to the general acceptance of this formulation.

More studies, including a broader range of doses and different types of sugar ester combined with DE, should be carried out in future to better understand this DE/sC8

interaction. More studies to understand the changes that prevent particle attachment may also be beneficial to help understand the mode of action. Further analysis of the formulations impact on the physical properties of grain should also be carried out by other evaluation methods, such as friction coefficients. Further bioassays with a wider range of doses and different type of sugar esters could be carried out in future to elucidate and establish the DE/ sC8 relationship.

References

- Akoh, C. C. and B. G. Swanson (1990). Optimized Synthesis of Sucrose Polyesters: Comparison of Physical Properties of Sucrose Polyesters, Raffinose Polyesters and Salad Oils. Journal of Food Science **55**(1): 236-243.
- Alexander, P., J. A. Kitchener and H. V. A. Briscoe (1944a). Inert dust insecticides: Part II. The nature of effective dusts. Annals of Applied Biology **31**(2): 150-156.
- Alexander, P., J. A. Kitchener and H. V. A. Briscoe (1944b). Inert dust insecticides: Part III. The effect of dusts on stored products pests other than *Calandra granaria*. Annals of Applied Biology **31**(2): 156-159.
- Altieri, M. A. (2002). Agroecology: the science of natural resource management for poor farmers in marginal environments. Agriculture, Ecosystems & Environment **93**(1-3): 1-24.
- Armitage, D. M., D. A. Collins, D. A. Cook and J. Bell (1999). The efficacy of silicaceous dust alternatives to organophosphorus compounds for the control of storage mites. Proceedmqe of the 7th International Working Conference on Stored-product Protection Beijing, P. R. China, Sichuan Publishing House of Science and Technology.
- Arthur, F. H. (2000). Toxicity of Diatomaceous Earth to Red Flour Beetles and Confused Flour Beetles (Coleoptera: Tenebrionidae): Effects of Temperature and Relative Humidity. Journal of Economic Entomology **93**(2): 526-532.
- Arthur, F. H. (2002). Survival of *Sitophilus oryzae* (L.) on wheat treated with diatomaceous earth: impact of biological and environmental parameters on product efficacy. Journal of Stored Products Research **38**(3): 305–313.
- Athanassiou, C. G., N. G. Kavallieratos, L. P. Economou, C. B. Dimizas, B. J. Vayias, S. Tomanović and M. Milutinović (2005). Persistence and Efficacy of Three Diatomaceous Earth Formulations Against *Sitophilus oryzae* (Coleoptera:Curculionidae) on Wheat and Barley. Journal of Economic Entomology **98**(4): 1404-1412.
- Athanassiou, C. G., N. G. Kavallieratos, F. C. Tsaganou, B. J. Vayias, C. B. Dimizas and C. T. Buchelos (2003). Effect of grain type on the insecticidal efficacy of SilicoSec against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Crop Protection **22**(10): 1141–1147.

ATSE (2002). Pesticide Use in Australia. Victoria, Australia, Australian Academy of Technology Science and Engineering.

Attique, M. N. R., A. Khaliq and A. H. Sayyed (2006). Could resistance to insecticides in *Plutella xylostella* (Lep., Plutellidae) be overcome by insecticide mixtures? J. Appl. Entomol. **130**(2): 122–127.

Ayala-Bravo, H. A., D. Quintanar-Guerrero, A. Naik, Y. N. Kalia, J. M. Cornejo-Bravo and A. Ganem-Quintanar (2003). Effects of sucrose oleate and sucrose laureate on in vivo human stratum corneum permeability. Pharmaceutical research **20**(8): 1267-1273.

Baczko, K., C. Nugier-Chauvin, J. Banoub, P. Thibault and D. Plusquellec (1995). A new synthesis of 6-O-acylsucroses and of mixed 6,6' -di-O-acylsucroses. Carbohydrate Research **269**(1): 79 - 88.

Beament, J. W. L. (1964). The Active Transport and Passive Movement of Water in Insects. Advances in Insect Physiology **2**: 67 -129.

Bianchi, F. J. J. A., C. J. H. Booij and T. Tscharntke (2006). Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. Proceedings of the Royal Society B: Biological Sciences **273**(1595): 1715-1727.

Bravo, A., S. S. Gill and M. Soberón (2007). Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. Toxicon **49**(4): 423–435.

Brown, J. B. and D. K. Kolb (1955). Applications of low temperature crystallization in the separation of the fatty acids and their compounds. Progress in the Chemistry of Fats and other Lipids **3**: 57-72.

Bullen, K. (2007). Insect Control in Stored Grain. Q. Department of Primary Industries and Fisheries, DPI&F, QLD.

Carlson, S. D. and H. J. Ball (1962). Mode of Action and Insecticidal Value of a Diatomaceous Earth as a Grain Protectant. Journal of Economic Entomology **55**(6): 964-970.

- Carvalho, F. P. (2006). Agriculture, pesticides, food security and food safety. Environmental Science & Policy **9**(7-8): 685–692.
- Casida, J. E. (1993). Insecticide action at the GABA-gated chloride channel: Recognition, progress, and prospects. Archives of Insect Biochemistry and Physiology **22**(1-2): 13–23.
- Casida, J. E., D. W. Gammon, A. H. Glickman and L. J. Lawrence (1983). Mechanisms of Selective Action of Pyrethroid Insecticides. Annual Review of Pharmacology and Toxicology **23**: 413-438.
- Casida, J. E. and G. B. Quistad (1998). Golden Age of Insecticide Research: Past, Present, or Future? Annual Review of Entomology **43**: 1-16.
- CGC (2013). Official Grain Grading Guide. C. I. Services. Canada, Canadian Grain Commission.
- Champ, B. R. and C. E. Dyte (1977). FAO Global Survey of Pesticide Susceptibility of Stored Grain Pests. FAO Plant Protection Bulletin **25**(2): 49-67.
- Chanbang, Y., F. H. Arthur, G. E. Wilde and J. E. Throne (2007). Efficacy of diatomaceous earth to control *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) in rough rice: Impacts of temperature and relative humidity. Crop Protection **26**(7): 923–929.
- Chapman, R. F. (2013). Insects : structure and function. New York, Cambridge University Press.
- Chauvin, C. and D. Plusquellec (1991). A new chemoenzymatic synthesis of 6'-O-acylsucroses. Tetrahedron Letters **32**(29): 3495–3498.
- Chortyk, O. T., G. J. Pomonis and A. W. Johnson (1996). Syntheses and Characterizations of Insecticidal Sucrose Esters. Journal of Agricultural and Food Chemistry **44**(6): 1551-1557.
- Chou, T.-C. (2010). Drug Combination Studies and Their Synergy Quantification Using the Chou-Talalay Method. Cancer Res **70**(2): 440-446.

- Colette, W. A., L. K. Almas and G. L. Schuster (2001). Evaluating the Impact of Integrated Pest Management on Agriculture and the Environment in the Texas Panhandle. Western Agricultural Economics Association Annual Meetings. Logan Utah.
- Collins, D. A. and D. A. Cook (2006). Laboratory studies evaluating the efficacy of diatomaceous earths, on treated surfaces, against stored-product insect and mite pests. Journal of Stored Products Research **42**(1): 51-60.
- Cruces, M. A., F. J. Plou, M. Ferrer, M. Bernabé and A. Ballesteros (2001). Improved synthesis of sucrose fatty acid monoesters. Journal of the American Oil Chemists' Society **78**(5): 541-546.
- Davies, P. E., L. S. J. Cook and D. Goenarso (1994). Sublethal responses to pesticides of several species of Australian freshwater fish and crustaceans and rainbow trout. Environmental Toxicology and Chemistry **13**(8): 1341–1354.
- Dayan, F. E., C. L. Cantrell and S. O. Duke (2009). Natural products in crop protection. Bioorganic & Medicinal Chemistry **17**(12): 4022–4034.
- de Lisle, A. L. (1970). Animal feed supplement and method of eliminating worms from animals. U. S. Patent. United States of America, Phoenix Gems, Inc.,.
- Denholm, I. and M. W. Rowland (1992). Tactics for Managing Pesticide Resistance in Arthropods: Theory and Practice. Annual Review of Entomology **37**: 91-112.
- Deshpande, P. S., T. D. Deshpande, R. D. Kulkarni and P. P. Mahulikar (2013). Synthesis of Sucrose–Coconut Fatty Acids Esters: Reaction Kinetics and Rheological Analysis. Industrial & Engineering Chemistry Research **52**(43): 15024-15033.
- Dolley, T. P. and P. R. Moyle (2003). History and Overview of the U.S. Diatomite Mining Industry, with Emphasis on the Western United States. U. S. G. S. U.S. Department of the Interior. **Bulletin 2209–E**.
- Duus, J. Ø., C. H. Gotfredsen and K. Bock (2000). Carbohydrate Structural Determination by NMR Spectroscopy: Modern Methods and Limitations. Chemical Reviews, **100**(12): 4589–4614.
- Dyte, C. E. (1974). Problems Arising from Insecticide Resistance in Storage Pests. EPPO Bulletin **4**(3): 275–286.

Dyte, C. E. and D. Halliday (1985). Problems of development of resistance to phosphine by insect pests of stored grains. EPPO Bulletin **15**(1): 51–57.

Ebeling, W. (1964). Permeability of Insect Cuticle. he Physiology of Insecta M. Rockstein. New York, Academic Press Inc. **3**: 508-523.

Ebeling, W. (1971). Sorptive dusts for pest control. Annual Review of Entomology **16**: 123-158.

Edde, P. A. (2012). A review of the biology and control of *Rhyzopertha dominica* (F.) the lesser grain borer. Journal of Stored Products Research **48**: 1-18.

Emerson, G. M., N. McL Gray, G. A. Jelinek, D. Mountain and H. J. Mead (1999). Organophosphate poisoning in perth, western australia, 1987–1996. The Journal of Emergency Medicine **17**(2): 273–277.

Entosol (2015). Dryacide.

Fabritius, H., C. Sachs, D. Raabe, S. Nikolov, M. Friák and J. Neugebauer (2011). Chitin in the Exoskeletons of Arthropoda: From Ancient Design to Novel Materials Science. Chitin Formation and Diagenesis. N. S. Gupta. London New York, Springer Dordrecht Heidelberg 35-60.

FAO (2009). How to Feed the World in 2050. High Level Expert Forum. Rome, Agricultural Development Economics Division, Economic and Social Development Department.

FAOSTAT (2013a). Total World's Cereals (excluding beer) Supply 1961 - 2009. <http://faostat3.fao.org/home/index.html#DOWNLOAD>, FAO.

FAOSTAT (2013b). World Crops Production, Cereals Total 1961 - 2012. <http://faostat3.fao.org/home/index.html#DOWNLOAD>, FAO.

Farone, W. A., T. Palmer and G. J. Puterka (2002). Polyol ester insecticides and method of synthesis. . U. S. Patent. United State of America, Ava Chemical Ventures L.L.C.

Faulde, M. K., M. Tisch and J. J. Scharninghausen (2006). Efficacy of modified diatomaceous earth on different cockroach species (Orthoptera, Blattellidae) and silverfish (Thysanura, Lepismatidae). **79**(3): 155-161.

Fernandez, M. I., B. W. Woodward and B. E. Stromberg (1998). Effect of diatomaceous earth as an anthelmintic treatment on internal parasites and feedlot performance of beef steers. Animal Science **66**(03): 635-641.

Ferrer, M., M. A. Cruces, M. Bernabé, A. Ballesteros and F. J. Plou (1999). Lipase-catalyzed regioselective acylation of sucrose in two-solvent mixtures. Biotechnology and Bioengineering **65**(1): 10-16.

Fields, P. and Z. Korunic (2000). The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored-product beetles. Journal of Stored Products Research **36**(1): 1–13.

Fields, P. G. (1998). Diatomaceous earth: advantages and limitations. Proceedings of the 7th International Working Conference on Stored-Product Protection, Beijing, China, Sichuan Publishing House of Science and Technology.

Fields, P. G., S. Allen, Z. Korunic, A. McLaughlin and T. E. Stathers (2002). Standardized testing for diatomaceous earth. Proceedings of the 8th International Working Conference on Stored-Product Protection, Wallingford, United Kingdom, CAB International.

Fitremann, J., Y. Queneau, J.-P. Maître and A. Bouchu (2007). Co-melting of solid sucrose and multivalent cation soaps for solvent-free synthesis of sucrose esters. Tetrahedron Letters **48**(23): 4111–4114.

Flinn, P. W., D. W. Hagstrum, C. Reed and T. W. Phillips (2010). Insect population dynamics in commercial grain elevators. Journal of Stored Products Research **46**(1): 43–47.

Flint, M. L. (2012). IPM in practice : principles and methods of integrated pest management Oakland, University of California Division of Agriculture and Natural Resources.

Frost, R., O. Carmody, Y. Xi and S. Kokot (2007). Surface characterisation of selected sorbent materials for common hydrocarbon fuels Surface Science **601**(9): 2066-2076.

Fukuto, T. R. (1990). Mechanism of action of organophosphorus and carbamate insecticides. Environmental Health Perspectives **87**: 245–254.

Germinara, G. S., A. D. Cristofaro and G. Rotundo (2009). Antennal olfactory responses to individual cereal volatiles in *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae). Journal of Stored Products Research **45**(3): 195–200.

Gillilan, J. (2012). Improvement of U.S. EPA Minimum Risk Essential Oils' Pesticide Activity Through Surfactant Enhancement and Synergy, The Ohio State University. **Doctorate of Philosophy**.

Godfray, H. C. J., J. R. Beddington, I. R. Crute, L. Haddad, D. Lawrence, J. F. Muir, J. Pretty, S. Robinson, S. M. Thomas and C. Toulmin (2010). Food Security: The Challenge of Feeding 9 Billion People. Science **327**(5967): 812-818.

Golob, P. (1997). Current status and future perspectives for inert dusts for control of stored product insects. Journal of Stored Products Research **33**(1): 69-79.

Gottlieb, H. E., V. Kotlyar and A. Nudelman (1997). NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities. J. Org. Chem. **62**: 7512-7515.

Griffin, W. C. (1949). Classification of Surface-Active Agents by 'HLB'. Journal of the Society of Cosmetic Chemists **1**(5): 311-326.

GTA (2014). GTA Wheat Standards 2014/2015. Australia, Grain Trade Australia.

Gupta, R. K., K. James and F. J. Smith (1983). Sucrose esters and sucrose ester/glyceride blends as emulsifiers. Journal of the American Oil Chemists' Society **60**(4): 862-869.

Hackman, R. H. (1964). Chemistry of the insect cuticle. The Physiology of Insecta. M. Rockstein. New York, Academic Press. **3**: 471-506.

