Pests and diseases of truffles and their host trees in Australia

Stewart Learmonth, Celeste Linde, Anne Mitchell, Alan Davey, Alison Mathews, Ainsley Seago and Helen Collie
September 2019

AgriFutures™ Emerging Industries

Pests and diseases of truffles and their host trees in Australia

Stewart Learmonth, Celeste Linde, Anne Mitchell, Alan Davey, Alison Mathews, Ainsley Seago and Helen Collie

September 2019

AgriFutures Australia Publication No. 19-040
AgriFutures Australia Project No. PRJ—009832

ISBN 978-1-76053-060-0
ISSN 1440-6845

Pests and diseases of truffles and their host trees in Australia

Publication No. 19040
Project No. PRJ-009832

Researcher Contact Details

Name: Alison Mathews
Address: Locked Bag 7
Department of Primary Industries and Regional Development
Manjimup
Western Australia 6258.

Phone: (08) 9777 0000
Email: alison.mathews@dpird.wa.gov.au

In submitting this report, the researcher has agreed to AgriFutures Australia publishing this material in its edited form.

AgriFutures Australia Contact Details

Building 007, Tooma Way
Charles Sturt University
Locked Bag 588
Wagga Wagga NSW 2650

02 6923 6900
info@agrifutures.com.au
www.agrifutures.com.au

Electronically published by AgriFutures Australia at www.agrifutures.com.au in September 2019.

AgriFutures Australia is the new trading name for Rural Industries Research & Development Corporation (RIRDC), a statutory authority of the Federal Government established by the Primary Industries Research and Development Act 1989.

For the fledgling Australian truffle industry little was known of the pests that attack truffles. This project was undertaken to assist the relatively new Australian agriculture industry of truffle production identify key pest and disease threats and investigate ways in which the key agents may be managed to remove or minimise their adverse effects on the quality, and quantity of truffles. The project also sought to extend the foundation laid by others on pests and diseases that might affect the health of the host trees as well as truffles.

Australian truffle growers present this high value culinary product in a washed state ready for both domestic and export markets. Sound whole truffles are prized in these markets. Any damage by pests or disease results in increased cost to prepare and grade truffles as well as growers receiving a reduced return for damaged truffles because they are trimmed and downgraded. The project sought to enable growers to have the tools to identify and monitor key pests when truffles are forming underground as the first step in reducing damage.

This project provides pictorial and descriptive information on the most common pests and diseases of both the host trees and the truffles themselves. The information also includes the more common beneficial and benign agents that occur in truffle orchards and highlights key exotic pests and diseases growers need to be aware of. Recommendations on the likelihood that any particular pest or disease detected in truffle orchards will require intervention are given. Relevant monitoring methods to help growers decide on the need to instigate management plans to protect trees and truffles from damage were developed within this project or from other relevant studies and experience of the research team. Recommendations for integrated pest and disease management options for the more important pest agents are provided.

Through this project truffle growers (and associated industry consultants and companies) now have a reference source to be able to identify and monitor key pests and diseases that may be limiting production or their ability to produce truffles of high quality. Quantifying damage to truffles in the grading room is the prerequisite to taking action in the orchard to protect future crops. While the outcomes of this project extend the knowledge on the identification and to some extent, management of key pest agents, more work needs to be done both in terms of refining management options as well as having a better understanding of the factors involved in enhancing the health of the host trees.

The outcomes and outputs of this research project will benefit truffle growers across Australia as well as those companies and individuals that support them, and those who sell, value add and buy their product.As well as funding provided by AgriFutures Australia, other sources included the Department of Primary Industries and Regional Development of Western Australia, The Australian Truffle Growers' Association, Truffle Producers of Western Australia, Australian National University and the Department of Primary Industries of New South Wales.

This report is an addition to AgriFutures Australia's diverse range of over 2000 research publications and it forms part of our Emerging Industries arena, which aims to support new and emerging rural industries.

Most of AgriFutures Australia's publications are available for viewing, free downloading or purchasing online at: agrifutures.com.au.

Michael Beer
General Manager, Business Development
AgriFutures Australia

Acknowledgments

We are grateful for the financial and in-kind support to conduct this project provided by AgriFutures Australia, the Department of Primary Industries and Regional Development Western Australia, the Australian Truffle Growers' Association, Truffle Producers of Western Australia, Australian National University, Manjimup Underground, Advyron R. S., the Department of Primary Industries New South Wales and The Truffle and Wine Co. WA.

We thank the many truffle growers who participated in the baseline survey. The participation rate was extremely high which gave us the confidence we were undertaking a project of relevance for the industry.

By the very nature of truffle production, research such as was involved in this project can be undertaken only in commercial truffle orchards. The co-operation of the many truffle growers in allowing access to their orchards by the research team is gratefully acknowledged.

Identifications of invertebrates was provided by the following personnel and their assistance is gratefully acknowledged: Dr Matthew Binns - earwigs; Josh Douglas - slaters; Dr Penny Greenslade – springtails; Dr Michael Nash – slugs; Dr Ainsley Seago – truffle related and other beetles; Andras Szito – many groups of invertebrates; Julianne Warlock - millipedes.

Information on pest management was provided by Peter Dal Santo of AgAware Consulting P/L at grower workshops and in general discussion by Chris Sharpe of DPIRD.

Data analysis was provided by Andrew Vanburgel of DPIRD.

Helpful comments on technical content and the composition of the report were provided by Svet Micic and Diana Fisher, Judy Rose and Paige Wilson of DPIRD.

Ingenuity and effort in the field and laboratory was provided by Alan Jacob of DPIRD.

Gavin Booth of Australian Truffle Traders provided impetus to set up the project and support while the project was being undertaken.

Abbreviations

ACT – Australian Capital Territory

ANU – Australian National University

APVMA – Australian Pesticides and Veterinary Medicines Authority

ATGA – Australian Truffle Growers' Association

DPIRD – Department of Primary Industries and Regional Development, Western Australia

EPPRD – Emergency Plant Pest Response Deed

IPDM – Integrated Pest and Disease Management

NSW – New South Wales

NSW DPI – New South Wales Department of Primary Industries

QLD – Queensland

SA – South Australia

TAS – Tasmania

TPWA – Truffle Producers of Western Australia

VIC – Victoria

WA – Western Australia

Contents

List of Tables

List of Figures

Executive Summary

Background

The truffle industry is relatively new to Australia with the first truffle produced only 20 years ago. Production is becoming significant - in 2014 around 8 tonnes was harvested with at least 85% of truffles exported. Australia is now the fourth largest truffle producing country in the world. Over the next 10 years, truffle production is set to increase dramatically as existing plantings enter the productive phase. Further expansion in the longer term will occur with new plantings and existing orchards expanding.

With the exception of the Northern Territory, all Australian states and mainland territories produce truffles. Currently, about three-quarters of Australian truffles are produced in Western Australia. There is one dominant truffle type, the black truffle (*Tuber melanosporum*), which is harvested during winter and counter seasonal to those truffles produced in the northern hemisphere, the main market for Australian truffles. There are about 300 truffle producers across Australia and orchards vary in size from just a few hundred trees to over 40,000 trees. The area under truffles in Australia is about 700 ha, and from mature trees, about 12 tonnes were harvested in 2018, with a farm gate value of about $A12m. When the multiplying factor of truffles as an enhancement to food and the festivals it engenders such as the Canberra Truffle Festival, the Melbourne Truffle Festival and Manjimup Truffle Kerfuffle, it is worth considerably more. An estimate of the current total economic impact of the industry is estimated at $54M (based on a commonly used multiplier of 4.5).

Invertebrate pests and diseases of truffles and truffle host trees cause significant losses to the industry across Australia. Pests, diseases and sometimes competition from ground floor plants adversely affect tree establishment and ongoing tree health. In addition, pests cause direct damage to truffles as well as inducing rots.

Prior to this study, management of pests and diseases has been undertaken largely on an *ad hoc* basis with little input from specialists. Correct identification of the actual agents involved and therefore how best to manage them has been lacking. This has been exacerbated with many growers not having an agriculture background.

This project sought to engage with growers through their membership of Associations, both national and regional. This 'mapping' activity was successfully conducted in Western Australia and through the project expanded the grower list for eastern Australia.

Such a contactable grower base provides both government and industry organisations with the potential to determine the nature and significance of pests and diseases as well as being able to involve growers directly with a rapid response to exotic threats.

Thus this project had the capacity to contribute to a more cohesive national industry and at the same time directly contribute to lifting productivity and economic growth through more efficient management of pests, diseases and weeds.

Aims/objectives

This project sought to develop a database of growers and their truffle orchards in Australia. To document and identify the range of pests, diseases and weeds that occur in Australian truffle orchards a database was compiled through a questionnaire to growers and from subsequent field activities. Furthermore, an information package was compiled for growers on best management practice to minimise the impact of these pests and diseases.

Information on endemic pests and diseases identified in this project would be cross-referenced with overseas information from an ongoing literature review and contact with overseas colleagues. This information will be available to Plant Health Australia who have produced a biosecurity manual for the truffle industry.

Clarification of pests that occur in Australian truffle orchards would be applicable in quarantine aspects of Australia’s involvement in the international trading of truffles.

A further objective of this project was to minimise adverse effects of pests and diseases on tree and truffle health, specifically factors that may reduce market value of truffles. By enhancing the production of quality truffles, Australian truffle growers themselves as well as companies and individuals that are involved in either a direct or indirect way with the industry will benefit.

Methods used

An outline of the key tasks and deliverables was subdivided into the broad categories with associated sub-tasks of:

- Completing a national grower survey to obtain baseline information on e.g. number of growers, area of production, truffle host species grown and observed pest and diseases
- Ensuring Tech Transfer by a number of means to implement a two-way exchange with truffle growers
- Conducting field studies to develop monitoring and management techniques for key pests and diseases
- Producing an Australian Truffle Orchard IPDM Manual and an Australian Truffle Orchard Pest Identification Guide
- Completing the Final Report for the project.

Results/key findings

The database of Australian truffle growers was achieved with partial success. Information regarding the truffle industry in Western Australia was very accurate because of the concentration of growers in the south west and a high proportion of growers being members of TPWA. The information regarding the truffle industry in eastern Australia was not as accurate due to the wide area over which truffle growers are located and not all growers are members of ATGA. Databases of information on growers who are members of ATGA and TPWA are held by these associations while information on some but not all non-members is held confidentially by DPIRD.

The data generated on the Australian truffle industry from the national survey was facilitated by an 84% participation rate by growers contacted to take part. The information on results of the survey collated in this report is therefore considered to be a good reflection of the industry.

Through the range of tech transfer modes established for growers, including the establishment of a dedicated reporting area in a MyPestGuide phone App. to receive personal advice on pest and disease identification and articles on the DPIRD web site, an effective dialogue was established. This was enhanced by conducting regular monitoring for one year on nine truffle orchards across Australia as well as annual field visits. A further twenty-three truffle orchards were involved with monitoring demonstrations and management studies. Numerous field days were attended by members of the project team as well as giving presentations annually at the national ATGA Conference and Manjimup Truffle Kerfuffle.

The information on pests and diseases gathered during the project was collated into two separate publications – “Australian Truffle Orchards Integrated Pest and Disease Management Manual” and “Australian Truffle Orchards Pests and Diseases Identification Guide”. The ‘Manual’ will be available on the DPIRD web site and revised periodically. The ‘Guide’ will be printed in hard copy by DPIRD for distribution to relevant parties. Updates will be reprinted as required. While the original objectives of the project included weeds as a pest agent in truffle orchards, it was quickly realised that to document the range and prevalence of weeds and investigate their management was beyond the scope of the resources of this project.

At the outset, the project sought to conduct investigations to manage what were considered to be major pests - slugs, snails and collembolans (springtails). Of these, only slugs were studied. In the early stages of the project snails and springtails were assessed as being of minor importance, especially in comparison to more important agents discovered as the project proceeded. However towards the end of the project, the view that springtails were minor pests was upgraded to be now considered worthy of further investigation. New agents identified during this project which have an adverse effect on truffle host trees and truffles include slaters, an obligate truffle feeding beetle, millipedes and some fungal tree diseases.

Investigations on the management of key pests and diseases concentrated on developing identification kits and monitoring methods for what are considered to be the key truffle pests: slugs, slaters and an obligate truffle-feeding beetle – ‘Australian truffle beetle’. Field studies confirmed the pest status of what are considered to be the major diseases of truffle host trees.

Information on endemic pests and diseases identified in this project can be cross-referenced with overseas information and the Plant Health Australia Biosecurity Manual for the truffle industry.

The main outputs for this project are the two publications mentioned above. These have the objectives of giving growers information and recommendations regarding pest identification, monitoring and some recommendations for management so they have the guidelines to implement them to produce quality truffles. Pest management options are dynamic depending on the pests present as well as the ways they may be managed. The project sought to draw on experience from other industries and initiate new management techniques that will no doubt be expanded upon and even changed as others conduct further studies. As growers implement and refine suggested management plans, they will become more familiar with current pests on their own farms. Furthermore, this research will increase awareness among growers of pest and disease problems in truffle orchards, thereby assisting with timely detection of newly evolved diseases or pest/disease incursions. The sooner new pests/diseases are discovered, the more likely eradication programs will be successful.

Implications for relevant stakeholders

To protect trees and truffles from pests and diseases, Australian truffle growers now have the basis for a tool kit to be built upon to monitor and manage pests and diseases. The reputation of the Australian truffle supply both at home and in overseas markets will be maintained if not enhanced through management to have healthy truffle tree hosts and implement in-orchard monitoring to reduce the level of damage to truffles. By becoming more familiar with pest agents in their orchards, there is a greater chance that growers will be able to detect exotic agents should they incur.

By increasing the viability and continuation of the industry through production of quality truffles, growers, marketers and consumers of truffles will all benefit. Financial reward from all phases of the industry will be maintained. Even the general public will benefit as an add-on component, from the increase in truffle production and associated increase in affordability of truffle products and associated agritourism / economic growth in the regions where truffles are grown.

The project sought to provide management guidelines for current pests and diseases of Australian truffle orchards. It is hoped that the communication established between project team members and truffle growers will ensure that growers will access and implement management guidelines, the main outputs from the project. Committees of both major truffle grower organisations ATGA and TPWA have been kept informed of project activities and therefore are well placed to act as conduits for such information for any new or existing growers not aware of the project outputs and outcomes.

For other pest agents, some management solutions were identified but more investigations are required. These are highlighted in the Recommendations section of this report.

The range of pests and disease documented during the project forms the basis for a reasonable level of confidence that important exotic pests and diseases of both host trees and truffles do not occur in Australia. This provides background information that supports the fact that the industry is a signatory to an EPPRD deed.

Investigations carried out in this project have some relevance for other industries where the same pest agents are important. This is particularly the case for broadacre agriculture in Australia where liaison with researchers would benefit both industries; for example, slugs, slaters and to a lesser extent, millipedes are of increasing concern as establishment pests in broadacre agriculture as that sector has moved towards adoption of minimum tillage techniques.

With the clarification of pests and diseases important to the truffle industry, companies supplying inputs for management either as advice or goods, will be better placed to help growers achieve improvements in crop management and crop protection.

While there are some differences in the suite of pest agents that occur in truffle industries in other countries, there will undoubtedly be some relevance for techniques to be tested in Australia. Now that the Australian industry has made some contribution to the better understanding of pest agents and their management, researchers and growers in other countries may have more interest in sharing ideas with their Australian counterparts to improve pest management to the advantage of all.