Hardstone, M. C. and J. G. Scott (2010). A review of the interactions between multiple insecticide resistance loci. Pesticide Biochemistry and Physiology **97**(2): 123–128.

Hassan, A. R. and K. Bakshi (2005). Pest Management, Productivity and Environment: A Comparative Study of IPM and Conventional Farmers of Northern Districts of Bangladesh. Pakistan Journal of Social Sciences **3**(8): 1007-1014.

- Hill, D. S. (2008). *Pests of Crops in Warmer Climates and Their Control*, Springer: 59-78.
- Hodges, R. J., J. C. Buzby and B. Bennett (2011). Foresight Project on Global Food and Farming Futures. Journal of Agricultural Science **149**(S1): 37–45.
- Hollis, S., C. McDonald and J. Rader (2015). Methods for Treating Arthropods. U. S. Patent. United States OMS Investments, Inc. **20150017116**.
- Hu, J. S., D. B. Gelman, M. E. Salvucci, Y. P. Chen and M. B. Blackburn (2010). Insecticidal activity of some reducing sugars against the sweet potato whitefly, *Bemisia tabaci*, Biotype B. Journal of Insect Science **10**(203).
- Husband, F. A., D. B. Sarney, M. J. Barnard and P. J. Wilde (1998). Comparison of foaming and interfacial properties of pure sucrose monolaurates, dilaurate and commercial preparations. Food Hydrocolloids **12**(2): 237-244.
- Imgarcade.com (2015). Images For (Diatomaceous Earth Microscopic View). Retrieved June 13th 2015.
- IRAC (2010). Mode of Action Classification. I. R. A. Committee, Insecticide Resistance Action Committee.
- Ishaaya, I., A. R. Horowitz and R. Nauen (2007). Insecticides Design Using Advanced Technologies. Netherlands, Springer Berlin Heidelberg.
- Jackson, D., R. Severson, V. Sisson and M. Stephenson (1991). Ovipositional response of tobacco budworm moths (Lepidoptera: Noctuidae) to cuticular labdanes and sucrose esters from the green leaves of *Nicotiana glutinosa* L. (Solanaceae). Journal of Chemical Ecology **17**(12): 2489-2506.
- Jackson, K. and D. Webley (1994). Effects of Dryacide® on the physical properties of grains, pulses and oilseeds. Proceedings of the 6th International Working Conference on Stored-product Protection - Volume 2, Canberra Australia, CABI, London.
- Johnson, P., S. Cenkowski and J. Paliwal (2011). Bulk Density and Angle of Repose of Distiller's Spent Grain Under Different Drying Methods and Soluble Concentrations. CSBE/SCGAB 2011 Annual Conference. Inn at the Forks, Winnipeg, Manitoba, The Canadian Society for Bioengineering.

Juraske, R., P. Fantke, A. C. R. Ramírez and A. González (2012). Pesticide residue dynamics in passion fruits: Comparing field trial and modelling results. Chemosphere **89**(7): 850-855.

Kaleemullah, S. and J. J. Gunasekar (2002). Moisture-dependent Physical Properties of Arecanut Kernels. Biosystems Engineering **82**(3): 331–338.

Kalkan, F. and M. Kara (2011). Handling, frictional and technological properties of wheat as affected by moisture content and cultivar. Powder Technology **213**(1-3): 116–122.

Kaur, R., D. I. Schlipalius, P. J. Collins, A. J. Swain and P. R. Ebert (2012). Inheritance and relative dominance, expressed as toxicity response and delayed development, of phosphine resistance in immature stages of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). Journal of Stored Products Research **51**: 74–80.

Kavallieratos, N. G., C. G. Athanassiou, F. D. Mpakou and A. E. Mpassoukou (2007). Factors Affecting Laboratory Bioassays with Diatomaceous Earth on Stored Wheat: Effect of Insect Density, Grain Quantity, and Cracked Kernel Containment. Journal of Economic Entomology **100**(5): 1724-1731.

Kavallieratos, N. G., C. G. Athanassiou, F. G. Pashalidou, N. S. Andris and Ž. Tomanović (2005). Influence of grain type on the insecticidal efficacy of two diatomaceous earth formulations against *Rhyzopertha dominica* (F) (Coleoptera: Bostrichidae). Pest Management Science **61**(7): 660–666.

Kim, J. E., J. J. Han, J. H. Yoon and J. S. Rhee (1998). Effect of salt hydrate pair on lipase-catalyzed regioselective monoacylation of sucrose. Biotechnology and Bioengineering **57**(1): 121-125.

Kogan, M. (1998). Integrated Pest Management: Historical Perspectives and Contemporary Developments. Annual Review of Entomology **43**: 243-270.

Koiwai, A., F. Suzuki, T. Matsuzaki and N. Kawashima (1983). The fatty acid composition of seeds and leaves of *Nicotiana* species. Phytochemistry **22**(6): 1409-1412.

Korunic, Z. (1998). Diatomaceous earths, a group of natural insecticides. Journal of Stored Products Research **34**(2-3): 87-97.

- Korunic, Z., S. Cenkowski and P. Fields (1998). Grain bulk density as affected by diatomaceous earth and application method. Postharvest Biology and Technology **13**(1): 81-89.
- Korunic, Z., P. G. Fields, M. I. P. Kovacs, J. S. Noll, O. M. Lukow, C. J. Demianyk and K. J. Shibley (1996). The effect of diatomaceous earth on grain quality. Postharvest Biology and Technology **9**: 373-387.
- Ku, M. A. and Y. D. Hang (1995). Enzymatic synthesis of esters in organic medium with lipase from *Byssoschlamys fulva*. Biotechnology Letters **17**(10): 1081-1084.
- Kunert, A., A. A. Naz, O. Dedek, K. Pillen and J. Le´on (2007). AB-QTL analysis in winter wheat: I. Synthetic hexaploid wheat (*T. turgidum* ssp. *dicoccoides* × *T. tauschii*) as a source of favourable alleles for milling and baking quality traits. Theoretical and Applied Genetics **115**(5): 683-695.
- Lalonde, D. I. V. and A. W. A. Brown (1954). The effect of insecticides on the action potentials of insect nerve. Canadian Journal of Zoology **32**(2): 74-81.
- Le Coent, A. L., M. Tayakout-Fayolle, F. Couenne, S. Briancon, J. Lieto, J. Fitremann-Gagnaire, Y. Queneau and A. Bouchu (2003). Kinetic parameter estimation and modelling of sucrose esters synthesis without solvent. Chemical Engineering Science **58**(2): 367-376.
- le Patourel, G. N. J. and J. Singh (1984). Toxicity of amorphous silicas and silica-pyrethroid mixtures to *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Journal of Stored Products Research **20**(4): 183–190.
- Legal, L., B. Moulin and J. M. Jallon (1999). The Relation between Structures and Toxicity of Oxygenated Aliphatic Compounds Homologous to the Insecticide Octanoic Acid and the Chemotaxis of Two Species of *Drosophila*. Pesticide Biochemistry and Physiology **65**(2): 90–101.
- LeHen-Ferrenbach, C. and K. Hill (2010). Sugar-Based Surfactants. Fundamentals and Applications. C. C. Ruiz, CRC Press.
- Li, S., Z. Song, Z. Liu and S. Bai (2008). Characterization and insecticidal activity of sucrose octanoates. Agronomy for Sustainable Development **28**(2): 239-245.

- Liu, Z. (1999). Purification processes of crude sucrose ester. Journal of Wuhan Polytechnic University(3): 11-14.
- Lockey, K. H. (1988). Lipids of the insect cuticle: origin, composition and function. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry **89**(4): 595–645.
- Lorini, I. and H. Beckel (2006). Efficacy of “diatomaceous earth” to control the main stored grain pests. Proceedings of the 9th International Working Conference on Stored Product Protection, Sao Paulo, Brazil, Research Information Ltd.
- Luz, C., J. Rodrigues and L. F. N. Rocha (2012). Diatomaceous earth and oil enhance effectiveness of *Metarhizium anisopliae* against *Triatoma infestans*. Acta Tropica **122** 29–35.
- Lyon, R. L. (1965). Structure and toxicity of insecticide deposits for control of bark beetles. U. S. D. o. Agriculture. Washington, USA, USDA. **1343**.
- Manlay, R. J., C. Feller and M. J. Swift (2007). Historical evolution of soil organic matter concepts and their relationships with the fertility and sustainability of cropping systems. Agriculture, Ecosystems & Environment **119**(3–4): 217–233.
- Marshall, D. R., D. J. Mares, H. J. Moss and F. W. Ellison (1986). Effects of Grain Shape and Size on Milling Yields in Wheat. II*. Experimental Studies. Australian Journal of Agricultural Research **37**(4): 331-342.
- McKenzie, C. L. and G. J. Puterka (2004). Effect of sucrose octanoate on survival of nymphal and adult *Diaphorina citri* (Homoptera: Psyllidae). Journal of Economic Entomology **97**(3): 970-975.
- McLaughlin, A. (1994). Laboratory trials on desiccant dust insecticides. Proceedings of the 6th International Working Conference on Stored Product Protection, Canberra, Australia, CAB International, Wallingford, United Kingdom.
- Michaud, J. P. and C. L. McKenzie (2004). Safety of a novel insecticide, sucrose octanoate, to beneficial insects in Florida citrus. Florida Entomologist **87**(1): 6-9.
- MountSylviaDiatomite (2015). Absorbacide. 2015.

- Narahashi, T. (1981). Mode of action of chlorinated hydrocarbon pesticides on the nervous system. Toxicology of Halogenated Hydrocarbons Toxicology of Halogenated Hydrocarbons M. A. Q. Khan and R. H. Stanton, Pergamon Press Inc. : 222–242.
- Nath, N. S., I. Bhattacharya, A. G. Tuck, D. I. Schlipalius and P. R. Ebert (2011). Mechanisms of Phosphine Toxicity. Journal of Toxicology **2011**: 494168.
- Nicolaou, K. C. and H. J. Mitchell (2001). Adventures in Carbohydrate Chemistry: New Synthetic Technologies, Chemical Synthesis, Molecular Design, and Chemical Biology. Angewandte Chemie International Edition **40**(9): 1576-1624.
- Noble-Nesbitt, J. (1970). Structural aspects of penetration through insect cuticles. Pesticide Science **1**(5): 204–208.
- Oerke, E.-C., H.-W. Dehne, F. Schönbeck and A. Weber (1999). Crop Production and Crop Protection: Estimated Losses in Major Food and Cash Crops. Amsterdam, Netherlands, Elsevier.
- Oerke, E. C. (2006). Crop losses to pests. Journal of Agricultural Science **144**: 31-43.
- Osipow, L. and W. Rosenblatt (1967). Micro-emulsion process for the preparation of sucrose esters. Journal of the American Oil Chemists Society **44**(5): 307-309.
- Osipow, L., F. D. Snell, W. C. York and A. Finchler (1956). Methods of Preparation Fatty Acid Esters of Sucrose. Industrial & Engineering Chemistry **48**(9): 1459-1462.
- Patil, D. R., D. G. Rethwisch and J. S. Dordick (1991). Enzymatic synthesis of a sucrose-containing linear polyester in nearly anhydrous organic media. Biotechnology and Bioengineering **37**(7): 639-646.
- Pimentel, D., H. Acquay, M. Biltonen, P. Rice, M. Silva, J. Nelson, V. Lipner, S. Giordano, A. Horowitz and M. D'Amore (1992). Environmental and economic costs of pesticide use. BioScience **42**(10): 750-760.
- Pittendrigh, B. R., V. M. Margam, K. R. Walters Jr., L. D. Steele, B. P. Olds, L. Sun, J. Huesing, S. H. Lee and J. M. Clark (2014). Understanding Resistance and Induced Responses of Insects to Xenobiotics and Insecticides in the Age of “Omics” and Systems Biology. Insect Resistance Management Biology, Economics, and Prediction.

D. W. Onstad. Wilmington, DE, USA, Insect Resistance Management Science, DuPont Agricultural Biotechnology: 55-98.

Plat, T. and R. Linhardt (2001). Syntheses and applications of sucrose-based esters. Journal of Surfactants and Detergents **4**(4): 415-421.

Polat, T., H. G. Bazin and R. J. Linhardt (1997a). Enzyme Catalyzed Regioselective Synthesis of Sucrose Fatty Acid Ester Surfactants. Journal of Carbohydrate Chemistry **16**(9): 1319-1325.

Polat, T., H. G. Bazin and R. J. Linhardt (1997b). Enzyme Catalyzed Regioselective Synthesis of Sucrose Fatty Acid Ester Surfactants. Journal of Carbohydrate Chemistry **16**(9): 1319-1325.

Posner, E. S. and A. N. Hibbs (2005). Wheat Flour Milling St. Paul, Minnesota, USA, The American Association of Cereal Chemists Inc.

Potier, P., A. Bouchu, G. Descotes and Y. Queneau (2000). Proteinase N-catalysed transesterifications in DMSO–water and DMF–water: preparation of sucrose monomethacrylate. Tetrahedron Letters **41**(19): 3597–3600.

Potter, C. (1935). The biology and distribution of *Rhizopertha dominica* (Fab.). Transactions of the Royal Entomological Society of London **83**(4): 449-482.

Prusky, D. (2011). Reduction of the incidence of postharvest quality losses, and future prospects Food Security **3**(4): 463–474.

PulseAus (2013) Australian Pulse Standards 2013/2014. 1-98

Puterka, G. J., W. Farone, T. Palmer and A. Barrington (2003). Structure-Function Relationships Affecting the Insecticidal and Miticidal Activity of Sugar Esters. Journal of Economic Entomology **96**(3): 636-644.

Quarles, W. (1992). Diatomaceous earth for pest control. The IPM Practitioner **14**(5/6): 1-16.

Rabaron, A., G. Cave, F. Puisieux and M. Seiller (1993). Physical methods for measurement of the HLB of ether and ester non-ionic surface-active agents: H-NMR and dielectric constant. International Journal of Pharmaceutics **99**: 29-36.

Ridley, A. W., D. I. Schlipalius and G. J. Daglish (2012). Reproduction of phosphine resistant *Rhyzopertha dominica* (F.) following sublethal exposure to phosphine. Journal of Stored Products Research **48**: 106–110.

Riva, S., J. Chopineau, A. P. G. Kieboom and A. M. Klibanov (1988). Protease-catalyzed regioselective esterification of sugars and related compounds in anhydrous dimethylformamide. Journal of the American Chemical Society **110**(2): 584-589.

Robertson, J. L., H. K. Preisler, S. S. Ng, L. A. Hickie and W. D. Gelernter (1995). Natural Variation: A Complicating Factor in Bioassays with Chemical and Microbial Pesticides. Journal of Economic Entomology **88**(1): 1-10.