Recommendations

Australian truffle growers should be made aware of and be encouraged to adopt an integrated pest and disease management strategy as described in the Australian Truffle Orchard IPDM Manual which is complemented with the Australian Truffle Orchard Pest Identification Guide.

Australian truffle growers should be encouraged to maintain liaison with associations they belong to and other reliable sources of information on truffle pests and diseases, in order to keep the channels of communication open. Truffle growers should pro-actively seek out new and updated information on pest and disease agents and management.

Detailed research is required on:

- The biology and management of slugs; including cultural and biological control
- The biology and management of slaters; including cultural and biological control
- The biology and management of springtails; including cultural and biological control
- The biology, taxonomy and management of Australian truffle beetle (ATB); including cultural and biological control as well as research on lifecycle, current pest status in orchards distribution and risk analysis of further impact on industry.

The project team suggests that this research on ATB is best undertaken initially through a post graduate study because:

- ATB is a potentially very significant pest for the Australian truffle industry
- To date only one orchard is affected
- ATB and truffles is a most unusual crop/pest association requiring individual and detailed attention
- The biotic and abiotic causes of truffle rot.

Supplementary investigations to findings in the current project could be conducted on:

- Truffle host tree health and the impact of this on pests, diseases and truffle production
- The management of orchard establishment pests, in particular African black beetle and apple weevil
- The biology and management of millipedes; including cultural and biological control
- Management of dieback
- Clarification of the species of “powdery mildew” found in oaks and management strategies
- Investigate introducing filbert (hazelnut) blight resistant varieties to truffle orchards given the very likely event of this disease being introduced to Australia – this includes identifying suitable, cost effective varieties to import into Australia and any modifications to inoculation required
- Work with nurseries to develop seed selection protocols for the use of disease resistant seed genotypes, for example powdery mildew resistance in *Quercus robur*.

Introduction

The truffle industry is relatively new to Australia with the first truffle produced only 20 years ago. Production in 2014 was expected to be 8 tonnes with at least 85% of truffles exported. Statistics on the number of growers and the area under production in Australia are difficult to obtain, though estimates put the number of growers at approximately 250 and the area at 450ha. Commercial orchard size ranges from 200 trees to tens of thousands of trees. Over the next 10 years, truffle production is set to increase dramatically as existing plantings enter the productive phase. The area under truffle trees is increasing also with new orchards being planted and some expansion of existing orchards.

Invertebrate pests and diseases of truffles and truffle host trees cause significant losses to the industry across Australia. Tree establishment and ongoing vigour have been adversely affected by insect pests, and occasionally by diseases and competition with orchard floor plants. Pests cause direct damage to truffles resulting in lower yield as well as inducing truffle rot. These adverse effects represent an important management challenge to Australian growers relevant to both the establishment and ongoing maintenance of host trees as well as truffle production.

Prior to this study, management of pests and diseases has been undertaken largely on an *ad hoc* basis with little input from specialists. Correct identification of the actual agents involved and therefore how best to manage them has been lacking. This has been exacerbated with many growers not having an agriculture background.

This project included, at its core, the establishment of a database of growers' contact details across Australia. This 'mapping' activity has been successfully conducted in Western Australia, and it provides both government and industry organisations with the potential to determine the nature and significance of pests and diseases as well as being able to involve growers directly with a rapid response to incursions of exotic agents that threaten the Australian truffle industry.

The process of mapping the truffle orchards of eastern Australia has been a challenge in the past, but where this project deviates from previous efforts was that the mapping was in part an information disseminating activity where all sectors of the industry were engaged and had the opportunity to benefit. Through this process of mutual reward, growers who may not normally have been wanting to be involved with external communication, have collaborated in project activities and are therefore more likely to report suspected exotic incursions of pests and diseases.

Thus this project had the capacity to contribute to a more cohesive national industry and at the same time directly contribute to lifting productivity and economic growth through more efficient management of pests, diseases and weeds.

Objectives

Compiling truffle grower contacts and questionnaire survey

This project sought to develop a database of growers and their truffle orchards in Australia. To document and identify the range of pests, diseases and weeds that occur in Australian truffle orchards a database was compiled through a questionnaire to growers and from subsequent field activities. Furthermore, an information package was compiled for growers on best management practice to minimise the impact of these pests and diseases. This was undertaken in several inter-related steps.

A questionnaire survey of truffle growers with an emphasis on conducting this by phone was undertaken, first in WA and then in the eastern states to determine the range of pest and disease issues already identified by growers and to clarify their current pest management practises. The association of Truffle Producers of Western Australia (TPWA) already had a significant database of growers. A mapping project was undertaken in eastern Australia in liaison with the Australian Truffle Growers' Association (ATGA) to generate a database of growers before commencing the phone survey in eastern Australia.

Two-way contact with truffle growers

As well as the phone surveys, the project was to establish a conduit for growers to keep up to date with findings and provide information and questions to the project team throughout the life of the project. This included the facility for growers to have existing and potential new pests identified. Points of contact were identified and communicated to growers nationally. This conduit also provided the project team and industry with the potential for an early warning of significant new pest issues, assisting in the dissemination of urgent information to growers.

Two-way contact was achieved by setting up a dedicated area within the DPIRD developed MyPestGuide mobile phone App. As well as posting articles and e-newsletter on project activities on the DPIRD web site, project team members presented results and were available for one-on-one discussion at industry conferences and field walks and acted upon truffle growers' direct enquiries by phone or email.

Key pest and disease target areas

In order to obtain detailed information on the identification, seasonality and determinants of abundance of some of the more important pests in the truffle industry, focussed truffle orchard monitoring was planned to be undertaken in nine commercial orchards in both eastern Australia and Western Australia for one year.

From previous studies and experience in WA, slugs, snails and collembolans have been found to be important in adversely affecting truffle yield and quality. Their biology and management were investigated.

Through more intensive monitoring of some truffle orchards and conducting trials on the management of key pests, more detailed information on major pests was obtained. As new information was gathered, it was included as updates to information already available for growers.

Information on endemic pests and diseases identified in this project were cross-referenced with overseas information from an ongoing literature review and contact with overseas colleagues. This information was made available to Plant Health Australia who have produced a biosecurity manual for the truffle industry.

Methodology

At the commencement of the project, an outline of the key tasks and deliverables and the time each would be achieved was mapped and disseminated to project team members. This was subdivided into the broad categories with associated sub-tasks of:

- National grower questionnaire survey report
    - Western Australia
    - Eastern Australia
    - Combination of results
- Tech Transfer
    - Gathering information on identification, monitoring guidelines and management of pests and diseases of truffles and their host trees both from review of current information and new information generated as a result of activities of the project
    - Transferring this information via the DPIRD web site, six-monthly e-newsletters on project activities, talks to growers and publications both hard copy and electronic
- Pest management
    - Establishing a pest enquiry line for truffle growers
    - Undertaking field site studies based on results of the grower survey, developing monitoring protocols and procedures, selecting truffle orchard monitoring sites and undertaking the monitoring for one year at each site – seven in WA and two in the eastern states
    - Conducting field trials after developing protocols, reviewing the field study sites information, selecting sites and running trials
- Producing an "Australian Truffle Orchards Integrated Pest and Disease Management Manual" and "Australian Truffle Orchards Pests and Diseases Identification Guide". Australian Truffle Orchard IPDM Manual and an Australian Truffle Orchard Pest Identification Guide
- Complete the Final Report for the project.

More detail on methodology for each of these areas is included in the following chapters.

Chapter 1: Identifying the pests and diseases

Introduction

In Europe, reference to truffles dates back to at least the seventeenth century where many important truffle pests were identified (eg recent publications: Martin-Santafe et al, 2014; Morcillo et al, 2015). Pests and diseases of both the host trees and truffles are included in those reports, but most emphasis were placed on invertebrate pests of truffles with the European truffle beetle and truffle fly receiving the most attention (Arzone, 1971; García-Montero et al, 2004; Hochberg, et al, 2003; Pacioni, 1919).

Information on the pests and diseases of truffle host trees and truffles in Australia was largely available from the investigations by Snare (2006, 2010) on pests and diseases in hazelnut orchards for nut production, research by Eslick (2012, 2013) on truffle rots and surveys in truffle orchards by Seago (2014). Studies on related and specific pests of truffle host trees include studies on beetles that feed on subterranean fungal fruiting bodies (Houston and Bougher, 2010; Houston, 2011), oak leaf miner (Common, 1976; New, 1981; Bashford and Elliot, 1989) and reports on the occurrence of a new mite of hazelnuts (Ozman, 2000) in Australia (Anon., 2016).

Objectives

With few detailed studies specific to the pest and disease situation on truffles and their host trees for Australia, this project sought to clarify the agents involved and their importance.

This information formed the basis of studies on the subsequent activities involved with reducing their impact trough investigations on their management.

Methodology

Australian truffle growers were requested to share their experiences with pests and diseases of truffles and their host trees through a national questionnaire survey. More detailed information on the identity and importance of pests and diseases was obtained by combinations of on-going interaction with growers, undertaking regular monitoring for one year in truffle orchards across the country and surveys of diseases in truffle orchards where growers had identified problems with tree health.

References

Anon. 2016. Hazelnut mite. New South Wales Department of Primary Industries. *Primefact* 1453.

Arzone, A. 1971. Reperti ecologici ed etologici di Liodes cinnamomea Panzer vivente su *Tuber melanosporum Vittadini* (Coleoptera Staphylinoidea). (Ecological and ethological findings of *Liodes cinnamomea* Panzer living on *Tuber melanosporum* Vittadini (Coleoptera Staphylinoidea)). Centro di Entomologia Alpina e Forestale del Consiglio Nazionale delle Ricerche, Italy. **158**: 317-357.

Bashford, R., Elliott, H.J. 1989. Studies of the oak leafminer *Phyllonorycter messaniella* (Zeller) (Lepidoptera: Gracillariidae) in southern Tasmania. *Papers and Proceedings of the Royal Society of Tasmania.* **123**: 223-8.

Common, I. F. B. 1976. The oak leaf-miner, *Phyllonorycter messaniella* (Lepidoptera: Gracillariidae) established in Australia. *J. Aust. ent.* Soc. **15**: 471-473.
Eslick, H. 2012. Identifying the Cause of Rot in Black Truffles and Management Control Options. RIRDC Publication No. 12/005.

Eslick, H. 2013. Identification and management of the agent causing rot in black truffles - Part 2 Identifying the Cause of Rot in Black Truffles and Management Control Options. RIRDC Publication No. 12/005.
Houston, T.F., Bougher, N.L. 2010. Records of hypogeous mycorrhizal fungi in the diet of some Western Australian bolboceratine beetles (Coleoptera: Geotrupidae, Bolboceratinae) *Australian Journal of Entomology.* **49**: 49–55.
Houston, T. F. 2011. Egg gigantism in some Australian earth-borer beetles (Coleoptera: Geotrupidae: Bolboceratinae) and its apparent association with reduction or elimination of larval feeding. *Australian Journal of Entomology*. **50**: 164–173.
New, T.R. 1981. The 0ak leaf-miner, *Phyllonorycter messaniella* (Zeller) (Lepidoptera: Gracillariidae) in Melbourne. *Aust. J. Zool.* **29**: 895-905.
Ozman, S.K. 2000. Some biological and morphological differences between gall and vagrant forms of *Phytoptus avellanae* nal. (Acari: Phytoptidae). *Internat. J. Acarol*. **26** (3): 215-9.
García-Montero, L.G, Pérez Andueza, G., Díaz P., Manjón J.L. 2004. Contribución al conociemiento ecológico de los dípteros fitógafos (Diptera: Heleomyzidae) de las trufas en España. (Contribution to the ecological knowledge of the phytophagous diptera (Diptera: Heleomyzidae) of truffles in Spain). *Bol San Veg Plagas*. **30**: 679-683.
Hochberg ME, Bertault G, Poitrineau K, Janssen A, 2003. Olfatory orientation of the truffle beetle, *Leiodes cinnamomea*. *Entomologia Experimentalis et Applicata* **109**: 147-153.
Houston, T.F. & Bougher, N.L. 2010 Records of hypogeous mycorrhizal fungi in the diet of some Western Australian bolboceratine beetles (Coleoptera: Geotrupidae, Bolboceratinae). *Australian Journal of Entomology.* **49**: 49–55.
Houston, T.F. 2011. Egg gigantism in some Australian earth-borer beetles (Coleoptera: Geotrupidae: Bolboceratinae) and its apparent association with reduction or elimination of larval feeding. *Australian Journal of Entomology***. 50**: 164–173.
Kirejtshuk, A.G., Lawrence, J. F. 1992. Review of the *Thalycrodes* complex of genera (Coleoptera: Nitidulidae) endemic to the Australian region. *J. Aust. ent. Soc.* **31:** 119-142**.**
Martin-Santafe, M., Perez-Fortea, V, Pedro Zuriaga, P., Barriuso, J. 2014. Phytosanitary problems detected in truffle cultivation in Spain. A review. *Forest Systems*. **23** (2): 307-316.
Morcillo, M., Sanchez, M., Vilanova, X. 2015. Truffle Farming Today, a Comprehensive World Guide. Publisher: Micologia Forestal & Aplicada. ISBN 978-84-617-1307-3.
New, T.R. 1981. The Oak Leaf-Miner, *Phyllonorycter messaniella* (Zeller) (Lepidoptera: Gracillariidae), in Melbourne. *Aust. J. Zool.* **29,** 895-905.
Pacioni G, 1991. Effects of *Tuber* metabolites on the rhizospheric environment. Mycol Res. **95**: 1355-1358.
Seago, A. 2014. Australian truffle beetles and the insect fauna of truffieres. PowerPoint presentation.
Sebahat, K.O. 2000. Some biological and morphological differences between gall and vagrant forms of *Phytoptus avellanae* Nal. (Acari: Phytoptidae). *International Journal of Acarology*. **26**:3, 215-219.
Snare L., 2006. Pest and Disease Analysis in Hazelnuts. Project NT05002, NSW Department of Primary Industries. In. (HA Ltd, ed.).
Snare L. 2010. Hazelnut grower’s handbook. NSW Department of Primary Industries. 79pp.

1.1 Australian truffle growers' questionnaire survey

The report on the national truffle grower survey questionnaire has been produced as a separate document to this final report: *The Australian Truffle Industry – a snapshot*. A copy has been attached to the final report submitted to AgriFutures Australia. A summary of that report is presented below.

Summary

The report is about the current state of the Australian truffle industry. As part of the project to identify and manage the important pests and diseases in the truffle industry, a survey was undertaken to determine grower experiences with threats and impediments to production with regard to the impacts of pests and diseases of truffle host trees and/or truffles. This report presents the findings of that survey and a review of the industry size and production.

The report is targeted at truffle growers, consultants and other industry groups involved in the production and marketing of Australian truffles.

Aims and objectives

This research sought to understand the current nature of the truffle industry and to begin to document and identify the threats and impediments to quality production. These results then guided further research within the project on pests and diseases.

Methods used

A survey of truffle growers was conducted by phone and email in 2015-2016 with some emphasis on the 2015 truffle season. The survey asked growers about their experiences with pests and diseases, as well as orchard attributes and their management program. In addition, industry consultation was conducted to develop a current industry profile.

Results/key findings

The survey report presents the state of the current industry based on a survey of growers, on-going industry consultation, and review of reports of industry size and activity in the last five years. Growth of the industry is determined by reference to a previous review of the industry (Lee, 2008).

Forty different pest or disease agents were reported by growers as occurring in Australian truffle orchards. These agents are described along with their environmental determinants and, in some instances, potential control measures. Tree diseases, tree pests and truffle pests are reported as potential production problems. Other organisms are reported as being below the threshold for the requirement for action.

Additional findings are that truffle growers are using a wide range of management practices to improve production. Management of the orchard floor with regard to weeds and leaf litter is highly variable, and it remains to be seen whether these variations influence the quantity and quality of truffles produced. Likewise, irrigation practices vary significantly. In a drying and warming climate, irrigation and the availability of irrigation water will be become an important factor in the success of truffle production. Other variations identified include planting density, use of fertilisers, and canopy management.

This research has enabled growers to see the range of situations and practices encountered by other growers and make more informed decisions regarding the management of pests and diseases as well as gaining an insight into methods used to reduce the presence of, and minimise susceptibility to, pests and diseases. This research has also benefitted the industry by providing initial grower observations of pests and diseases to plant disease specialists to conduct more in-depth assessment.

Implications for relevant stakeholders

Truffle growers are becoming aware of the potential of pests to cause economic loss. This study is the first step in assisting growers with their management of pests and diseases in accordance with the principles of integrated pest and disease management (IPDM). Where available, management options are discussed, and more information has been available following further research associated with this project.

Truffles are a very high value food crop that have captured the imagination of food lovers. Significantly, truffles also provide a ‘hook’ for the agrifood tourism industry.

An estimate of the size of the industry suggests the industry in Australia will grow significantly in the coming decade with implications for tourism, export stakeholders and biosecurity.

Recommendations

Truffle growers are encouraged to consider the findings of this report and become aware of the potential pests and diseases known to occur in their region, and to utilise the associated Australian Truffle Orchard Pest Identification Guide and the Australian Truffle Orchard IPDM Manual which are outputs of this project.

Reference

Lee, B. 2008. Taking stock of the Australian truffle industry. Rural Industries Research and Development Corporation (Australia) Project no. PRJ-002643. **ISBN** 1741517133. 47 pp.

1.2 Regular invertebrate pest monitoring in truffle orchards

Introduction

Few detailed studies to identify the invertebrates that inhabit Australian truffle orchards have been undertaken. Seago (2014) conducted surveys in eastern Australian truffle orchards but did not relate the occurrence of invertebrates to damage to host trees or truffles. More information on the pest status of invertebrates was reported by Snare (2006). In his research, Eslick (2012, 2013) provided detail on the importance of slugs and an indication that springtails might be damaging truffles. Apart from these studies, orchardists have relied upon general information available from other areas of horticulture for identification of any pest agents and where required, how to protect trees and truffles from damage.

The Australia wide questionnaire survey of truffle orchardists conducted in this project (see Chapter 1.1), had a component on the identity and severity of invertebrates. The responses required confirmation on the ground and complementary investigations to identify other pests, and beneficial and neutral agents present.

Objectives

With limited information available on the range of invertebrates that occur in Australian truffle orchards, a monitoring program was planned.

Clarification of the range of invertebrates present and an indication of their pest status was required in order to prioritise which pests would be the subject of more in-depth study with an emphasis on developing targeted monitoring techniques and management methods. Information on the more abundant and important agents of both trees and truffles would be collated into an Integrated Pests and Diseases Management Manual and Identification Guide for reference by growers to help identify, monitor and manage pests of both truffles and their host trees.

Methodology

Fortnightly monitoring with ground traps

Monitoring was undertaken at roughly fortnightly intervals over a year in nine orchards across Australia – two in eastern mainland Australia and seven in Western Australia. This number in the two regions was selected on the basis of the maturity of the industries in each region and their relative contribution to the national harvest of truffles. Logistic constraints of personnel and the wide geographic extent of truffle orchards across Australia including those in Tasmania, restricted the location of sites that could be included.

Details of the orchards selected for regular monitoring including the nearest town are given in Table 1.2.1.

An emphasis was placed on monitoring potential pests of truffles. Considering that slugs and springtails had been identified as important pests, the monitoring methods were selected accordingly. To get a representative sample of invertebrates occurring in each orchard, ten paired traps of two types were used - a pitfall trap (see Fig. 1.2.1) together with initially a concrete roof tile which was replaced soon after the monitoring commenced with a 20 cm square ceramic ‘bathroom tile’. These were placed at two series of five locations within each orchard. Each series was placed along two adjacent rows, with traps alternating between each row in a zig-zag fashion. The monitored rows were around one-third and two-thirds of the way across the orchard block.

Table 1.2.1 Details of Australian truffle orchards selected for regular monitoring to clarify the identity and abundance of invertebrates present

Site	Planting Year	Producing truffles?	Soil type	Truffle trees (%)
NSW1	2008	YES	Shale/Loam	Hazelnut/oak = 75/25
NSW2	2002	YES	Basalt/granite/sand	Hazelnut/oak = 75/25
WA1	2000	YES	Sandy/clay	Hazelnut/oak = 66/34
WA2	2010	NO	Sand to loam	Hazelnut/oak = 50/50
WA3	2009	NO	Ironstone/gravel & peaty sand	Oak = 100
WA4	2009	NO	Loam & gravel	Hazelnut/oak = 1/99
WA5	1997	YES	Sandy loam	Hazelnut/oak = 95/5
WA6	1999	YES	Peaty sand	Hazelnut/oak = 80/20
WA7	2013	YES	Loam	Hazelnut/oak = 90/10

Fig. 1.2.1. Pitfall traps used to monitor invertebrates in truffle orchards consisted of a pipe sunk into the soil along the tree row to a depth of about 20 cm in which a 10cm diameter funnel attached to a 300 mL screw cap plastic container with a gauze base for water drainage. The trap was protected from falling leaves, and acorns and hazelnuts with a lattice 'wall' around the top of the trap and corflute roof.

In some orchards, a piece of truffle was placed in every second pitfall trap of the ten trap series per orchard, to determine if trapping efficiency could be improved.

A score sheet was developed to score invertebrates present and to record any pests or diseases of trees encountered while checking the traps. In addition, an identification sheet of colour photographs of the major invertebrates or groups of invertebrates likely to be encountered was prepared for use by personnel undertaking the monitoring and posted on the DPIRD web site. Truffle orchardists were notified of this identification guide via publication of electronic newsletters.

Any unidentified invertebrates or diseases found during the monitoring were collected and sent to relevant specialists to identify. The occurrence of diseases of both truffles and their host trees that were observed by the project team while conducing monitoring in orchards as well as enquiries from growers, was referred to Dr Celeste Linde for follow up. In some cases, plant samples were dispatched for disease identification or Celeste would visit orchards in the eastern states as well as WA during visits to present talks. Results of these studies are presented in Chapter 1.3.

Invertebrates of host trees from observations in truffle orchards and grower enquiries

In addition to responses from orchardists in the national grower questionnaire and this regular monitoring in nine truffle orchards, enquiries from growers provided another source of information on the identity, distribution, abundance and to some extent the pest status of invertebrates of Australian truffle orchards. The communication methods outlined in Chapter 3 on Tech Transfer provide details of these conduits for growers to communicate with the project team.

Invertebrates from truffle damage assessments

One objective of the project was to quantify the damage to truffles by invertebrates which is described in Chapter 1.4. Many of the agents associated with this damage were the same as those recorded during the pitfall trap and tile monitoring described above. To clarify their importance, the abundance of invertebrates in orchard where damage assessments were undertaken was monitored. In addition to this, invertebrate pests found when harvesting truffles as well as agents detected during the much closer examination of the same truffles in the grading room, were identified.

Results

Fortnightly monitoring with ground traps

A wide range of invertebrates was recorded from the regular monitoring in nine truffle orchards in NSW and WA. All categories of pest and beneficial invertebrates were represented in all orchards where the monitoring was conducted. The most abundant pest and beneficial invertebrates recorded in individual orchards is shown in Table 1.2.2.

Table 1.2.2 For each of the two truffle orchards in NSW and seven in WA monitored for about one year, the most abundant invertebrates are indicated with '✓', but all invertebrates were represented in all orchards. The *footnote under the table explains the abbreviations

		Predatory beetles		Earwigs		Snails					
Site	**ABB**	**Carabs**	**Staphs**	**EE**	**Pred**	**GS**	**SPS**	**Slugs**	**Slaters**	**Millipedes**	**Springtails**
NSW1	-	✓	✓	-	-	-	-	✓	✓	✓	-
NSW2	-	✓	✓	-	-	-	-	✓	✓	✓	-
WA1	-	✓	✓	✓	✓	-	-	✓	✓	✓	✓
WA2	✓	✓	✓	✓	✓	-	✓	✓	✓	-	✓
WA3	✓	✓	✓	✓	✓	-	-	-	✓	✓	✓
WA4	✓	✓	✓	✓	✓	-	-	-	✓	-	✓
WA5	-	✓	✓	✓	✓	-	-	-	✓	-	✓
WA6	-	✓	✓	✓	✓	✓	✓	✓	✓	-	✓
WA7	-	✓	✓	-	-	-	-	-	-	-	✓

*Abbreviations: ABB = African black beetle; Predatory beetles: Carabs – Family Carabidae, Staphs = Family Staphylinidae; Earwigs: EE = European earwig, Pred = predatory earwigs; Snails: GS = garden snail, SPS = small pointed snail.

The data collected highlighted differences in the levels of abundance recorded for these most abundant invertebrates from either tiles or pitfall traps, and whether pitfall traps were more efficient if truffle pieces were placed in the trap. These differences are shown in the following series of graphs before using data collected from the more appropriate monitoring method to indicate differences in seasonal abundance among the truffle orchards. Results for each orchard are labelled as per table 1.2.2. Two blocks were monitored separately in orchard WA5 and results have been labelled 5.1 and 5.2 accordingly in some graphs below.

Difference in invertebrate abundance between tile and pitfall traps

Despite the fact that African black beetles are known to seek shelter under debris or in soil during the day, the number of beetles recorded for pitfall traps was consistently greater than that for tiles (Fig. 1.2.2).

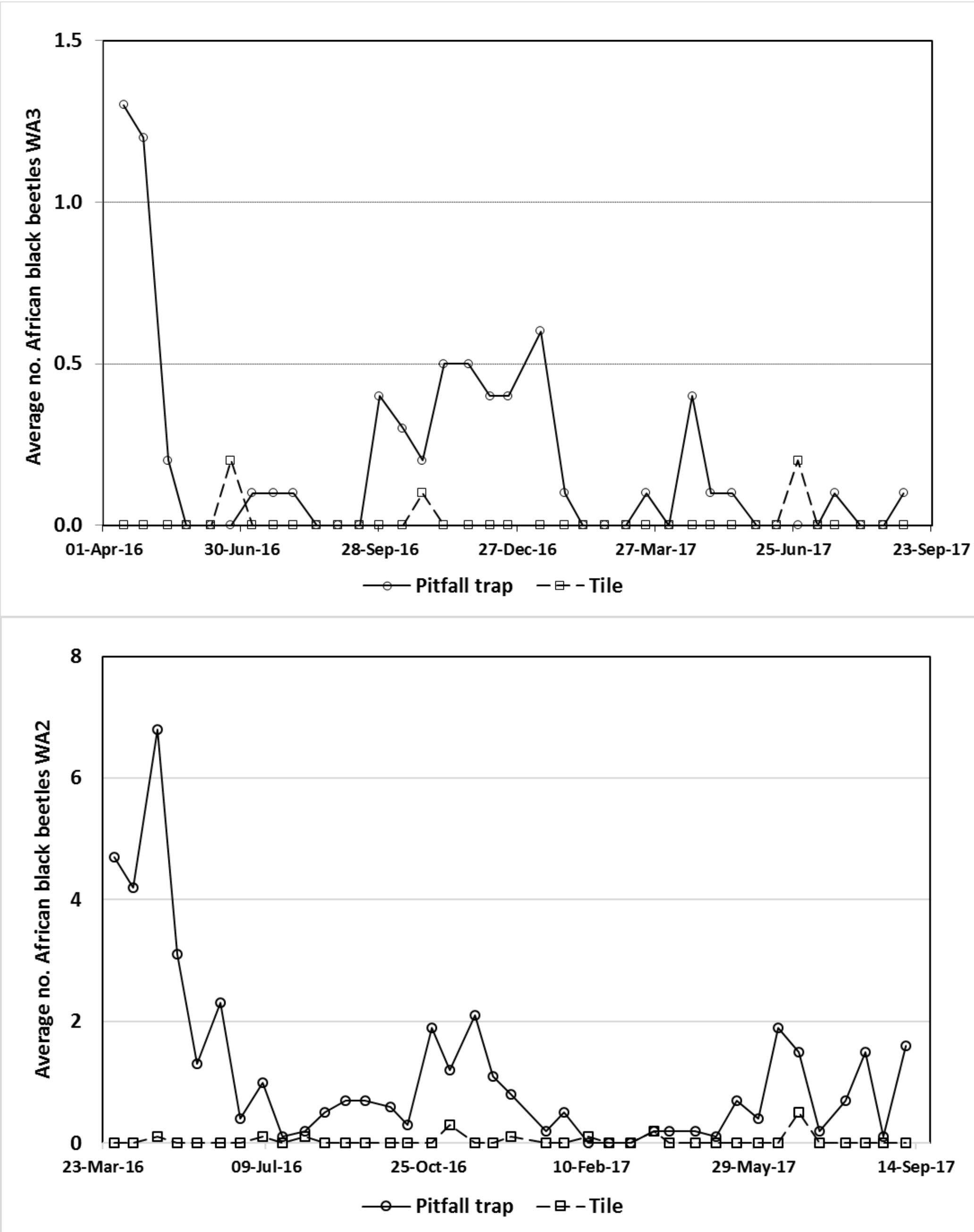
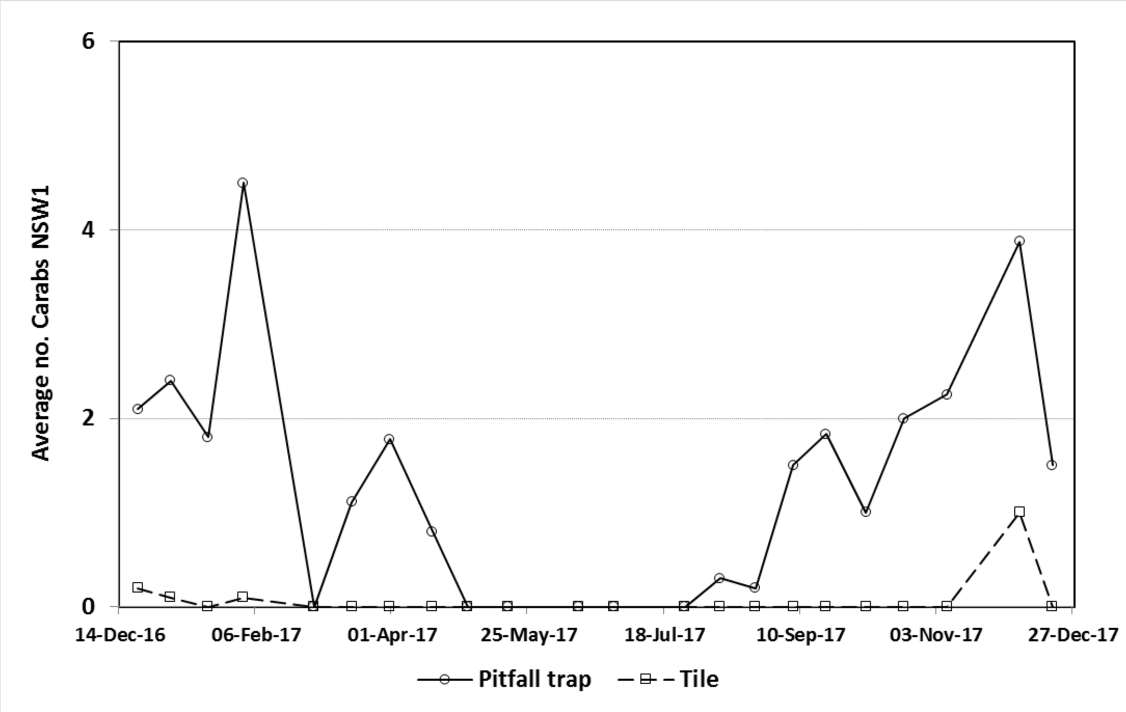


Fig. 1.2.2. Average number of African black beetles in pitfall traps and under tiles in two WA truffle orchards.