Ross, T. E. (1981). Diatomaceous earth as a possible alternative to chemical insecticides. Agriculture and Environment **6**(1): 43–51.

Roth, L. M. and E. R. Willis (1951). Hygroreceptors in Coleoptera. Journal of Experimental Zoology **117**(3): 451–487.

Roush, R. T. and J. A. McKenzie (1987). Ecological Genetics of Insecticide and Acaricide Resistance. Annual Review of Entomology **32**: 361-380.

Saez, A. and V. H. Fuentes Mora (2007). Comparison of the desiccation effects of marine and freshwater diatomaceous earths on insects. Journal of Stored Products Research **43**(4): 404-409.

Sahasrabudhe, M. R. and R. K. Chadha (1969). Chromatographic analysis of sorbitan fatty acid esters. Journal of the American Oil Chemists Society **46**(1): 8-12.

Schlipalius, D. I., Q. Cheng, P. E. B. Reilly, P. J. Collins and P. R. Ebert (2002). Genetic linkage analysis of the lesser grain borer *Rhyzopertha dominica* identifies two loci that confer high-level resistance to the fumigant phosphine. Genetics **161**(2): 773-782.

Schlipalius, D. I., N. Valmas, A. G. Tuck, R. Jagadeesan, L. Ma, R. Kaur, A. Goldinger, C. Anderson, J. Kuang, S. Zuryn, Y. S. Mau, Q. Cheng, P. J. Collins, M. K. Nayak, H. J. Schirra, M. A. Hilliard and P. R. Ebert (2012). A core metabolic enzyme mediates resistance to phosphine gas. Science **338**(6108): 807-810.

Schmitz, A., A. Sehrbrock and H. Schmitz (2007). The analysis of the mechanosensory origin of the infrared sensilla in *Melanophila acuminata* (Coleoptera; Buprestidae) adduces new insight into the transduction mechanism. Arthropod Struct Dev. **36**(3): 291-303.

Severson, R. F., R. F. Arrendale, O. T. Chortyk, C. R. Green, F. A. Thome, J. L. Stewart and A. W. Johnson (1985a). Isolation and characterization of the sucrose esters of the cuticular waxes of green tobacco leaf. Journal of Agricultural and Food Chemistry **33**(5): 870-875.

Severson, R. F., R. F. Arrendale, O. T. Chortyk, R. Green, F. A. Thome, J. L. Stewart and A. W. Johnson (1985b). Isolation and characterization of the sucrose esters of the cuticular waxes of green tobacco leaf. Journal of Agricultural and Food Chemistry **33**(5): 870-875.

Singh, M., J. R. Orsenigo and D. O. Shah (1984). Surface tension and contact angle of herbicide solutions affected by surfactants. Journal of the American Oil Chemists' Society **61**(3): 596-600.

Smith, C. M. and L. D. Goodhue (1942). Particle Size in Relation to Insecticide Efficiency. Industrial & Engineering Chemistry **34**(4): 490-493.

Soultani, S., S. Ognier, J.-M. Engasser and M. Ghouil (2003). Comparative study of some surface active properties of fructose esters and commercial sucrose esters. Colloids and Surfaces A: Physicochem. Eng. Aspects **227**(1): 35–44.

Stadler, T. and M. Buteler (2009). Modes of entry of petroleum distilled spray-oils into insects: a review. Bulletin of Insectology **62**(2): 169-177.

Stern, V. M., R. F. Smith, R. van den Bosch and K. S. Hagen (1959). The integrated control concept. Hilgardia **29**: 81-101.

Subramanyam, B. and R. Roesli (2000). Inert Dusts. Alternatives to Pesticides in Stored-Product IPM. B. Subramanyam and D. W. Hagstrum: 321-380.

Sunstein, C. R. (2003). The Cost-Benefit State: The Future of Regulatory Protection American Bar Association.

- Swamy, K. C. K., N. N. B. Kumar, E. Balaraman and K. V. P. P. Kumar (2009). Mitsunobu and Related Reactions: Advances and Applications. Chemical Reviews **109**(6): 2551-2651.
- Tabatabaeefar, A. (2003). Moisture-dependent physical properties of wheat. Int. Agrophysics **17**: 207–211.
- Teena, M., A. Manickavasagan, A. Mothershaw, S. El Hadi and D. S. Jayas (2013). Potential of Machine Vision Techniques for Detecting Fecal and Microbial Contamination of Food Products: A Review. Food and Bioprocess Technology **6**(7): 1621-1634.
- Troughton, J. H. and D. M. Hall (1967). Extracuticular Wax and Contact Angle Measurements on Wheat (*Triticum Vulgare* L.). Australian Journal of Biological Sciences **20**(3): 509 - 526.
- UN (2009). World Population To Exceed 9 Billion by 2050: Developing Countries to Add 2.3 Billion Inhabitants with 1.1 Billion Aged Over 60 and 1.2 Billion of Working Age. D. o. E. a. S. Affairs. New York, United Nations.
- Upadhyay, R. K. and S. Ahmad (2011). Management Strategies for Control of Stored Grain Insect Pests in Farmer Stores and Public Ware Houses. World Journal of Agricultural Sciences **7**(5): 527-549.
- USGS (2013). Mineral Commodity Summaries 2013. U. S. G. S. U.S. Department of the Interior. Virginia: 52-53.
- Vayias, B. J. and C. G. Athanassiou (2004). Factors affecting the insecticidal efficacy of the diatomaceous earth formulation SilicoSec against adults and larvae of the confused flour beetle, *Tribolium confusum* DuVal (Coleoptera: Tenebrionidae). Crop Protection **23**(7): 565–573.
- Visionary-Digital™ (2013). BK Plus Lab System. Retrieved 09/12/2014, from <http://www.duninc.com/IntegratedSystems1.html>.
- Vlahov, I. R., P. I. Vlahova and R. J. Linhardt (1997). Regioselective Synthesis of Sucrose Monoesters as Surfactants. Journal of Carbohydrate Chemistry **16**(1): 1-10.

Wagner, F. W., M. A. Dean, R. S. de la Motte and V. H. Stryker (1991). Separation and purification of sugar esters. U. S. Patent. United State of America, Nebraska Department Of Economic Development. **4,983,731**.

Wanasundara, U. N., P. K. J. P. D. Wanasundara and F. Shahidi (2005). Novel Separation Techniques for Isolation and Purification of Fatty Acids and Oil By-Products. Bailey's Industrial Oil and Fat Products. A. E. Bailey and F. Shahidi.

Wang, Q. (2008). Regioselective synthesis, characterization and physicochemical properties of sucrose esters. Faculty of Chemical, Environmental and Biological Science and Technology. Dalian, Dalian University of Technology

Doctor of Philosophy 134.

Wasserthal, L. T. (1996). Interaction of Circulation and Tracheal Ventilation in Holometabolous Insects. Advances Insect Physiology **26**: 297-351.

WFP (2013, 2013). Types of Insect Pests. Retrieved 22 September 2013

Wigglesworth, V. B. (1944). Action of inert dusts on insects. Nature **153**(3886): 493-494.

Wigglesworth, V. B. (1945). Transpiration Through the Cuticle of Insects. The Journal of Experimental Biology **21**: 97-114.

Wigglesworth, V. B. (1957). The Physiology of Insect Cuticle. Annual Review of Entomology **2**: 37-54.

Wilson, C. and C. Tisdell (2001). Why farmers continue to use pesticides despite environmental, health and sustainability costs. Ecological Economics **() 39**: 449–462.

Woudenberg-van Oosterom, M., F. van Rantwijk and R. A. Sheldon (1996). Regioselective acylation of disaccharides in tert-butyl alcohol catalyzed by *Candida antarctica* lipase. Biotechnology and Bioengineering **49**(3): 328-333.

Xiaojie Liu, G. H., Changgui Yuan (2006). Advance in Research, Production and Application of Sucrose Ester. Food and Fermentation Industries **27**(11): 64.

Yuan, Y. and T. R. Lee (2013). Contact Angle and Wetting Properties. Surface Science Techniques,. G. Bracco and B. Holst. Berlin Heidelberg, Springer-Verlag: 3-34.

Chapter 6

Conclusions and Future Perspectives

This thesis advances knowledge on the application of sugar esters as a new type of insecticide in protecting stored grain from insect pests. The first major contribution of this thesis is the understanding of their effectiveness against LGB and the dosage required to cause significant mortality within the population. The second major contribution of this thesis is the enhancement of sugar esters' insecticidal activity by combining with DE. The third major contribution is to provide possible explanations of the mode of action of these formulations.

6.1. Conclusion

- i. Successful synthesis of insecticidal sugar esters using six different sugars (sorbitol and xylitol) and fatty acids (octanoic, decanoic and lauric acids) was achieved by following the one-pot system reported by Farone et al. This simple method allows up-scaling possible for mass production. Products were purified using a two-step washing procedure, which gave final yields of between 40 to 60%. Purified and unpurified products were characterised with FTIR which indicated that sugar esters were successfully synthesised, and that a significant amount of the product loss occurs due to entrapment within solid precipitates during the first purification step (~20%). Further analysis by proton NMR confirmed that sugar ester synthesis was successful, and that primarily monoesters were formed. NMR data also indicated the presence of unreacted sugar and fatty acids in the purified products. HLB values were determined by calculating the ratio of sugars to fatty acids in the sugar ester based on NMR results. Studies on the wetting properties of sugar esters on grain demonstrated various wetting behaviour when coated with different sugar esters. Laurate esters have the best surface wetting activity, reducing the contact angle of grain from

127° to 45° (sorbitol laurate) and 31° (xylitol laurate). Generally, esters prepared with xylitol yield slightly lower contact angles compared to sorbitol esters which might result from the shorter carbon chain of the xylitol sugar groups.

- ii. The insecticidal activity of prepared sugar ester were successfully evaluated by carrying out bioassays using these sugar esters, as well as unreacted sugar and fatty acid to account for any possible contribution from these impurities. Studies comparing the effect of different fatty acid chain lengths and choice of sugar have indicated that sorbitol octanoate was the most potent ester produced with mortality of 81%±2 against LGB after 14 days of exposure. Sorbitol esters prepared with octanoic acid were able to effectively suppress progeny formation over the 60 day study period, whereas the rest did not suppress progeny. Octanoic acid and sorbitol have both been shown to have insecticidal effects, so the combination as a sugar ester may likewise be having similar effects against *R. dominica*. This indicates that the shorter fatty acid chain length (in the sugar ester, residual octanoic acid or both) is critical to inducing progeny suppression in the insect.
- iii. Removal of unreacted sugars from synthesized crude sugar esters significantly increases insecticidal effectiveness, whereas removal of the fatty acids had no statistically significant effect. This makes the purification using ethanol and water to remove extra sugars the most preferred post-processing method.
- iv. Difference in insecticidal properties between different sugar esters was observed. Sorbitol esters were more effective than xylitol esters, while increasing carbon chain length of the fatty acid was generally correlated with lower insect mortality. Overall, insecticidal activity was correlated with higher HLB values. Higher HLB values for these materials correspond to good emulsification properties for emulsifying waxes and oils into water, whereas the smaller HLB values for these sugar esters correspond to a balance between hydrophilic and hydrophobic (good wetting properties). This is reflected in the results from contact angle tests where lowest HLB sugar esters give best wettability on grain. This in turn indicating that

modes of action relying on high wettability (such as suffocation) are not likely. Petri dish studies on the route of exposure gave no information as to whether sugar ester acts as a contact poison, kills by ingestion or has anti-feedant properties. Microscopic examination found no build up of sugar ester or obvious changes to the cuticle layer at up to 5000x magnification.

- v. Combining sorbitol octanoate with DE was found to enhance the efficacy of the sugar ester. By adding just 100 ppm of DE, sorbitol octanoate at 4000 ppm kills almost all of the insect population within 14 days (relative potency of 0.023 vs. Dryacide), while also suppressing progeny production. However, the combination index for this formulation suggested that there is no synergy effect on insecticidal toxicity between DE and sucrose octanoate. There is in fact a small antagonistic effect. This was hypothesized to be due to increased adhesion of DE to the grain because of the sticky sugar ester. At this concentration of DE (100 ppm), grain quality was well maintained, with minor changes in bulk density from 82.62 ± 0.55 to 78.66 ± 0.52 and lowered angle of repose compared to grain treated by DE only (from $24^\circ \pm 2^\circ$ to $31^\circ \pm 1^\circ$). This small effect on grain properties will facilitate market acceptance of the formulation, which has a lower chance of causing damage to grain handling machinery.
- vi. Optical and electron microscopy showed that accumulation of DE particles tends to occur on the pronotum and the lower part of elytra; most likely a result of LGB crawling movement through dusted grains. The quantity of accumulated DE is positively correlated with the dosage applied to the grain. No signs of abrasion were observed on insect cuticle surfaces at 5000 times magnification. Hence it is likely that the mode of action for DE is through desiccation.

6.2. Future direction

This study confirmed that there is considerable potential of sugar esters to be used as new type of pesticides for pest control. While the lethal dose for the most effective sugar ester trialled here is quite high, combination with DE appears to yield an improvement in lethal dose. Should insect pest become insensitive to the treatment, higher doses may be acceptable due to the low mammalian toxicity and the biodegradable nature of sugar ester. By combining sugar ester with DE, the chances for resistance to develop is unlikely as both potentially having physical modes of action.

Suggestions for future study:

- i. This study was focused on one insect LGB and further study on other grain insects are required to evaluate potential of SE as new non-toxic insecticide.
- ii. The different temperatures and relative humidity are expected to have impact of efficacy which needs to be investigated.
- iii. Synthesis of other sugar esters, using different types of sugars and fatty acids, could also be carried out to discover more efficient insecticides.
- iv. The delivery method can also be further looked at to improve upon the current two-step application method. One example is to use DE particles as a carrier for sugar ester, to allow the application to be more time and labour saving.
- v. Investigate the mode of action of these formulations further, to understand sugar esters' insecticidal properties. For example, techniques such as dye or radio-labelling may be required to assist in the tracking of compounds to reveal the underlying mechanism.
- vi. To explore combined action of SE including cuticular penetration and ingestions which could be another potential angle for development new agents.

- vii. Further study is also needed to understand how sorbitol octanoate inhibits progeny production, such as by having an impact on LGB's egg laying habits or progeny development, as well as the underlying mechanism.
- viii. Thirdly, more studies are required to further quantify the impact of sugar ester application on grain's overall physical properties, i.e. influence at different grain moisture contents.
- ix. Studies should also be carried out to understand if any genetic changes are involved or it is purely physical activity, and to find if development of resistance is possible.
- x. More studies in developing new formulation combined with DE and other no toxic pesticides (botanicals, hormone growth factors etc).