With respect to monitoring for the abundance of predatory beetles belonging to the families Carabidae and Staphylinidae, pitfall traps also collected more insects than tiles (Fig. 1.2.3).



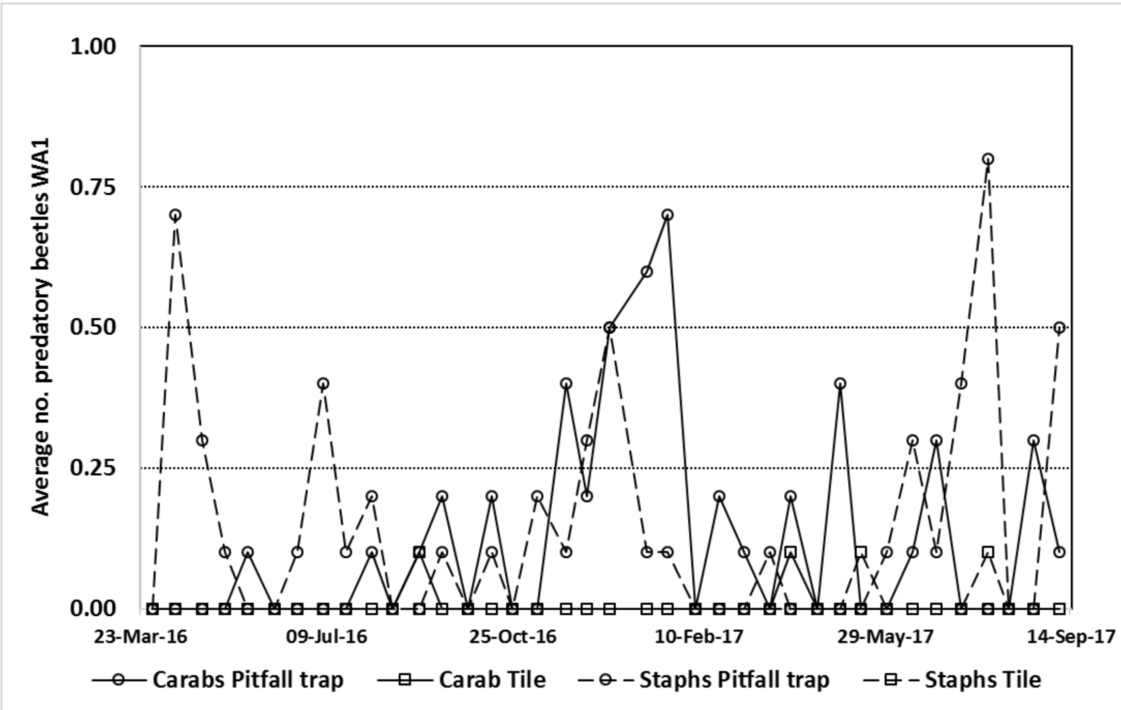
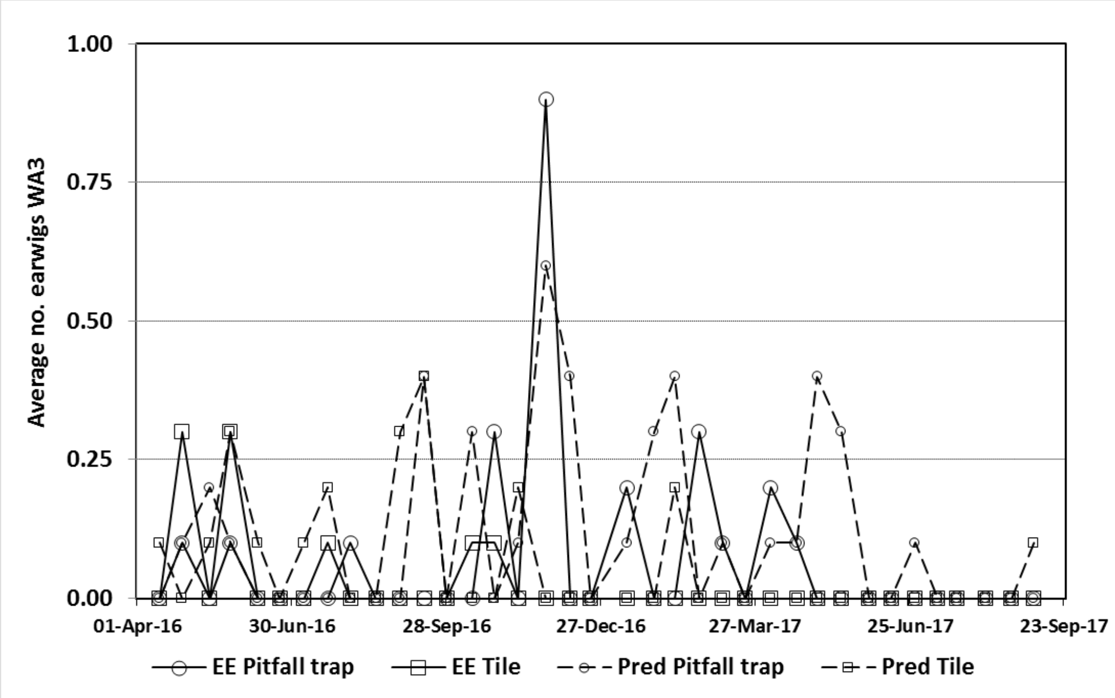


Fig. 1.2.3. Average number of predatory beetles in pitfall traps and under tiles in a NSW and a WA truffle orchard.

With respect to monitoring for the abundances of European earwigs and predatory earwigs, pitfall traps also collected more insects than tiles (Fig. 1.2.4).



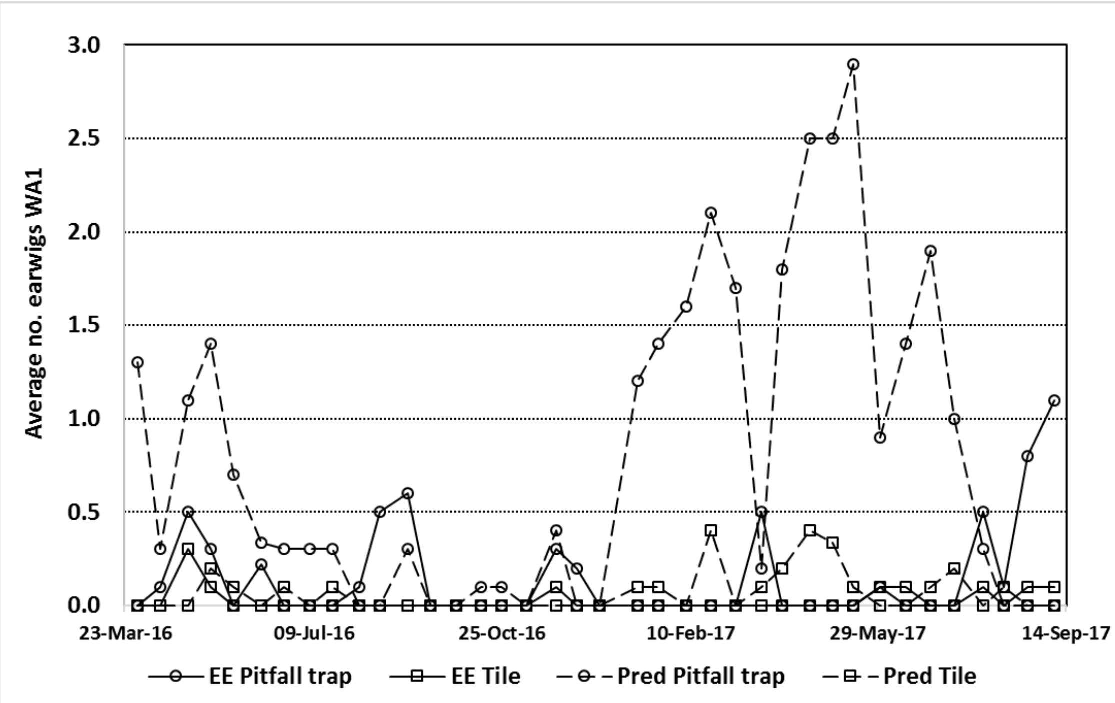


Fig. 1.2.4. Average number of European earwigs and predatory earwigs in pitfall traps and under tiles in two WA truffle orchards.

With respect to monitoring for the abundances of garden snails, pitfall traps collected more than tiles (Fig. 1.2.5).

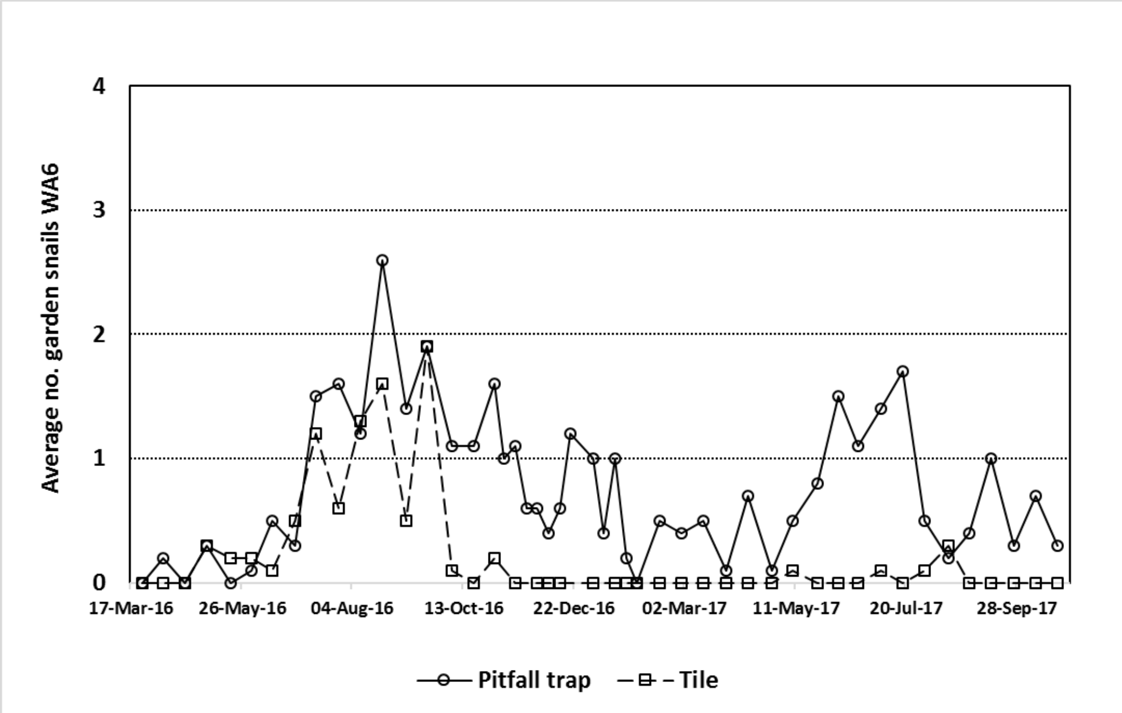


Fig. 1.2.5. Average number of garden snails in pitfall traps and under tiles in a WA truffle orchard.

With respect to monitoring for the abundances of small pointed snails, with the exception of a period of about two months in winter/early spring in the first year of monitoring, more were recorded in pitfall traps than tiles (Fig. 1.2.6).

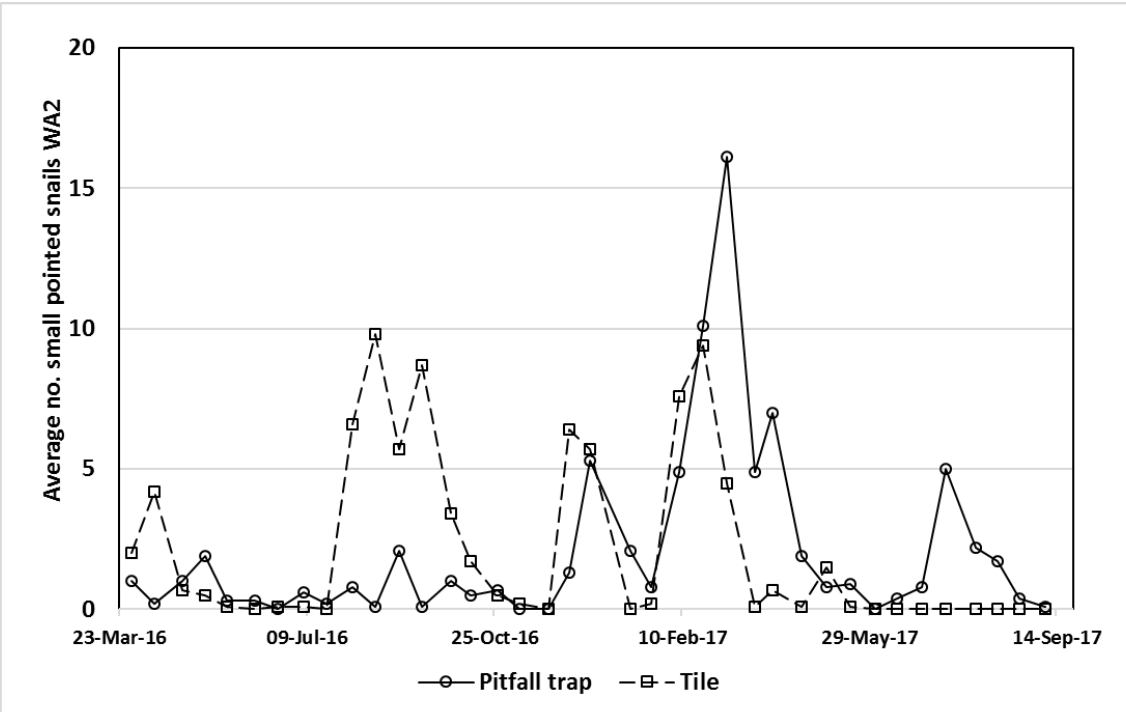


Fig. 1.2.6. Average number of small pointed snails in pitfall traps and under tiles in a WA truffle orchard.

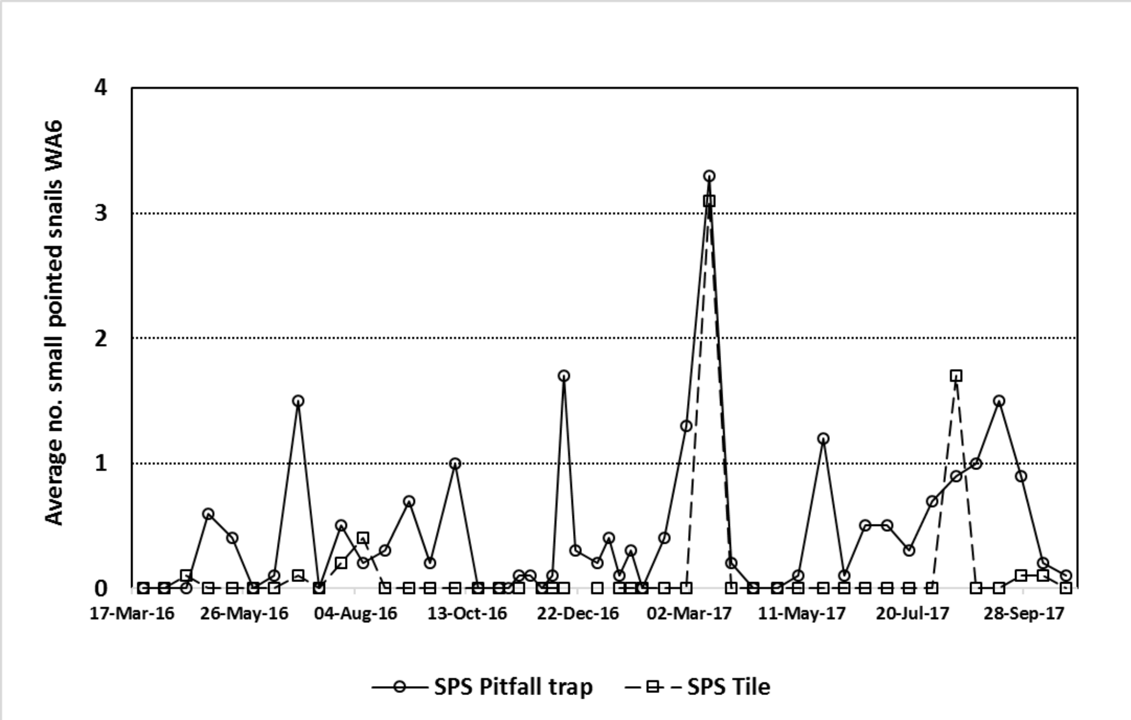


Fig. 1.2.6.(continued) Average number of small pointed snails in pitfall traps and under tiles in a WA truffle orchard.

With respect to monitoring for the abundances of slugs, there was no clear trend as to whether more were recorded for either pitfall traps or tiles (Fig. 1.2.7). It was unexpected that pitfall traps would be seen as a preferred refuge over tiles, but in some orchards, many slugs moved into pitfall traps over the two week monitoring interval.

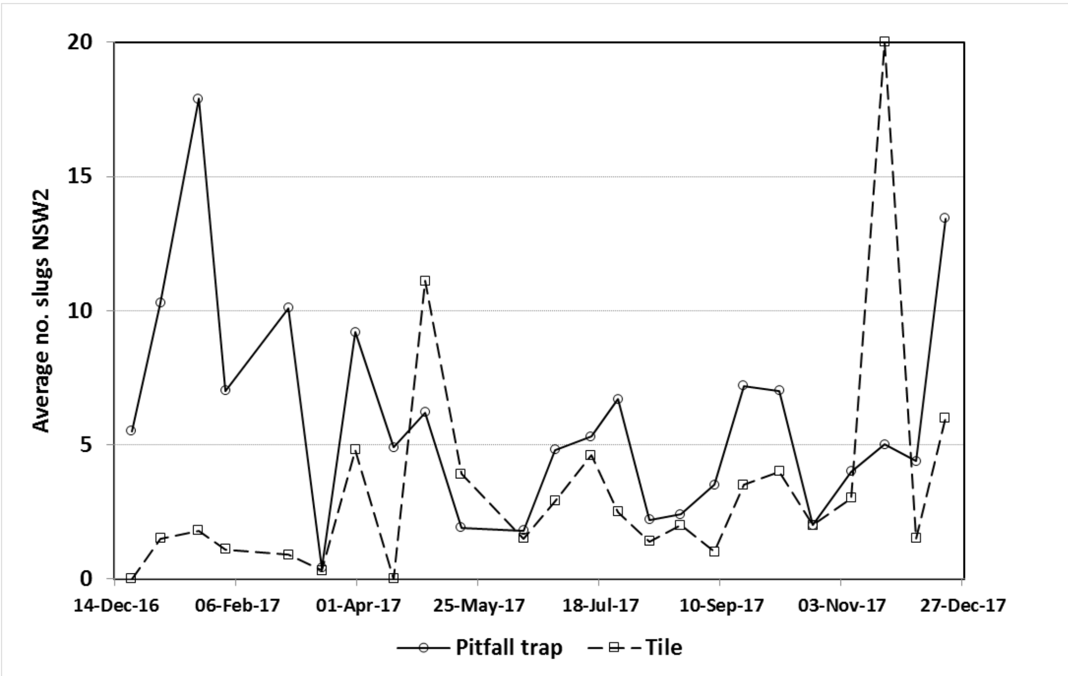


***Fig. 1.2.7*.** Average number of slugs in pitfall traps and under tiles in a NSW and a WA truffle orchard.

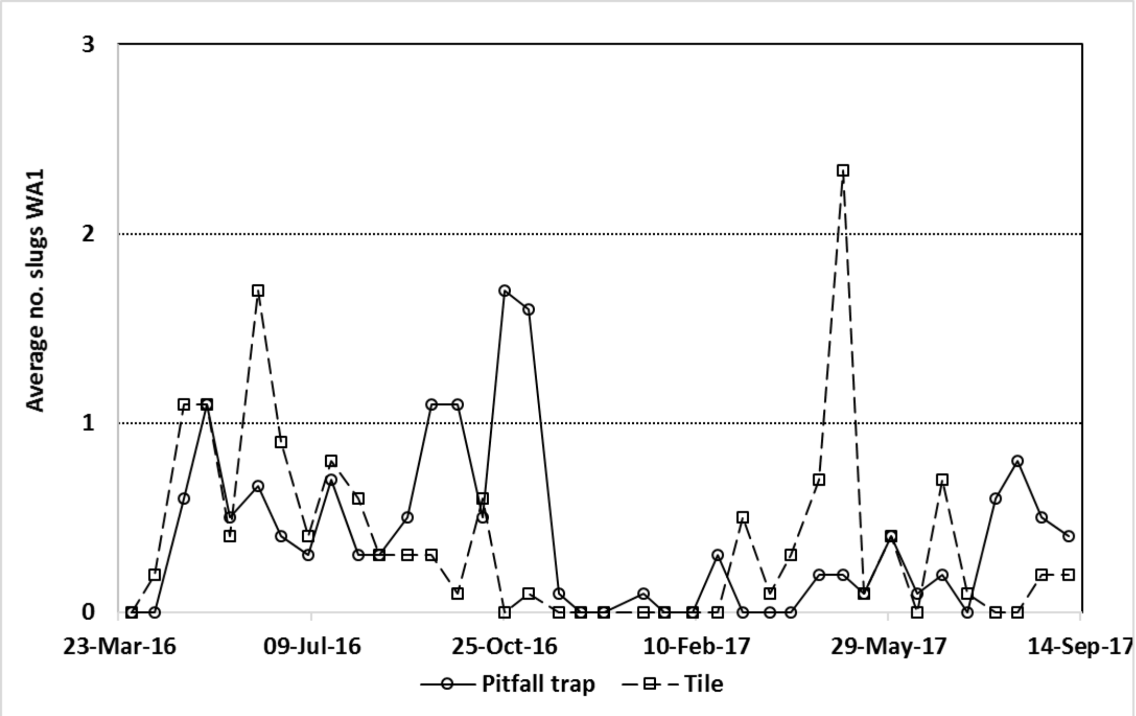
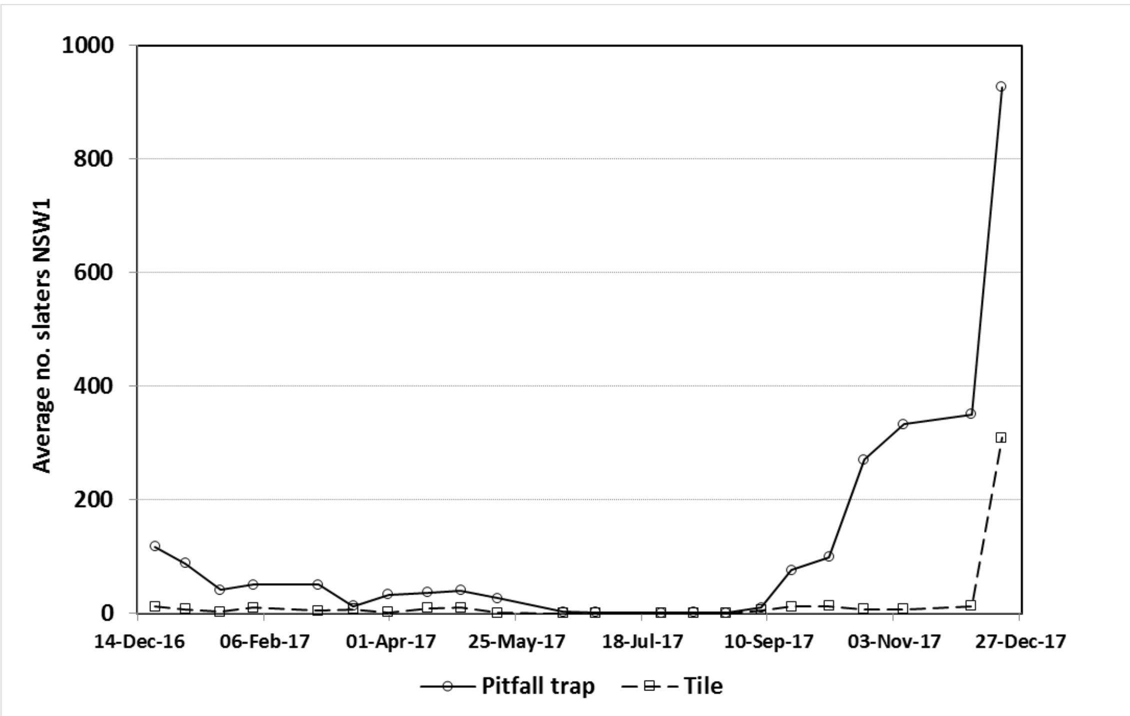


Fig. 1.2.7.(continued) Average number of slugs in pitfall traps and under tiles in a NSW and a WA truffle orchard.

With respect to monitoring for the abundances of slaters, there was a clear trend in the NSW 1 orchard for more slaters to be recorded in pitfall traps (Fig. 1.2.8). For the second site in NSW, for most of the monitoring period tiles and pitfall traps showed similar numbers of



slaters were recorded.

Fig. 1.2.8 Average number of slaters in pitfall traps and under tiles in a NSW and a WA truffle orchard.

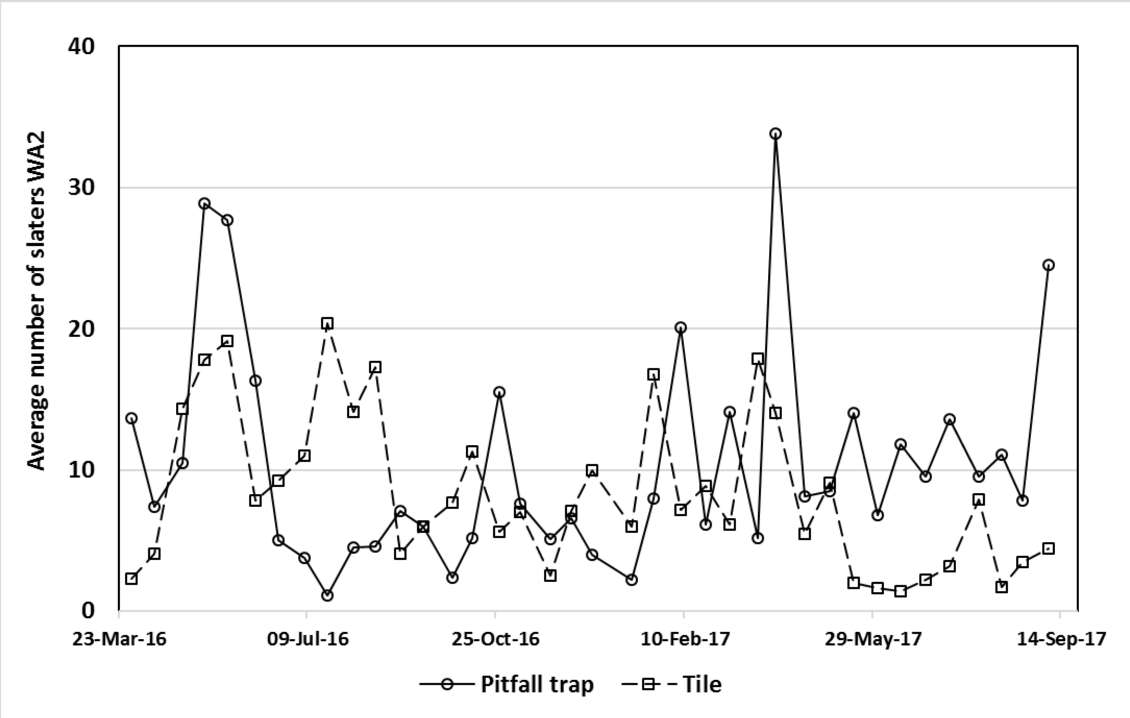


Fig. 1.2.8(continued) Average number of slaters in pitfall traps and under tiles in a NSW and a WA truffle orchard.

With respect to monitoring for the abundances of millipedes, many more were recorded in pitfall traps than for tiles (Fig. 1.2.9). This was unexpected considering a nocturnal invertebrate like millipedes would be inclined to seek shelter under a tile.

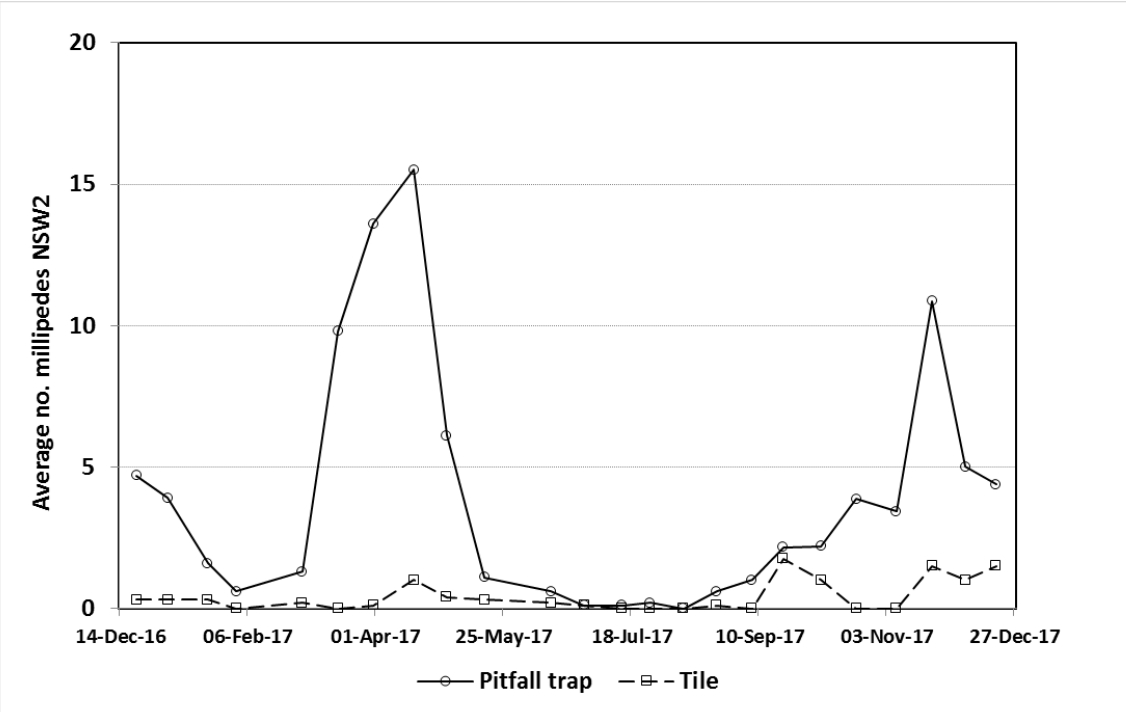


Fig. 1.2.9. Average number of millipedes in pitfall traps and under tiles in a NSW and a WA truffle orchard.