Appendix 1

IRAC MoA Classification v 7.3, February 2014		
See section 6.4 for further information on sub-groups. See section 6.3 for criteria for descriptors of the quality of MoA information.		
Main Group and Primary Site of Action	Chemical Sub-group or exemplifying Active Ingredient	Active Ingredients
1 Acetylcholinesterase (AChE) inhibitors Nerve action {Strong evidence that action at this protein is responsible for insecticidal effects}	1A Carbamates	Alanycarb, Aldicarb, Bendiocarb, Benfuracarb, Butocarboxim, Butoxycarboxim, Carbaryl, Carbofuran, Carbosulfan, Ethiofencarb, Fenobucarb, Formetanate, Furathiocarb, Isoprocarb, Methiocarb, Methomyl, Metolcarb, Oxamyl, Pirimicarb, Propoxur, Thiodicarb, Thiofanox, Triazamate, Trimethacarb, XMC, Xylylcarb
	1B Organophosphates	Acephate, Azamethiphos, Azinphos-ethyl, Azinphos-methyl, Cadusafos, Chlorethoxyfos, Chlorfenvinphos, Chlormephos, Chlorpyrifos, Chlorpyrifos-methyl, Coumaphos, Cyanophos, Demeton-S-methyl, Diazinon, Dichlorvos/ DDVP, Dicrotophos, Dimethoate, Dimethylvinphos, Disulfoton, EPN, Ethion, Ethoprophos, Famphur, Fenamiphos, Fenitrothion, Fenthion, Fosthiazate, Heptenophos, Imicyafos, Isofenphos, Isopropyl O-(methoxyaminothio-phosphoryl) salicylate, Isoxathion, Malathion, Mecarbam, Methamidophos, Methidathion, Mevinphos, Monocrotophos, Naled, Omethoate, Oxydemeton-methyl, Parathion, Parathion-methyl, Phenthoate, Phorate, Phosalone, Phosmet, Phosphamidon, Phoxim, Pirimiphos- methyl, Profenofos, Propetamphos, Prothiofos, Pyraclofos, Pyridaphenthion, Quinalphos, Sulfotep, Tebupirimfos, Temephos, Terbufos, Tetrachlorvinphos, Thiometon, Triazophos, Trichlorfon, Vamidothion
2 GABA-gated chloride channel antagonists Nerve action {Strong evidence that action at this protein is responsible for insecticidal effects}	2A Cyclo diene organochlorines	Chlordane, Endosulfan
	2B Phenylpyrazoles (Fiproles)	Ethiprole, Fipronil
3 Sodium channel modulators Nerve action {Strong evidence that action at this protein is responsible for insecticidal effects}	3A Pyrethroids Pyrethrins	Acrinathrin, Allethrin, d- <i>cis-trans</i> Allethrin, d- <i>trans</i> Allethrin, Bifenthrin, Bioallethrin, Bioallethrin S-cyclopentenyl isomer, Bioresmethrin, Cycloprothrin, Cyfluthrin, <i>beta</i> -Cyfluthrin, Cyhalothrin, <i>lambda</i> -Cyhalothrin, <i>gamma</i> -Cyhalothrin, Cypermethrin, <i>alpha</i> -Cypermethrin, <i>beta</i> -Cypermethrin, <i>theta</i> -cypermethrin, <i>zeta</i> -Cypermethrin, Cyphenothrin, (1 <i>R</i>)- <i>trans</i> - isomers], Deltamethrin, Empenthrin (EZ)-(1 <i>R</i>)- isomers], Esfenvalerate, Etofenprox, Fenpropathrin, Fenvalerate, Flucythrinate, Flumethrin, <i>tau</i> -Fluvalinate, Halfenprox, Imiprothrin, Kadethrin, Permethrin, Phenothrin [(1 <i>R</i>)- <i>trans</i> - isomer], Prallethrin, Pyrethrins (pyrethrum), Resmethrin, Silafluofen, Tefluthrin, Tetramethrin, Tetramethrin [(1 <i>R</i>)-isomers], Tralomethrin, Transfluthrin,

IRAC MoA Classification v 7.3, February 2014

See section 6.4 for further information on sub-groups.
See section 6.3 for criteria for descriptors of the quality of MoA information.

Main Group and Primary Site of Action	Chemical Sub-group or exemplifying Active Ingredient	Active Ingredients
	3B DDT Methoxychlor	DDT Methoxychlor
4 Nicotinic acetylcholine receptor (nAChR) agonists Nerve action {Strong evidence that action at one or more of this class of protein is responsible for insecticidal effects}	4A Neonicotinoids	Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Nitenpyram, Thiacloprid, Thiamethoxam,
	4B Nicotine	Nicotine
	4C Sulfoxaflor	Sulfoxaflor
	4D Butenolides	Flupyradifurone
5 Nicotinic acetylcholine receptor (nAChR) allosteric activators Nerve action {Strong evidence that action at one or more of this class of protein is responsible for insecticidal effects}	Spinosyns	Spinetoram, Spinosad
6 Chloride channel activators Nerve and muscle action {Strong evidence that action at one or more of this class of protein is responsible for insecticidal effects}	Avermectins, Milbemycins	Abamectin, Emamectin benzoate, Lepimectin, Milbemectin
7 Juvenile hormone mimics Growth regulation {Target protein responsible for biological activity is unknown, or uncharacterized}	7A Juvenile hormone analogues	Hydroprene, Kinoprene, Methoprene
	7B Fenoxycarb	Fenoxycarb
	7C Pyriproxyfen	Pyriproxyfen

IRAC MoA Classification v 7.3, February 2014

See section 6.4 for further information on sub-groups.
See section 6.3 for criteria for descriptors of the quality of MoA information.

Main Group and Primary Site of Action	Chemical Sub-group or exemplifying Active Ingredient	Active Ingredients
8 * Miscellaneous non-specific (multi-site) inhibitors	8A Alkyl halides	Methyl bromide and other alkyl halides
	8B Chloropicrin	Chloropicrin
	8C Sulfuryl fluoride	Sulfuryl fluoride
	8D Borates	Borax
	8E Tartar emetic	Tartar emetic
9 Modulators of Chordotonal Organs Nerve action {Target protein responsible for biological activity is unknown, or uncharacterized}	9B Pymetrozine	Pymetrozine
	9C Flonicamid	Flonicamid
10 Mite growth inhibitors Growth regulation {Target protein responsible for biological activity is unknown, or uncharacterized}	10A Clofentezine Hexythiazox Diflovidazin	Clofentezine, Hexythiazox, Diflovidazin
	10B Etoxazole	Etoxazole
11 Microbial disruptors of insect midgut membranes (includes transgenic crops expressing <i>Bacillus thuringiensis</i> toxins, however specific guidance for resistance management of transgenic crops is not based on rotation of modes of action)	11A <i>Bacillus thuringiensis</i> and the insecticidal proteins they produce	<i>Bacillus thuringiensis</i> subsp. <i>israelensis</i> <i>Bacillus thuringiensis</i> subsp. <i>aizawai</i> <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> <i>Bacillus thuringiensis</i> subsp. <i>tenebrionis</i> <i>B.t.</i> crop proteins: (* Please see footnote) Cry1Ab, Cry1Ac, Cry1Fa, Cry1A.105, Cry2Ab, Vip3A, mCry3A, Cry3Ab, Cry3Bb, Cry34Ab1/Cry35Ab1
	11B <i>Bacillus sphaericus</i>	<i>Bacillus sphaericus</i>

IRAC MoA Classification v 7.3, February 2014

See section 6.4 for further information on sub-groups.
See section 6.3 for criteria for descriptors of the quality of MoA information.

Main Group and Primary Site of Action	Chemical Sub-group or exemplifying Active Ingredient	Active Ingredients
12 Inhibitors of mitochondrial ATP synthase Energy metabolism {Compounds affect the function of this protein, but it is not clear that this is what leads to biological activity}	12A Diafenthiuron	Diafenthiuron
	12B Organotin miticides	Azocyclotin, Cyhexatin, Fenbutatin oxide
	12C Propargite	Propargite
	12D Tetradifon	Tetradifon
13 * Uncouplers of oxidative phosphorylation via disruption of the proton gradient Energy metabolism	Chlorfenapyr DNOC Sulfluramid	Chlorfenapyr DNOC Sulfluramid
14 Nicotinic acetylcholine receptor (nAChR) channel blockers Nerve action {Compounds affect the function of this protein, but it is not clear that this is what leads to biological activity}	Nereistoxin analogues	Bensultap, Cartap hydrochloride, Thiocyclam, Thiosultap-sodium
15 Inhibitors of chitin biosynthesis, type 0 Growth regulation {Target protein responsible for biological activity is unknown, or uncharacterized}	Benzoylureas	Bistrifluron, Chlorfluazuron, Diflubenzuron, Flucycloxuron, Flufenoxuron, Hexaflumuron, Lufenuron, Novaluron, Noviflumuron, Teflubenzuron, Triflumuron
16 Inhibitors of chitin biosynthesis, type 1 Growth regulation {Target protein responsible for biological activity is unknown, or uncharacterized}	Buprofezin	Buprofezin

IRAC MoA Classification v 7.3, February 2014

See section 6.4 for further information on sub-groups.
See section 6.3 for criteria for descriptors of the quality of MoA information.

Main Group and Primary Site of Action	Chemical Sub-group or exemplifying Active Ingredient	Active Ingredients
17 Moulting disruptor, Dipteran Growth regulation {Target protein responsible for biological activity is unknown, or uncharacterized}	Cyromazine	Cyromazine
18 Ecdysone receptor agonists Growth regulation {Strong evidence that action at this protein is responsible for insecticidal effects}	Diacylhydrazines	Chromafenozide, Halofenozide, Methoxyfenozide, Tebufenozide
19 Octopamine receptor agonists Nerve action {Good evidence that action at one or more of this class of protein is responsible for insecticidal effects}	Amitraz	Amitraz
20 Mitochondrial complex III electron transport inhibitors Energy metabolism {Good evidence that action at this protein complex is responsible for insecticidal effects}	20A Hydramethylnon	Hydramethylnon
	20B Acequinocyl	Acequinocyl
	20C Fluacrypyrim	Fluacrypyrim
21 Mitochondrial complex I electron transport inhibitors Energy metabolism {Good evidence that action at this protein complex is responsible for insecticidal effects}	21A METI acaricides and insecticides	Fenazaquin, Fenpyroximate, Pyrimidifen, Pyridaben, Tebufenpyrad, Tolfenpyrad
	21B Rotenone	Rotenone (Derris)

IRAC MoA Classification v 7.3, February 2014

See section 6.4 for further information on sub-groups.
See section 6.3 for criteria for descriptors of the quality of MoA information.

<i>Main Group and Primary Site of Action</i>	<i>Chemical Sub-group or exemplifying Active Ingredient</i>	<i>Active Ingredients</i>
22 Voltage-dependent sodium channel blockers Nerve action {Good evidence that action at this protein complex is responsible for insecticidal effects}	22A Indoxacarb	Indoxacarb
	22B Metaflumizone	Metaflumizone
23 Inhibitors of acetyl CoA carboxylase. Lipid synthesis, growth regulation {Good evidence that action at this protein is responsible for insecticidal effects}	Tetronic and Tetramic acid derivatives	Spirodiclofen, Spiromesifen, Spirotetramat
24 Mitochondrial complex IV electron transport inhibitors Energy metabolism {Good evidence that action at this protein complex is responsible for insecticidal effects}	24A Phosphine	Aluminium phosphide, Calcium phosphide, Phosphine, Zinc phosphide
	24B Cyanide	Cyanide
25 Mitochondrial complex II electron transport inhibitors Energy metabolism {Good evidence that action at this protein complex is responsible for insecticidal effects}	Beta-ketonitrile derivatives	Cyenopyrafen, Cyflumetofen
28 Ryanodine receptor modulators Nerve and muscle action {Good evidence that action at this protein complex is responsible for insecticidal effects}	Diamides	Chlorantraniliprole, Cyantraniliprole, Flubendiamide

IRAC MoA Classification v 7.3, February 2014

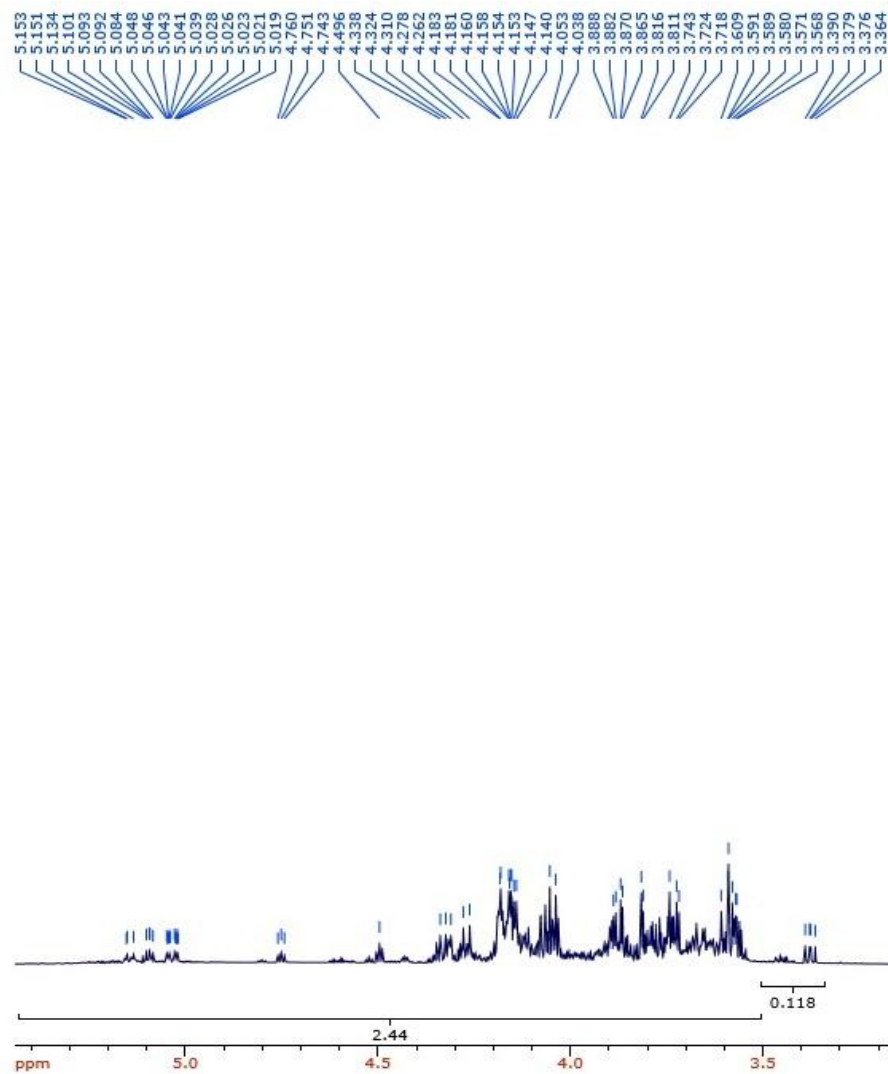
*See section 6.4 for further information on sub-groups.
See section 6.3 for criteria for descriptors of the quality of MoA information.*