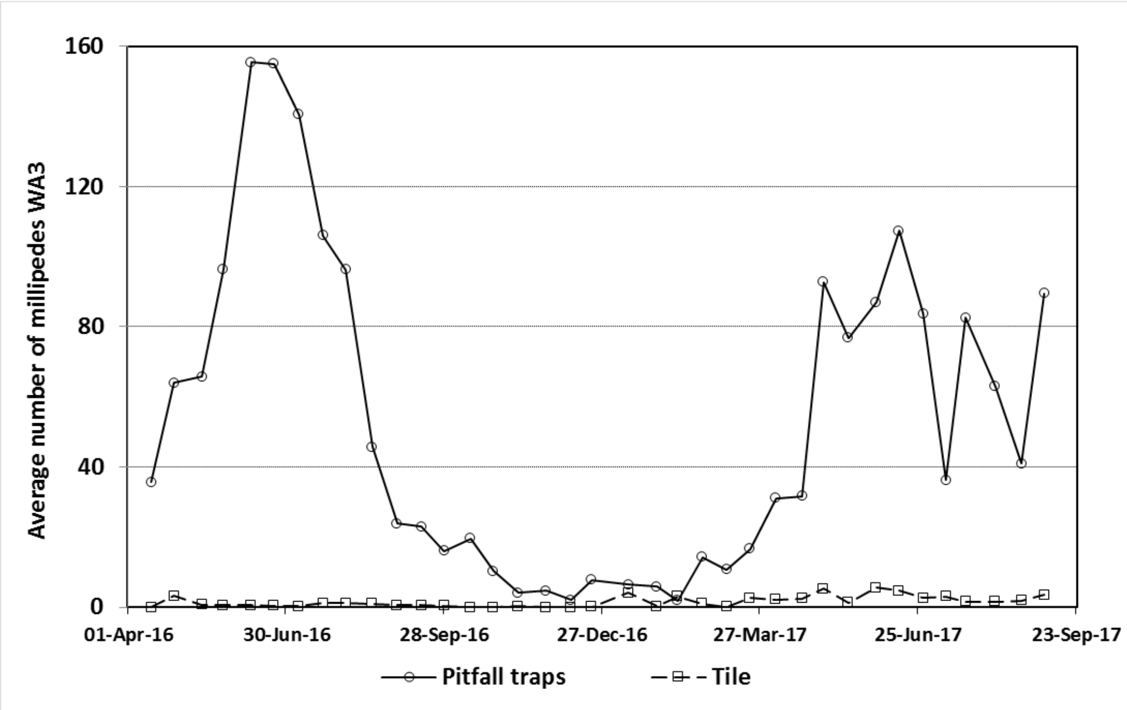


Fig. 1.2.9.(continued.) Average number of millipedes in pitfall traps and under tiles in a NSW and a WA truffle orchard.

With respect to monitoring for the abundances of springtails, more were recorded consistently in pitfall traps than for tiles (Fig. 1.2.10).

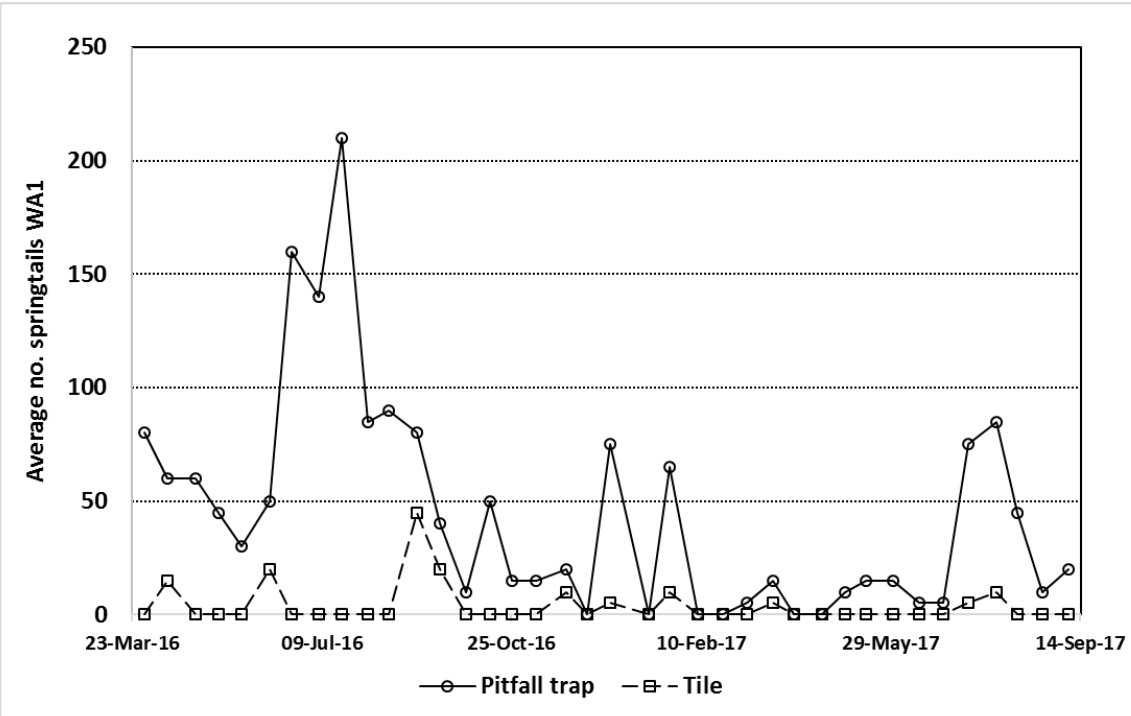


Fig. 1.2.10. Average number of springtails in pitfall traps and under tiles in two WA truffle orchards.

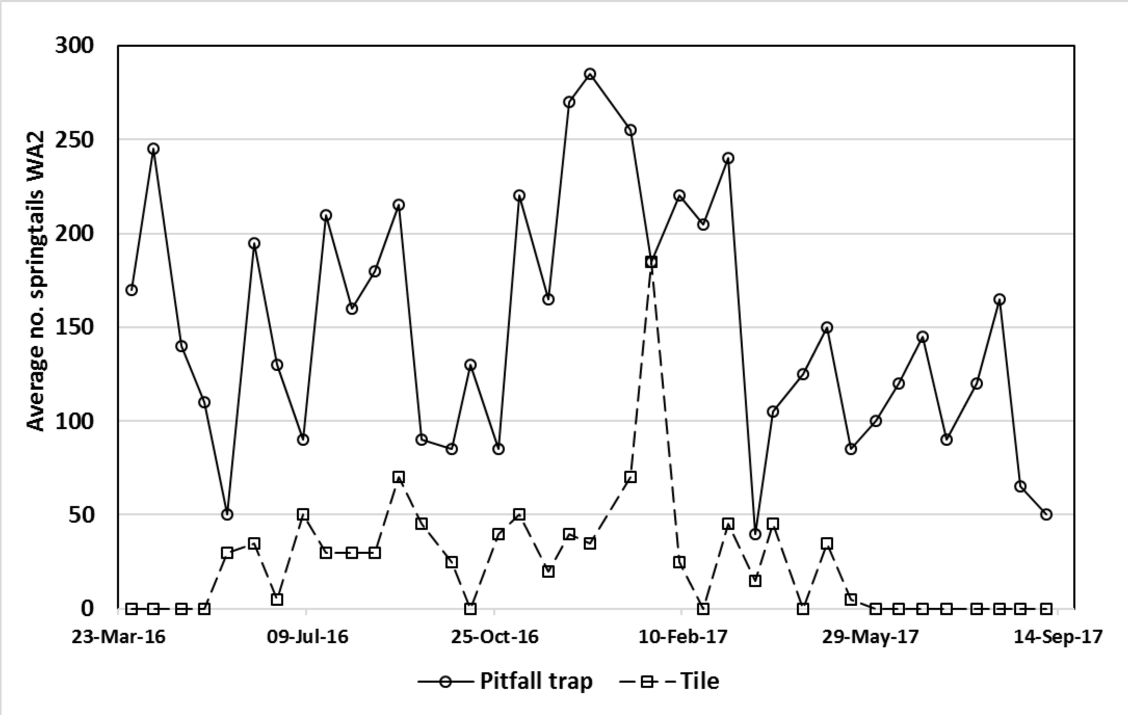


Fig. 1.2.10.(continued.) Average number of springtails in pitfall traps and under tiles in two WA truffle orchards.

Difference in invertebrate abundance between pitfall traps with and without truffle

When placing a piece of truffle in half the pitfall traps in some orchards, there appeared to be little effect on increasing the number of slaters (Fig. 1.2.11).

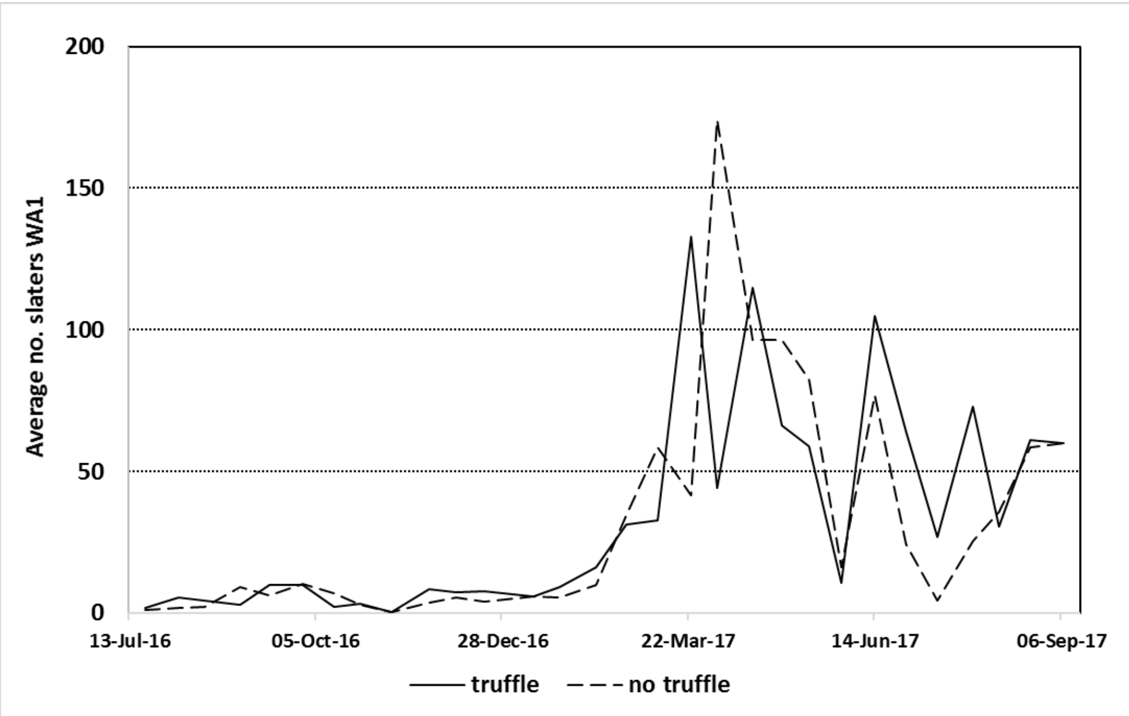


Fig. 1.2.11. Average number of slaters in pitfall traps either baited with a piece of truffle or not in two WA truffle orchards.

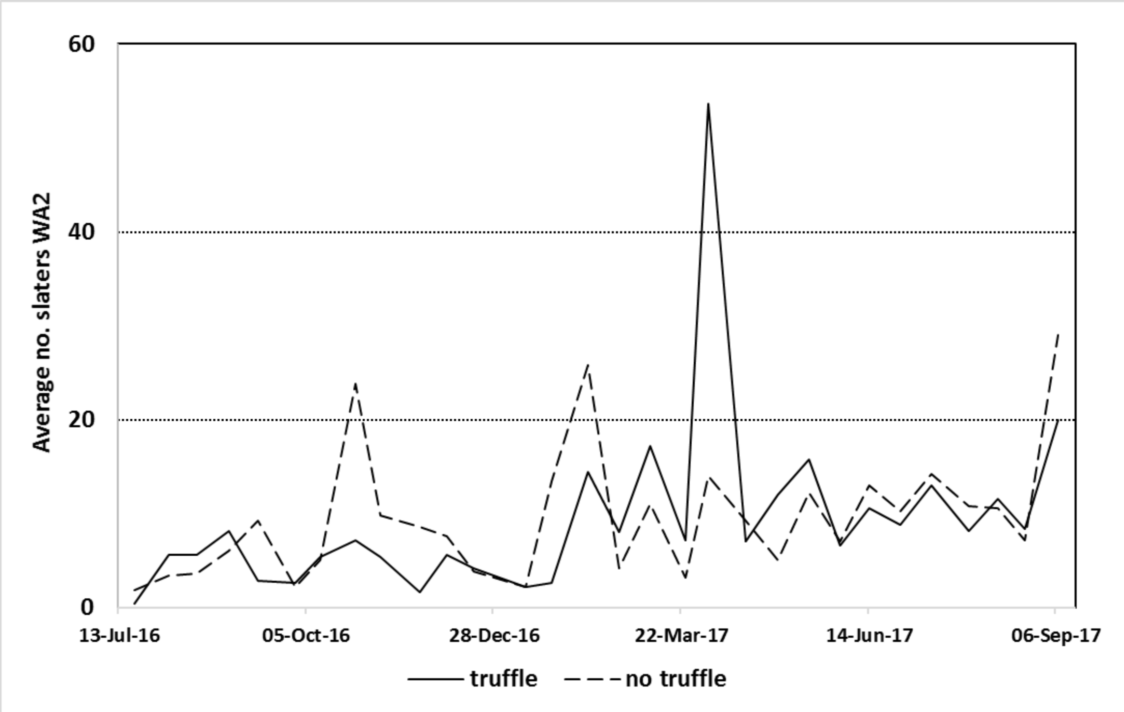


Fig. 1.2.11.(continued.) Average number of slaters in pitfall traps either baited with a piece of truffle or not in two WA truffle orchards.

The effect on the number of springtails recorded in truffle baited pitfall traps compared with traps with no truffle added was somewhat mixed but the overall effect was minor, with few extra springtails being attracted (Fig.1.2.12.).

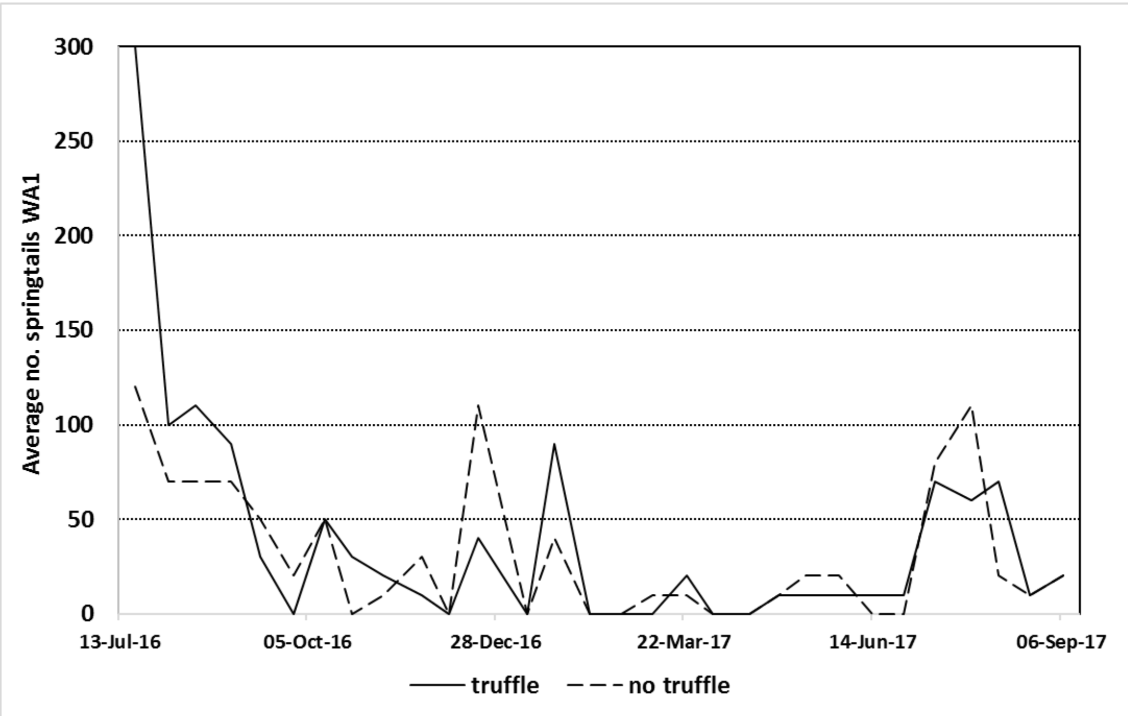


Fig. 1.2.12. Average number of springtails in pitfall traps either baited with a piece of truffle or not in two WA truffle orchards.

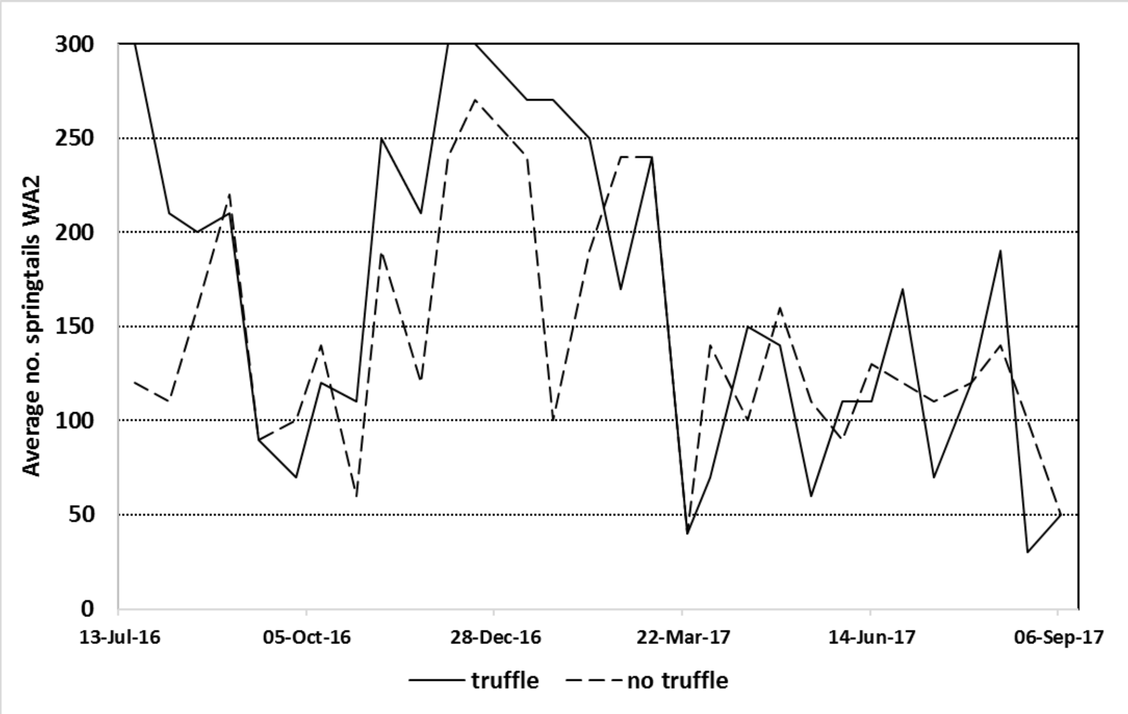


Fig. 1.2.12.(continued.) Average number of springtails in pitfall traps either baited with a piece of truffle or not in two WA truffle orchards.

From these comparisons, the most appropriate monitoring method for the year long study for the main pests and the predatory insects was pitfall traps. The addition of truffle pieces seemed to have little impact on numbers recorded.

Seasonal abundance of invertebrates

Some detail on each of the most abundant invertebrates (see Table 1.2.2) among the nine truffle orchards is presented in the following graphs. These indicate the seasonal abundance among the orchards for the more abundant invertebrates and possible relationships between predatory invertebrates and some of the other invertebrates are suggested.

The following series of graphs presents the results of seasonal abundance for all orchards of the main invertebrates for each as shown in Table 1.2.2.

African black beetle adults were recorded in low to moderate numbers in three WA truffle orchards (Fig. 1.2.13).

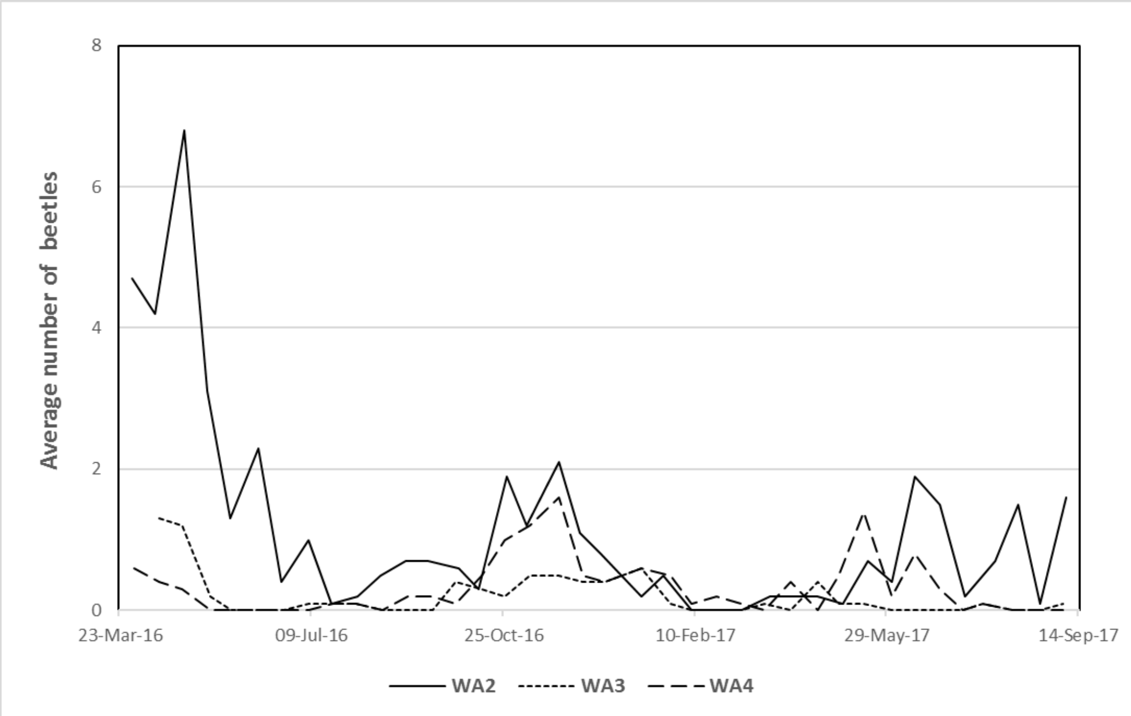
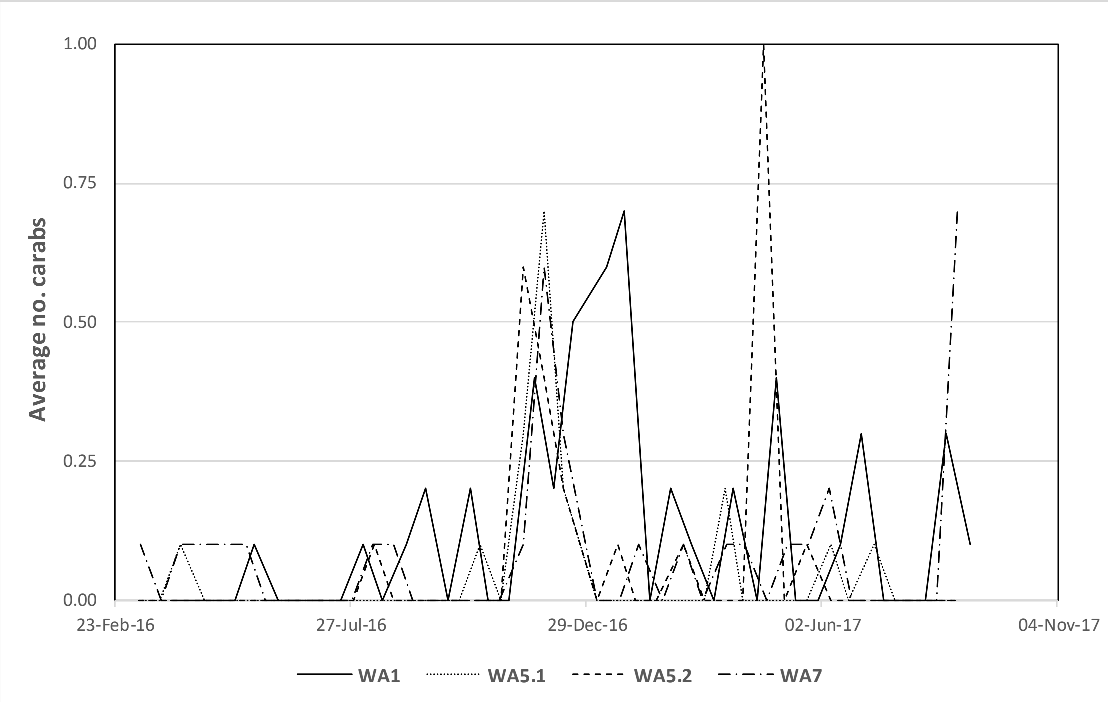


Fig. 1.2.13. The abundance of African black beetle adults in pitfall traps on three WA truffle orchards.

African black beetle has one generation each year. Adults overwinter and start laying eggs in spring. The next generation of adults emerges in summer. Results of the monitoring in the truffle orchards reflects this life cycle. The apparent reduction in abundance of adults during winter is a reflection of reduced activity in cooler weather rather than mortality.

Predatory beetles belonging to the Family Carabidae were recorded consistently but in low to numbers in all the WA truffle orchards monitored (Fig. 1.2.14). Beetles were most abundant in late winter to spring with some increase in three orchards during summer.



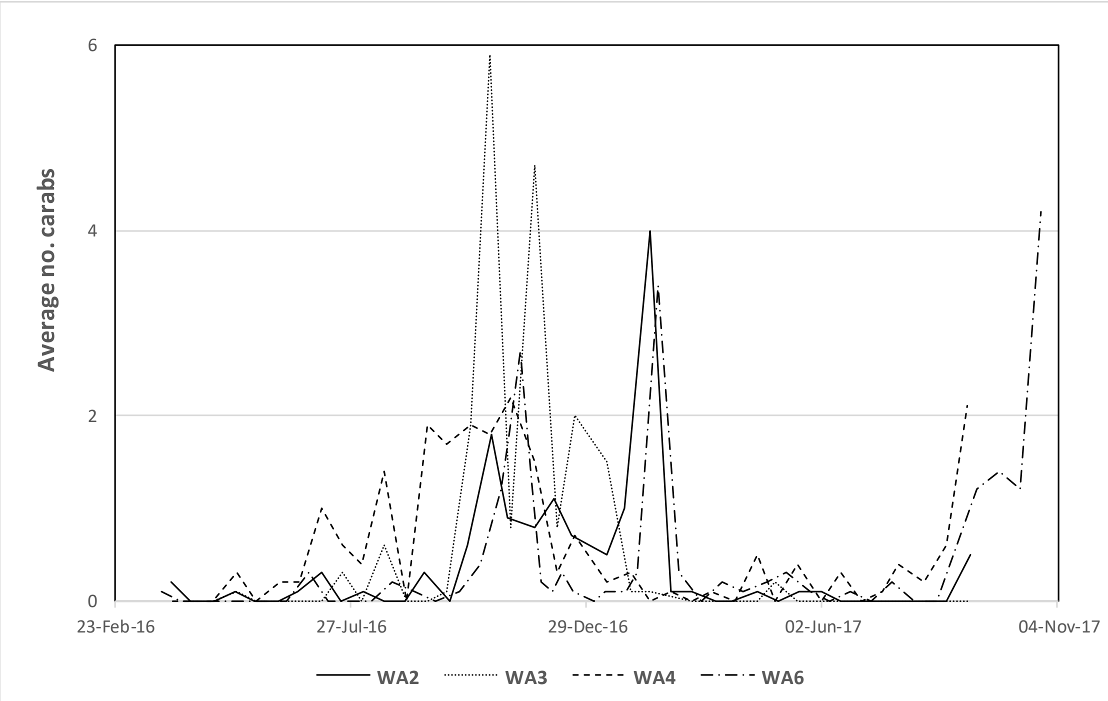


Fig. 1.2.14. The abundance of predatory beetles from Family Carabidae in pitfall traps on seven WA truffle orchards, including the two blocks from orchard WA5.

A similar situation of relatively low abundance of the other group of predatory beetles in Family Staphylinidae was recorded (Fig. 1.2.15). These were also most prevalent in WA truffle orchards. Unlike the carabid beetles, and apart from some occasions when numbers increased over a short period, the abundance of these beetles was somewhat more uniform throughout the year.