<i>Main Group and Primary Site of Action</i>	<i>Chemical Sub-group or exemplifying Active Ingredient</i>	<i>Active Ingredients</i>
UN * Compounds of unknown or uncertain MoA {Target protein responsible for biological activity is unknown, or uncharacterized}	Azadirachtin	Azadirachtin
	Benzoximate	Benzoximate
	Bifenazate	Bifenazate
	Bromopropylate	Bromopropylate
	Chinomethionat	Chinomethionat
	Cryolite	Cryolite
	Dicofol	Dicofol
	Pyridalyl	Pyridalyl
	Pyrifluquinazon	Pyrifluquinazon

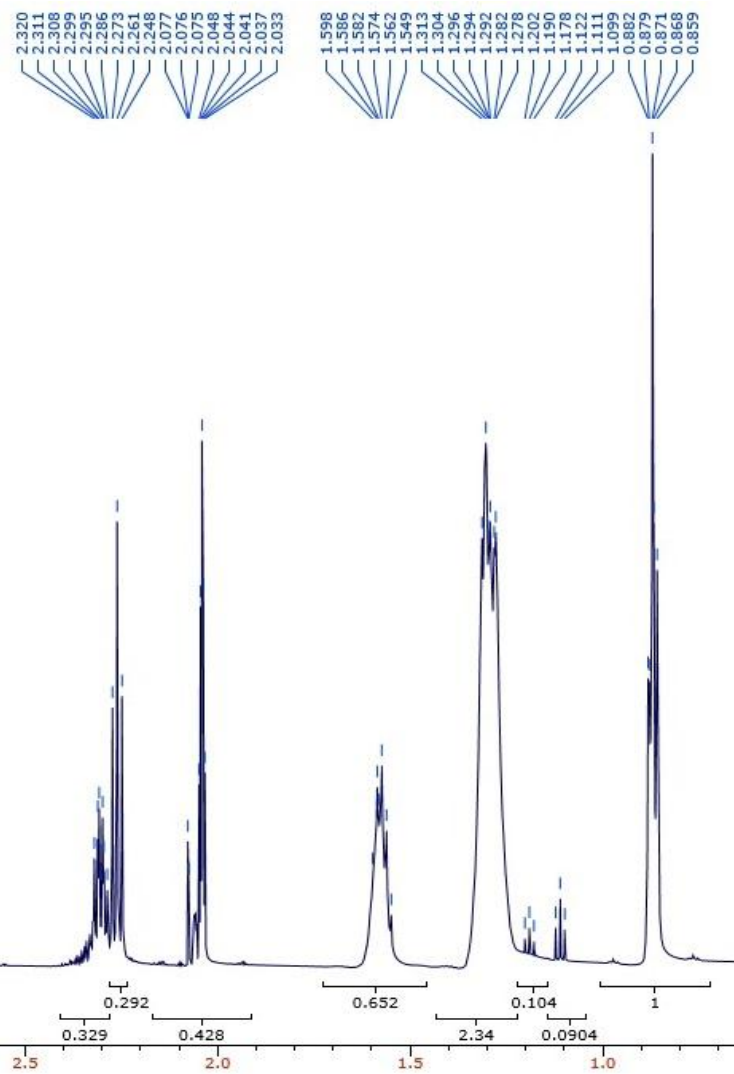
Table Notes:

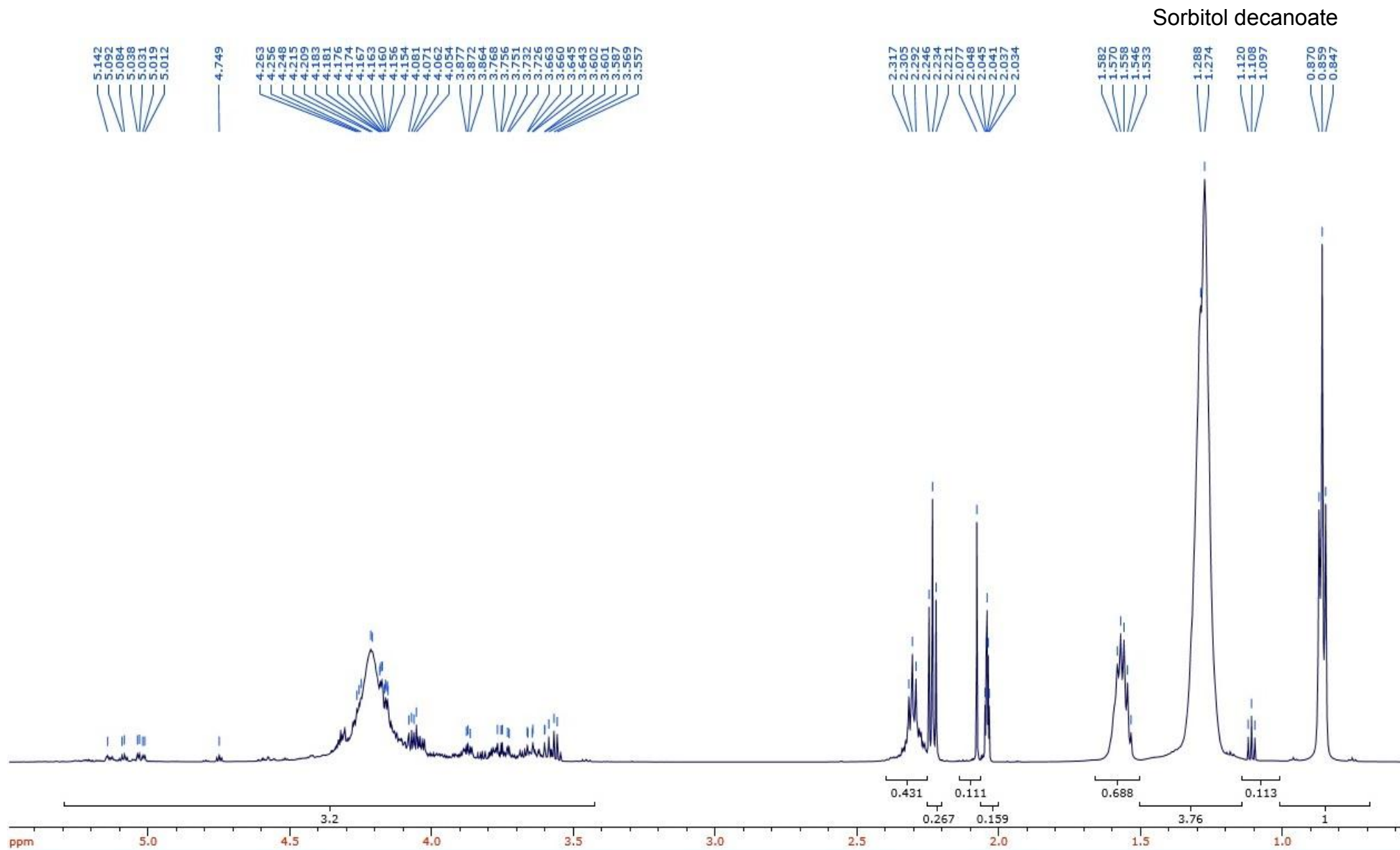
- a) Inclusion of a compound in the classification above does not necessarily signify regulatory approval.
- b) MoA assignments will usually involve identification of the target protein responsible for the biological effect, although groupings can be made where compounds share distinctive physiological effects and have related chemical structures.
- c) Groups 26 and 27 are unassigned at this time and have therefore been omitted from the table.
- d) A compound with an unknown or controversial MoA or an unknown mode of toxicity will be held in group 'UN' until evidence becomes available to enable that compound to be assigned to a more appropriate MoA class.
- e) Actives in groups marked with a * are thought not to share a common target site and therefore may be freely rotated with each other unless there is reason to expect cross-resistance. These groups are 8, 13, and UN.

Appendix 2 NMR spectra of sugar esters purified with ethanol and deionized water

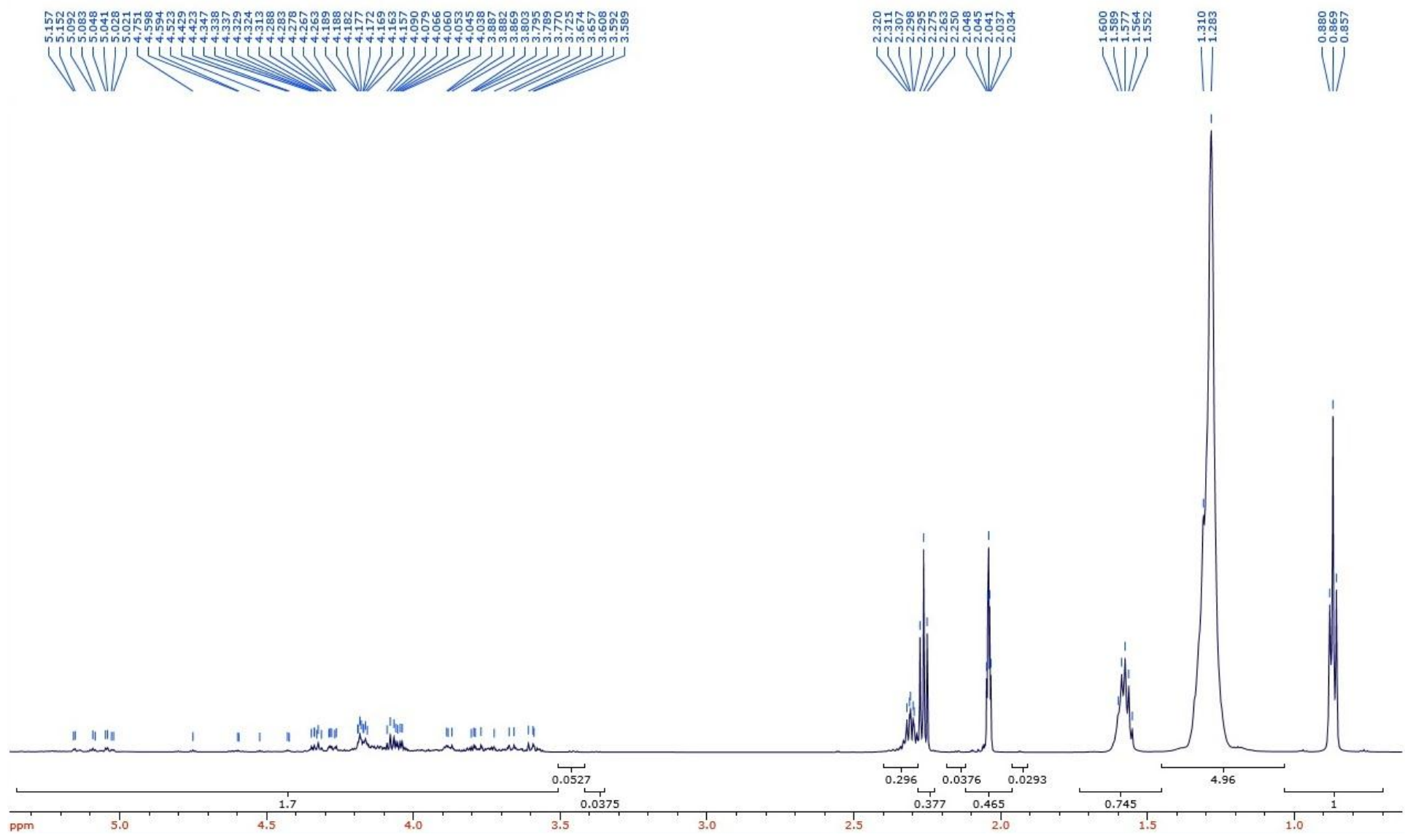


Sorbitol octanoate

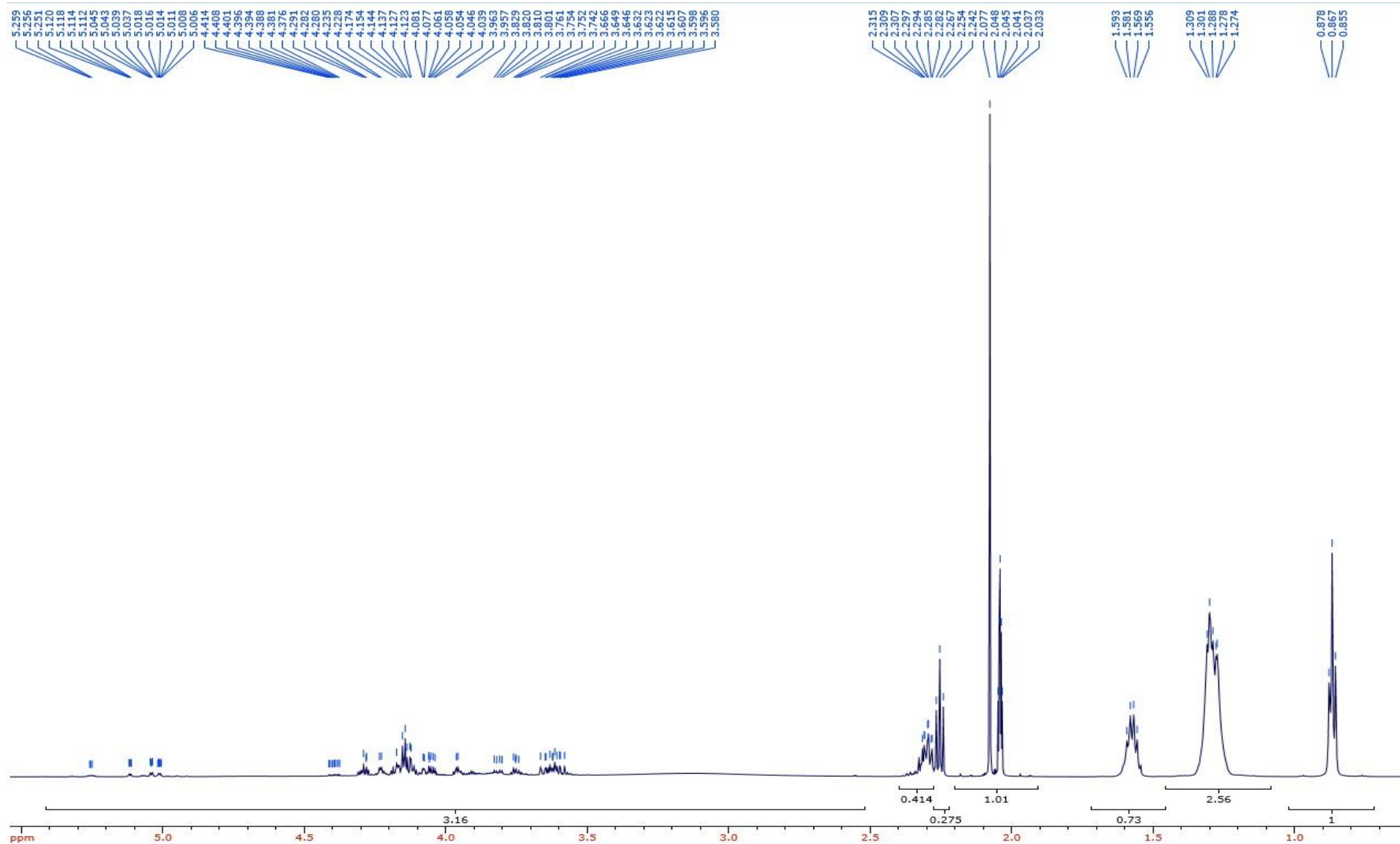


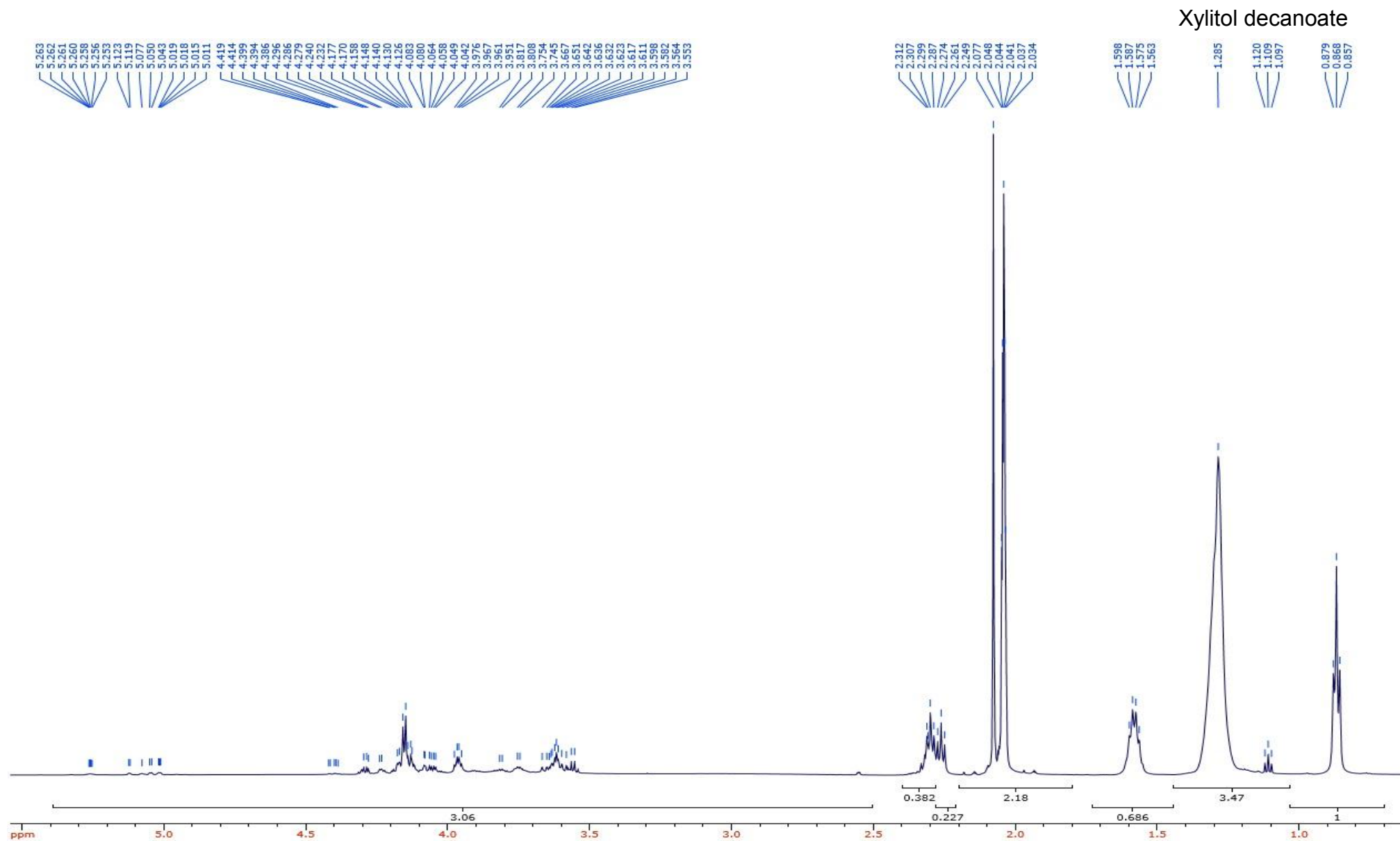


Sorbitol laurate

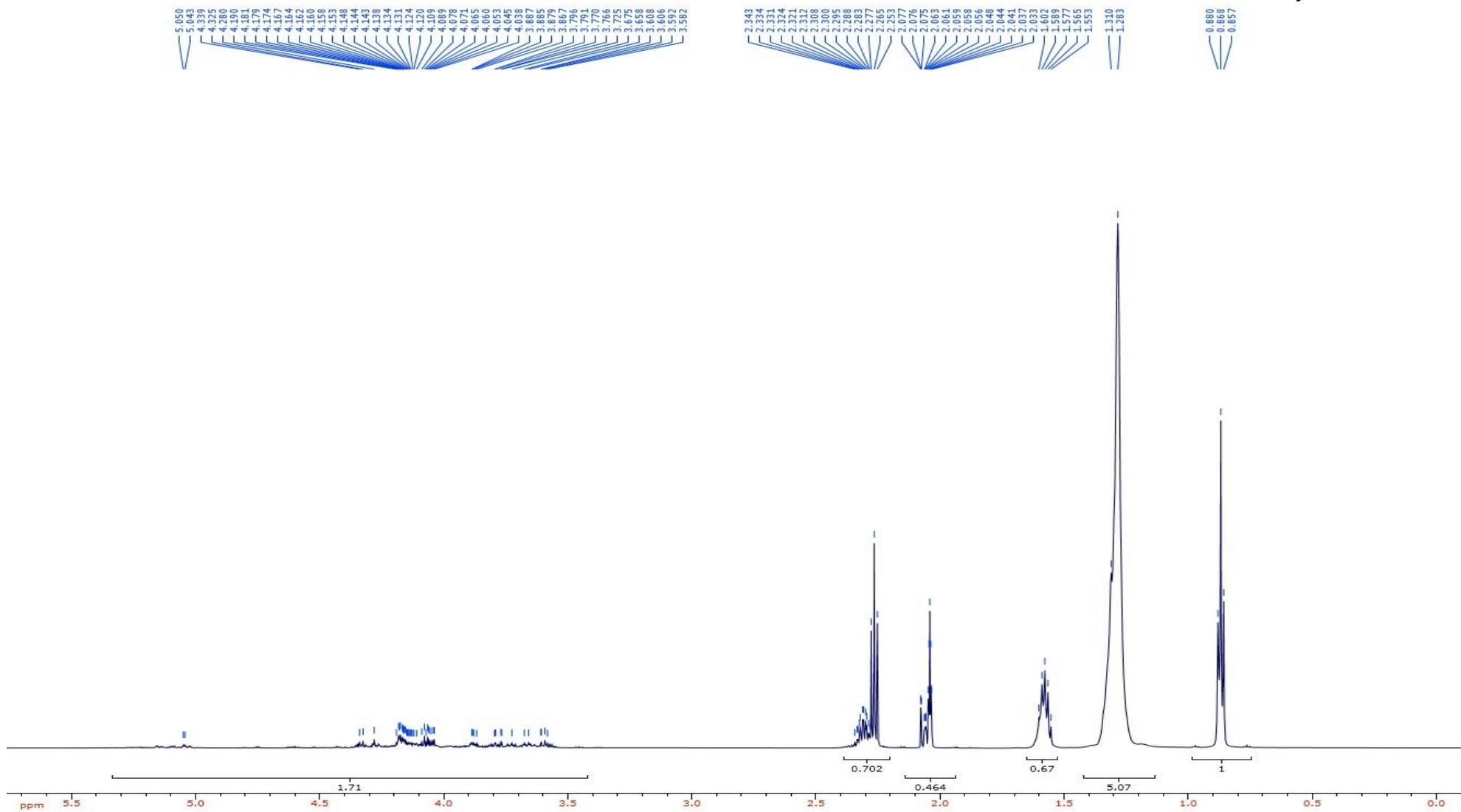


Xylitol octanoate





Xylitol laurate



Appendix 3 Supplementing bioassay data

Appendix 3a - Toxicity of sugar ester prepared with polyol and fatty acids having different chain length against *R. dominica* at concentration of 100, 400, 1000 and 4000ppm in 7 days.

Concentration (ppm)	Mean mortality (%) (7 days)†	REGWQ test, subset for alpha = 0.05		
		1	2	3
Negative Control	0±0	*		
sC8.100	0±0	*		
sC8.400	3±1	*		
sC8.1000	0±0	*		
sC8.4000	54±4			*
xC8.100	0±0	*		
xC8.400	0±0	*		
xC8.1000	0±0	*		
xC8.4000	6±2	*		
sC10.100	2±0	*		
sC10.400	2±1	*		
sC10.1000	1±0	*		
sC10.4000	10±2	*		

xC10.100	0±0	*	
xC10.400	0±0	*	
xC10.1000	0±0	*	
xC10.4000	28±3		*
sC12.100	0±0	*	
sC12.400	1±0	*	
sC12.1000	0±0	*	
sC12.4000	2±0	*	
xC12.100	2±0	*	
xC12.400	1±0	*	
xC12.1000	0±0	*	
xC12.4000	0±0	*	

†mean mortality % ± standard deviation, s=sorbitol, x=xylitol, C8=octanoic acid, C10=decanoic acid, C12=lauric acid

a). Sorbitol octanoate at 4000ppm concentration (sC8.4000) gave the highest percent mean mortality compared to all other treatments (REGWQ test, $p < 0.05$)

b). Bioassay carried out on bulk grain, done in triplicate

c). 30 adult (0-2 week old) *R dominica* were used in each replicate

d). Data subjected to Abbotts' correction and arcsine transformed prior to REGWQ test

Appendix 3b - Toxicity of sugar ester prepared with polyol and fatty acids having different chain length against *R. domonica* at concentration of 100, 400, 1000 and 4000ppm in 14 days.

Concentration (ppm)	Mean mortality (%) (14 days)†	REGWQ test, subset for alpha = 0.05			
		1	2	3	4
Negative Control	0±0	*			
sC8.100	1±0	*			
sC8.400	3±1	*			
sC8.1000	0±0	*			
sC8.4000	81±2				*
xC8.100	1±0	*			
xC8.400	0±0	*			
xC8.1000	0±0	*			
xC8.4000	30±3		*		
sC10.100	4±1	*			
sC10.400	2±1	*			
sC10.1000	1±0	*			
sC10.4000	16±3	*			
xC10.100	1±0	*			
xC10.400	1±0	*			

xC10.1000	0±0	*	
xC10.4000	50±5		*
sC12.100	0±0	*	
sC12.400	2±1	*	
sC12.1000	1±0	*	
sC12.4000	3±1	*	
xC12.100	2±0	*	
xC12.400	2±0	*	
xC12.1000	1±0	*	
xC12.4000	1±0	*	

†mean mortality % ± standard deviation, s=sorbitol, x=xylitol, C8=octanoic acid, C10=decanoic acid, C12=lauric acid

‡progeny ± standard deviation

- a). Sorbitol octanoate at 4000ppm concentration (sC8.4000) gave the highest percent mean mortality compared to all other treatments (REGWQ test, $p < 0.05$)
- b). Bioassay carried out on bulk grain, done in triplicate, 30 adult (0-2 week old) *R dominica* were used in each replicate
- c). Data subjected to Abbotts' correction and arcsine transformed prior to REGWQ test
- d). Mean mortality of 14 days are accumulation totals including mortality results of 7 days

Appendix 3c - Effect of purification on *R. dominica*'s mortality in 7 days at 1000 and 4000ppm

Sugar ester	Concentration (ppm)	Mean mortality (%) 7 Days†	REGWQ test, subset for alpha = 0.05		
			1	2	3
Negative Control	0	0±0	*		
sC8 crude	1000	1±0	*		
	4000	0±0	*		
sC8 two-steps	1000	0±0	*		
	4000	54±4			*
sC8 three-steps	1000	2±0	*		
	4000	23±4		*	
xC8 crude	1000	1±0	*		
	4000	7±2	*	*	
xC8 two-steps	1000	0±0	*		
	4000	6±2	*	*	
xC8 three-steps	1000	0±0	*		
	4000	13±2	*	*	

†mean mortality % ± standard deviation

a). Crude sorbitol octanoate after washing with ethanol, having concentration at 4000ppm (sC8.two-steps, 4000ppm) gave the highest percent mean mortality compared to all other treatments (REGWQ test, $p < 0.05$)

- b). Bioassay carried out on bulk grain, done in triplicate
- c). 30 adult (0-2 week old) *R dominica* were used in each replicate
- d). Data subjected to Abbotts' correction and arcsine transformed prior to REGWQ test
- e). Two-steps: washed to remove catalyst and unreacted sugars
- f). Three-steps: washed to remove catalyst, unreacted sugars and unreacted fatty acids

Appendix 3d - Effect of purification on *R. dominica*'s mortality in 14 days at 1000 and 4000ppm

Sugar ester	Concentration (ppm)	Mean mortality (%) 14 Days†	REGWQ test, subset for alpha = 0.05			
			1	2	3	4
Negative Control	-	0±0	*			
sC8 crude	1000	2±0	*			
	4000	4±1	*			
sC8 two-steps	1000	0±0	*			
	4000	81±2				*
sC8 three-steps	1000	2±0	*			
	4000	58±7			*	*
xC8 crude	1000	1±0	*			
	4000	9±2	*	*		
xC8 two-steps	1000	0±0	*			
	4000	30±3	*	*		
xC8 three-steps	1000	0±0	*			
	4000	36±4		*	*	

†mean mortality % ± standard deviation

a). Crude sorbitol octanoate after washing with ethanol, having concentration at 4000ppm (sC8.two-steps, 4000ppm) gave the highest percent mean mortality compared to all other treatments (REGWQ test, $p < 0.05$)

-
- b). Bioassay carried out on bulk grain, done in triplicate
 - c). 30 adult (0-2 week old) *R dominica* were used in each replicate
 - d). Data subjected to Abbotts' correction and arcsine transformed prior to REGWQ test
 - e). Two-steps: washed to remove catalyst and unreacted sugars
 - f). Three-steps: washed to remove catalyst, unreacted sugars and unreacted fatty acids

Appendix 3e - Toxicity of sorbitol octanoate, DE and their combinations against *R. dominica* expressed in mortality (7th day)

Treatment	Concentration (ppm)	Mean mortality (%) (7 Days)†	REGWQ test, subset for alpha = 0.05							
			1	2	3	4	5	6	7	
Negative Control	0	0±0	*							
DE	100	3±1	*							
	400	43±5			*	*				
	700	84±2					*	*	*	
	1000	94±1								*
sC8	1000	0±0	*							
	2000	2±0	*							
	4000	12±1	*	*						
sC8/DE	1000/100	0±0	*							
	1000/400	9±2	*	*						
	1000/700	24±0	*	*	*					
	1000/1000	32±3		*	*					
	2000/100	10±3	*	*						
	2000/400	64±1				*	*	*		
	2000/700	64±1				*	*	*		
	2000/1000	81±3					*	*	*	

4000/100	62±3	*	*		
4000/400	87±4			*	*
4000/700	87±2			*	*
4000/1000	88±2			*	*

†mean mortality % ± standard deviation

a). Bioassay carried out on bulk grain, done in triplicate

b). 30 adult (0-2 week old) *R dominica* were used in each replicate

c). Data subjected to Abbotts' correction and arcsine transformed prior to REGWQ test

Appendix 3f - Toxicity of sorbitol octanoate, DE and their combinations against *R. dominica* expressed in mortality (14th day)

Treatment	Concentration (ppm)	Mean mortality (%) (14 Days)†	REGWQ test, subset for alpha = 0.05						
			1	2	3	4	5	6	
Negative Control	0	0±0	*						
DE	100	29±4		*					
	400	84±1				*	*	*	
	700	98±1							*
	1000	98±1							*
sC8	1000	2±1	*						
	2000	2±0	*						
	4000	31±4		*					
sC8/DE	1000/100	4±2	*						
	1000/400	47±4		*	*				
	1000/700	68±3			*	*			
	1000/1000	74±2				*	*		
	2000/100	41±6		*					
	2000/400	97±2							*
	2000/700	94±1						*	*
	2000/1000	100±0							*

4000/100	96±0	*	*
4000/400	100±0		*
4000/700	99±0		*
4000/1000	100±0		*

†mean mortality % ± standard deviation

a). Bioassay carried out on bulk grain, done in triplicate

b). 30 adult (0-2 week old) *R dominica* were used in each replicate

c). Data subjected to Abbotts' correction and arcsine transformed prior to REGWQ test

Appendix 3g - Toxicity of sorbitol octanoate (sC8), DE and their combinations, including a positive control, Dryacide, against *R. dominica* expressed in mortality (7th and 14th day)

Treatment	Concentration (ppm)	Mean mortality (%) (7 Days)†	Mean mortality (%) (14 Days)†
Negative Control	0	0±0	0±0
DE	80	58±3	73±1
	160	78±6	84±4
	240	86±2	93±1
	320	98±1	100±0
	400	96±1	99±0
Dryacide	80	61±3	71±1
	160	57±2	67±1
	240	94±0	97±1
	320	97±1	100±0
	400	100±0	100±0
sC8	3000	4±1	8±2
	4000	10±2	12±2
	5000	10±2	24±0
	6000	31±6	43±6

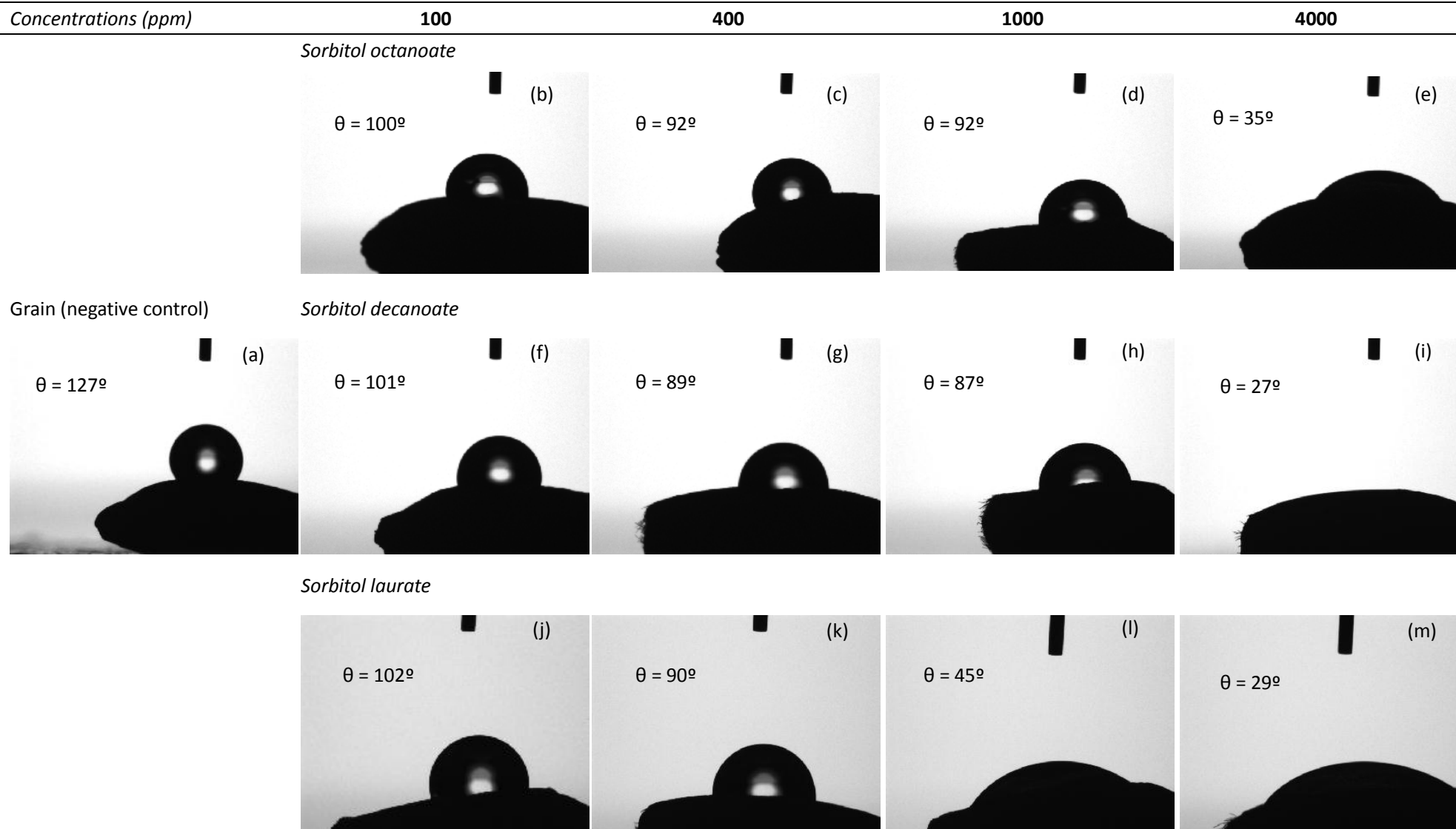
	7000	34±3	61±4
sC8/DE	800/20	6±1	8±1
	1600/40	4±1	11±0
	2400/60	19±4	31±4
	3200/80	26±3	51±3
	4000/100	69±2	96±0

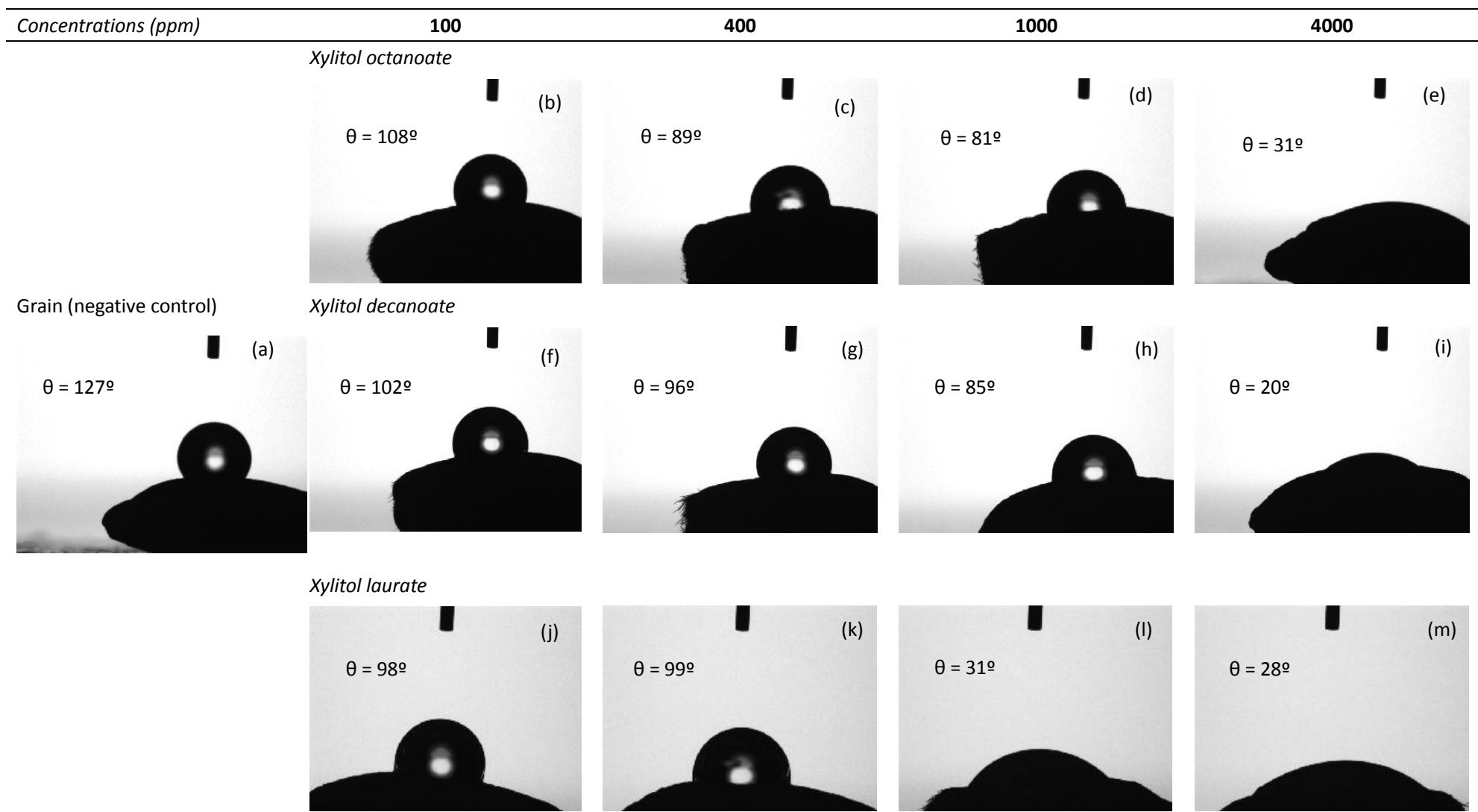
†mean mortality % ± standard deviation

a). Bioassay carried out on bulk grain, done in triplicate

b). 30 adult (0-2 week old) *R dominica* were used in each replicate

Appendix 4 Contact angles of Katana grains coated with different sugar esters at varied concentrations

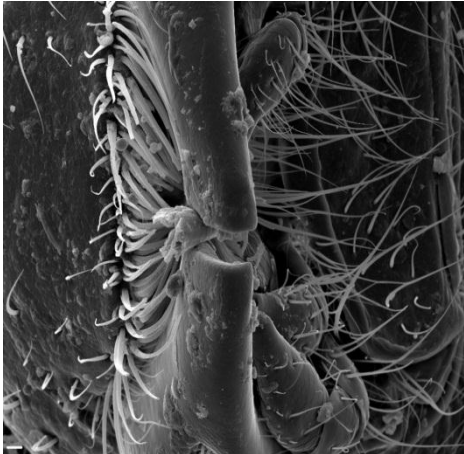




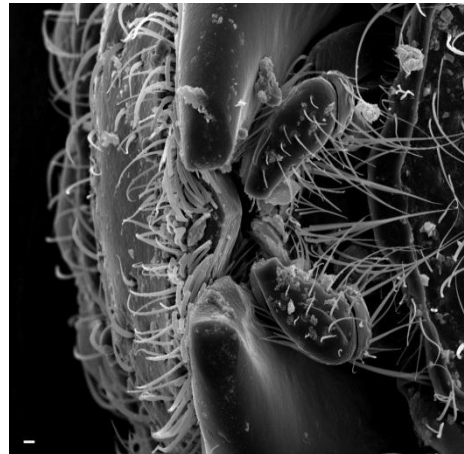
Appendix 5 SEM images showing insect parts including mandible, tibia, tarsal segments and abdominal sternites after treated by DE-sC8 combined formulation

Mandible of *R. dominica* treated by DE-sC8 combined formulation displayed at 1000x and 5000x magnification

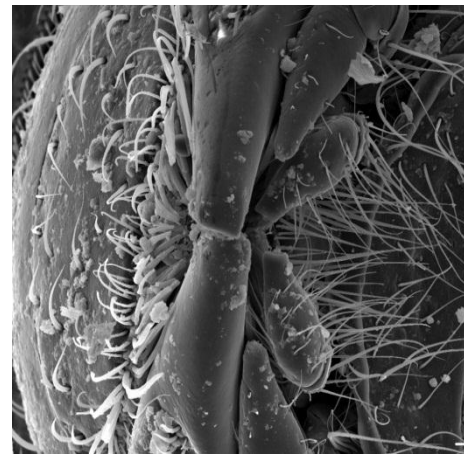
DE 100ppm



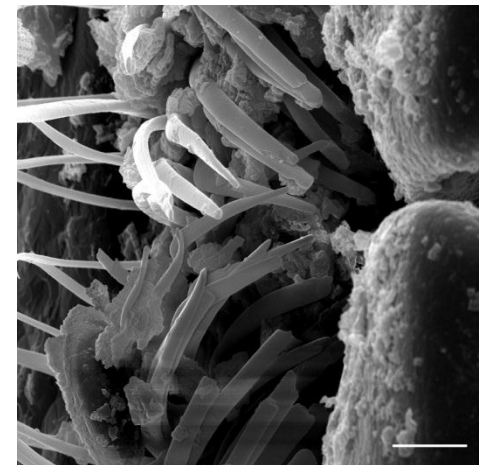
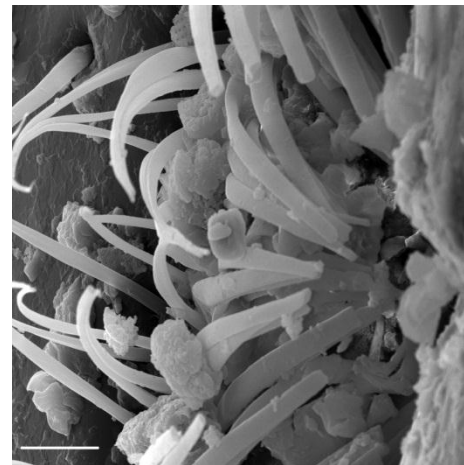
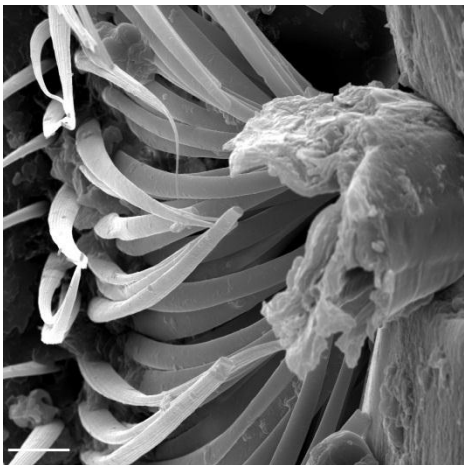
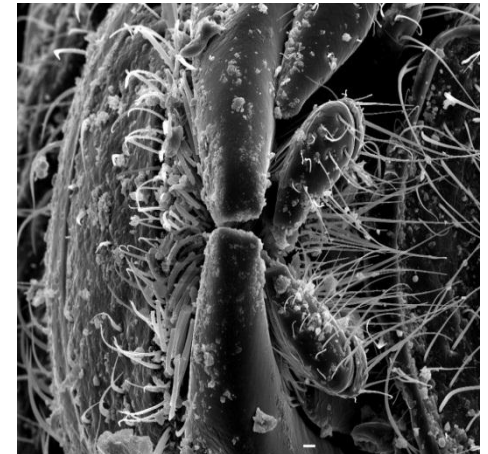
DE 400ppm



DE 700ppm



DE 1000ppm



(a). Image magnification, first row = 1000x, second row = 5000x; scale bar = 10 μ m.

(b) sC8 concentration at 4000ppm

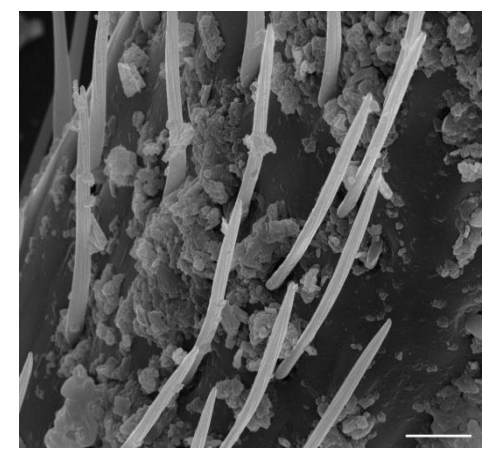
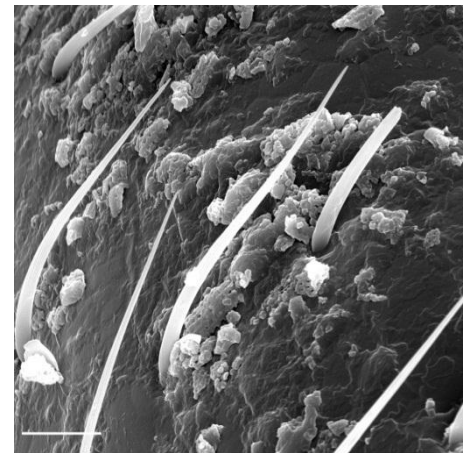
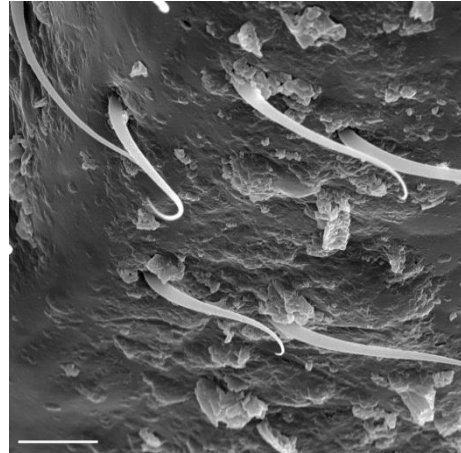
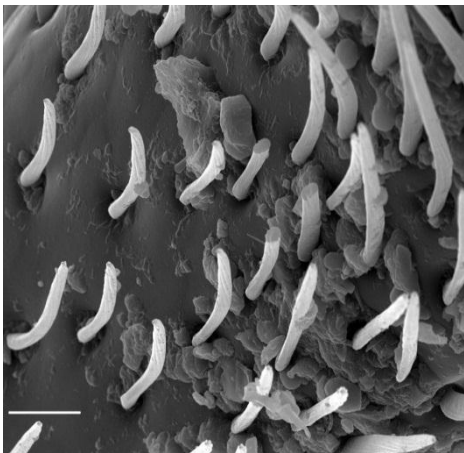
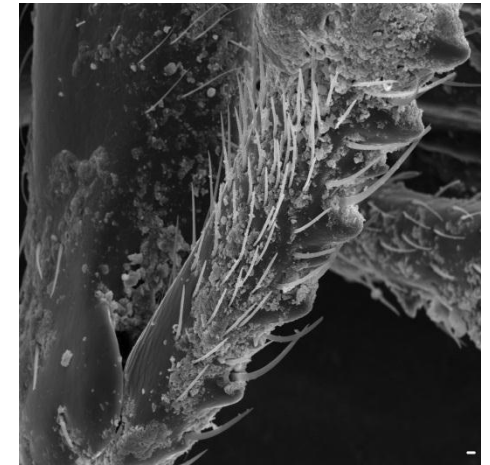
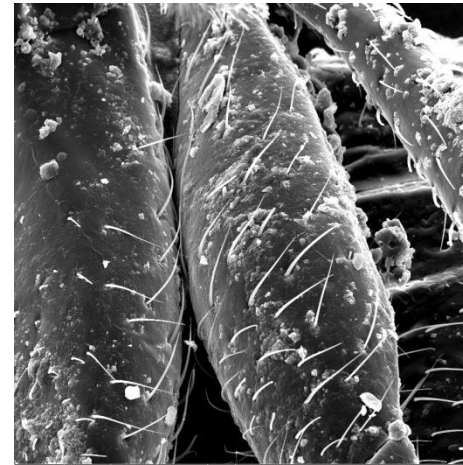
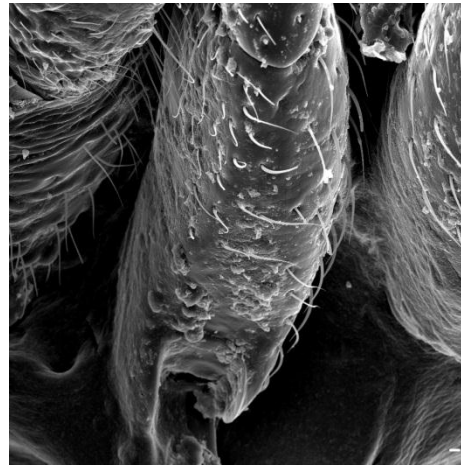
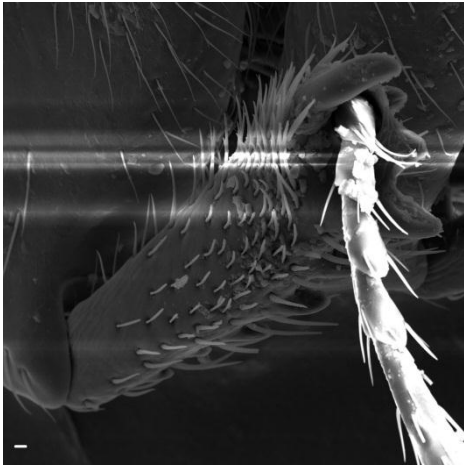
Tibia and tarsal segments of *R. dominica* treated by DE-sC8 combined formulation displayed at 1000x and 5000x magnification

DE 100ppm

DE 400ppm

DE 700ppm

DE 1000ppm



(a). Image magnification, first row = 1000x, second row = 5000x; scale bar = 10 μ m.

(b) sC8 concentration at 4000ppm

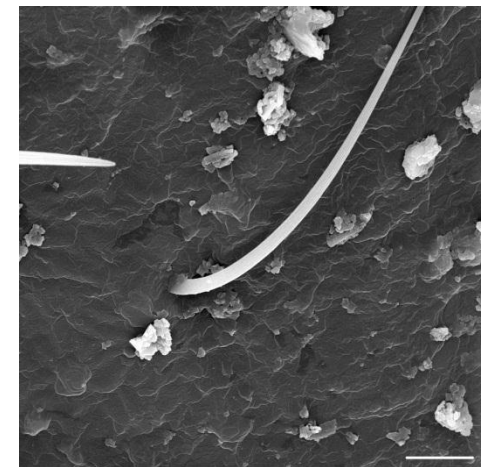
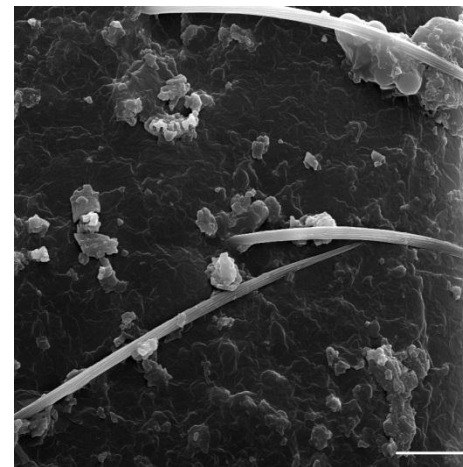
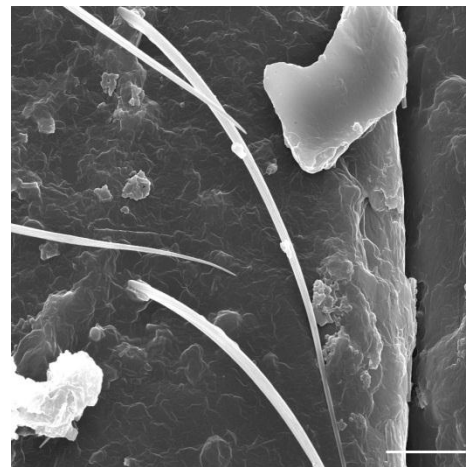
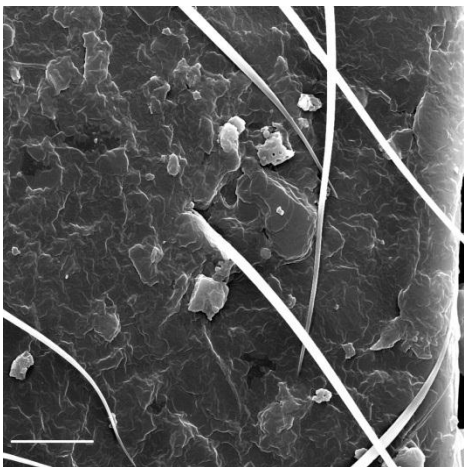
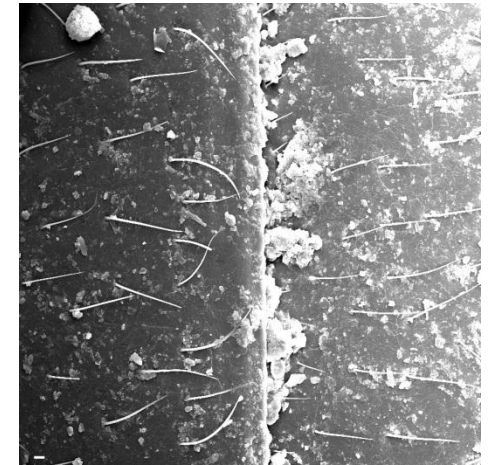
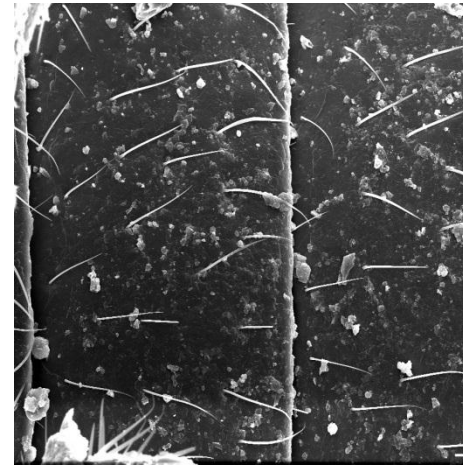
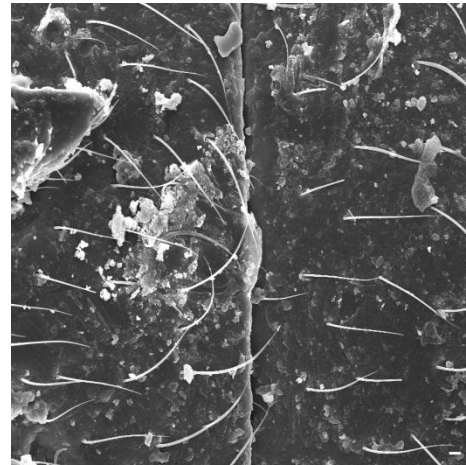
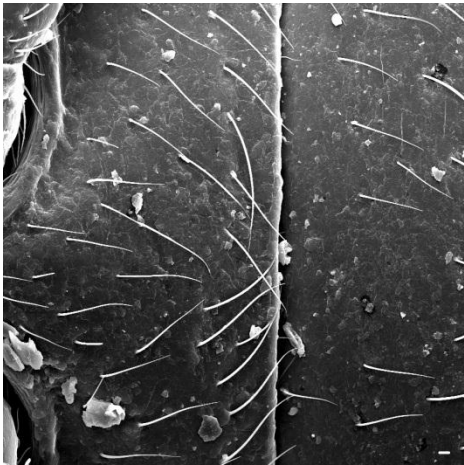
Abdominal sternites of *R. dominica* treated by DE-sC8 combined formulation displayed at 1000x and 5000x magnification

DE 100ppm

DE 400ppm

DE 700ppm

DE 1000ppm



(a). Image magnification, first row = 1000x, second row = 5000x; scale bar = 10 μ m.

(b) sC8 concentration at 4000ppm