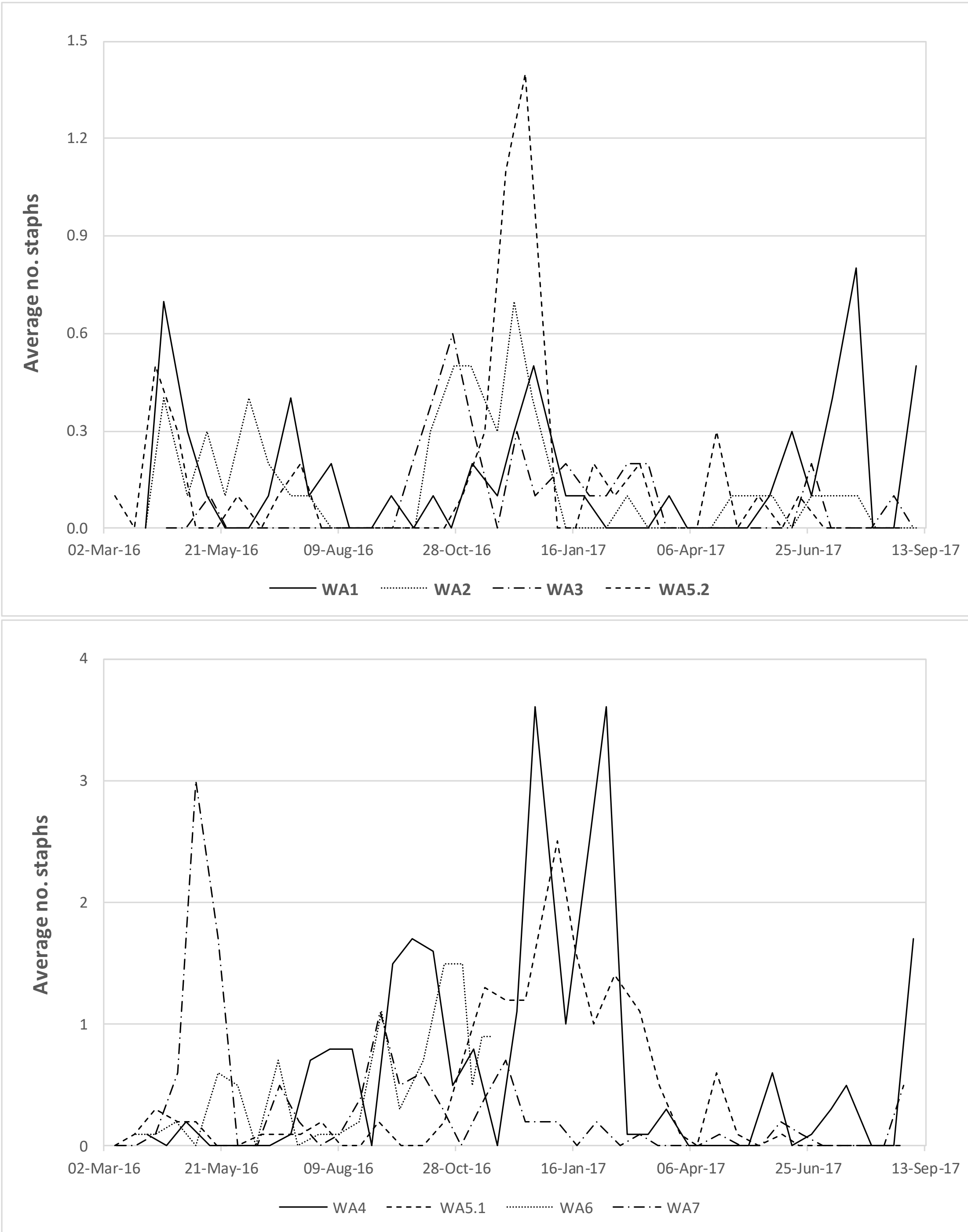


Fig. 1.2.15. The abundance of predatory beetles from Family Staphylinidae in pitfall traps on seven WA truffle orchards, including the two blocks from orchard WA5.

The abundance of European earwig and predatory earwigs is given in Fig. 1.2.16 for orchards in WA.

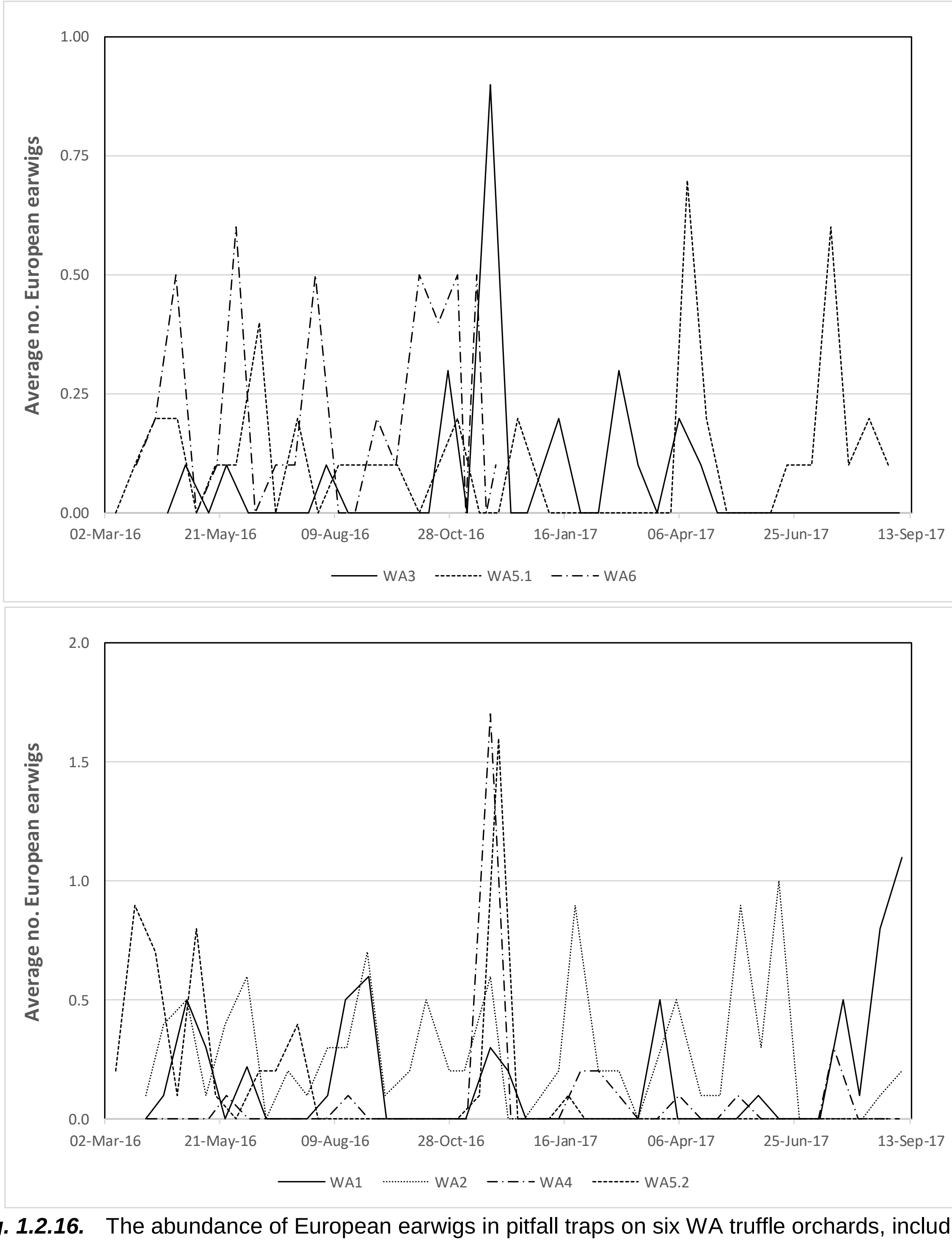
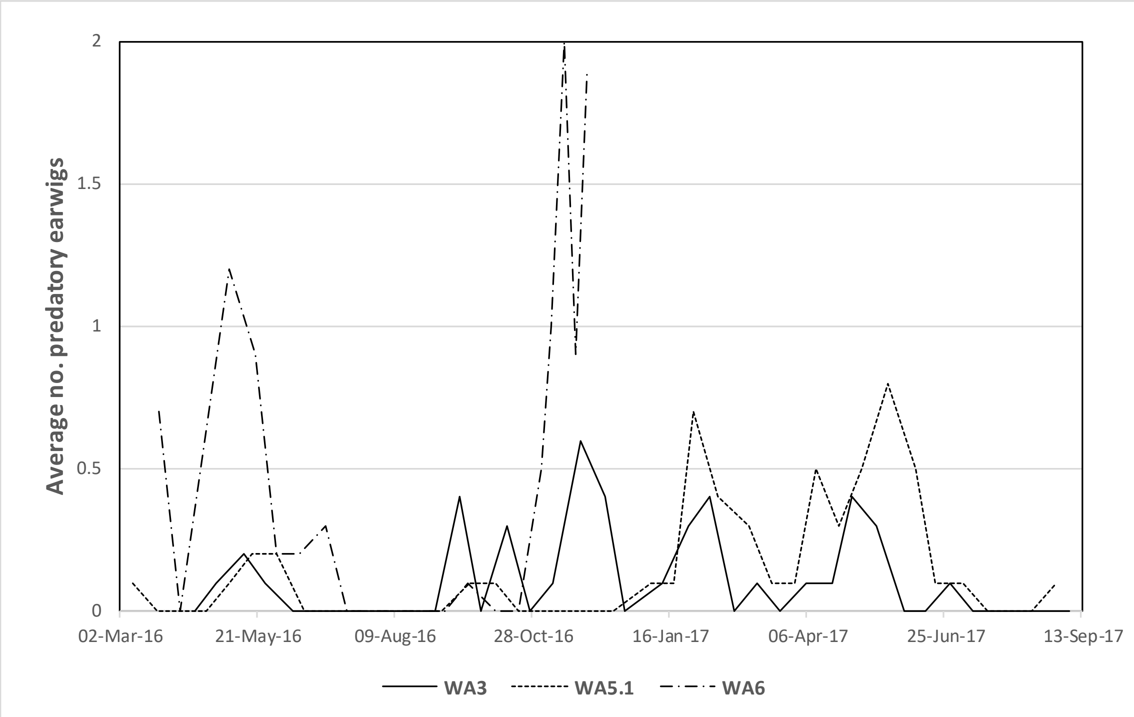


Fig. 1.2.16. The abundance of European earwigs in pitfall traps on six WA truffle orchards, including one orchard where two blocks were monitored.



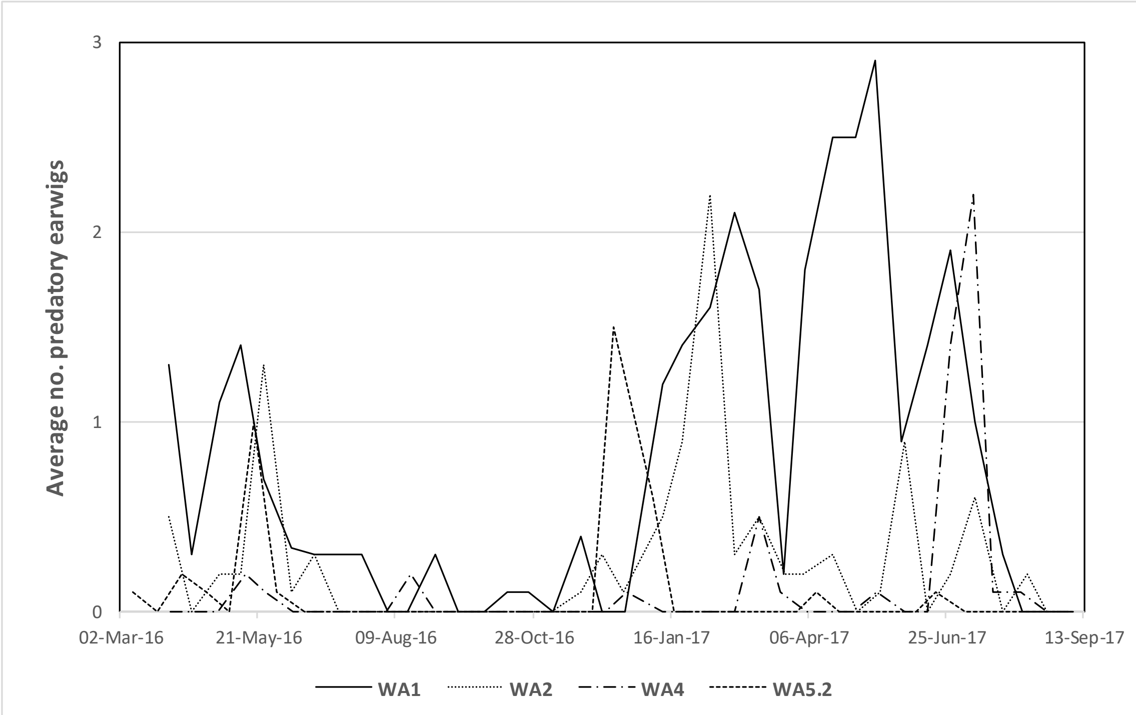
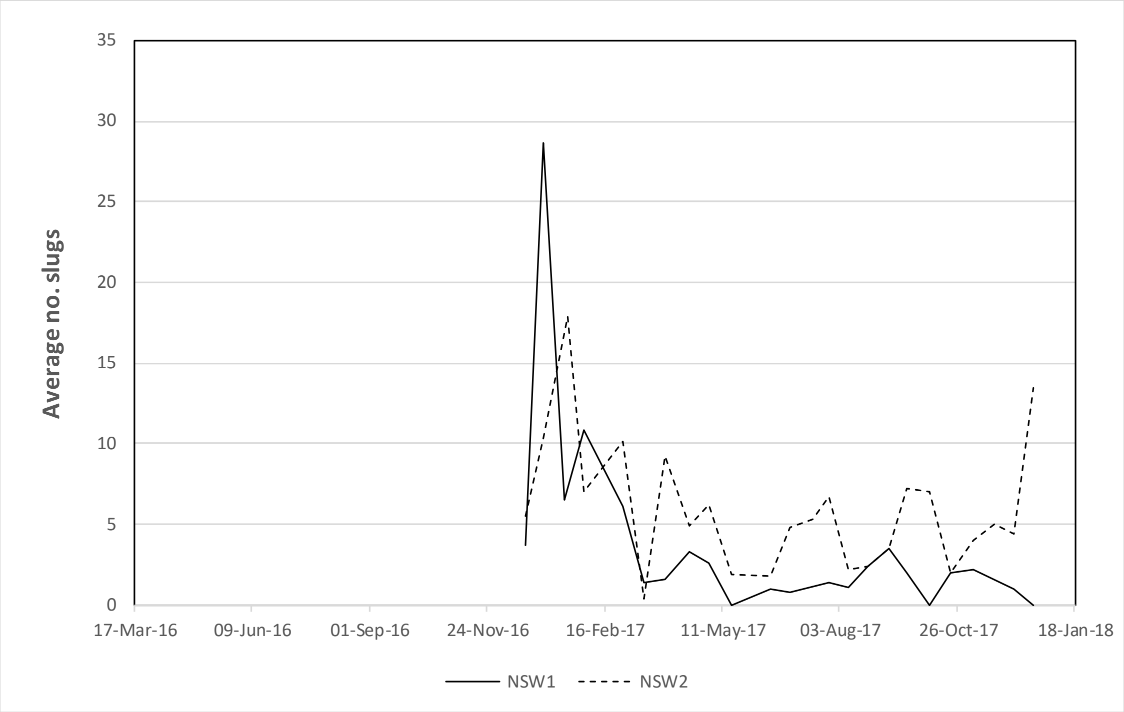


Fig. 1.2.16.(continued) The abundance of predatory earwigs in pitfall traps on six WA truffle orchards, including one orchard where two blocks were monitored.

European earwig is regarded as both beneficial in that soft bodied insects such as aphids are part of their food source, and a pest because they also feed on young or soft plant tissue. They feed on the ground and can also climb. They have been observed feeding on truffle, but are not regarded as a major pest of truffles. Predatory earwigs are primarily a ground dwelling insect and would likely also feed on soft bodied prey. There did not appear to be any major trend in the times of peak abundance for either European earwig or predatory earwigs.

The seasonal abundance of snails was indicated in the few orchards where they were recorded in any significant numbers in Fig. 1.2.5 and 1.2.6 for garden snail and small pointed snail respectively. Both species were most abundant in autumn through winter and small pointed snail was also abundant in late summer on some orchards.

The seasonal abundance of slugs (Fig. 1.2.17) indicated late winter to spring as a time of population increase and that either their abundance declines, or more likely slugs are less active over summer.



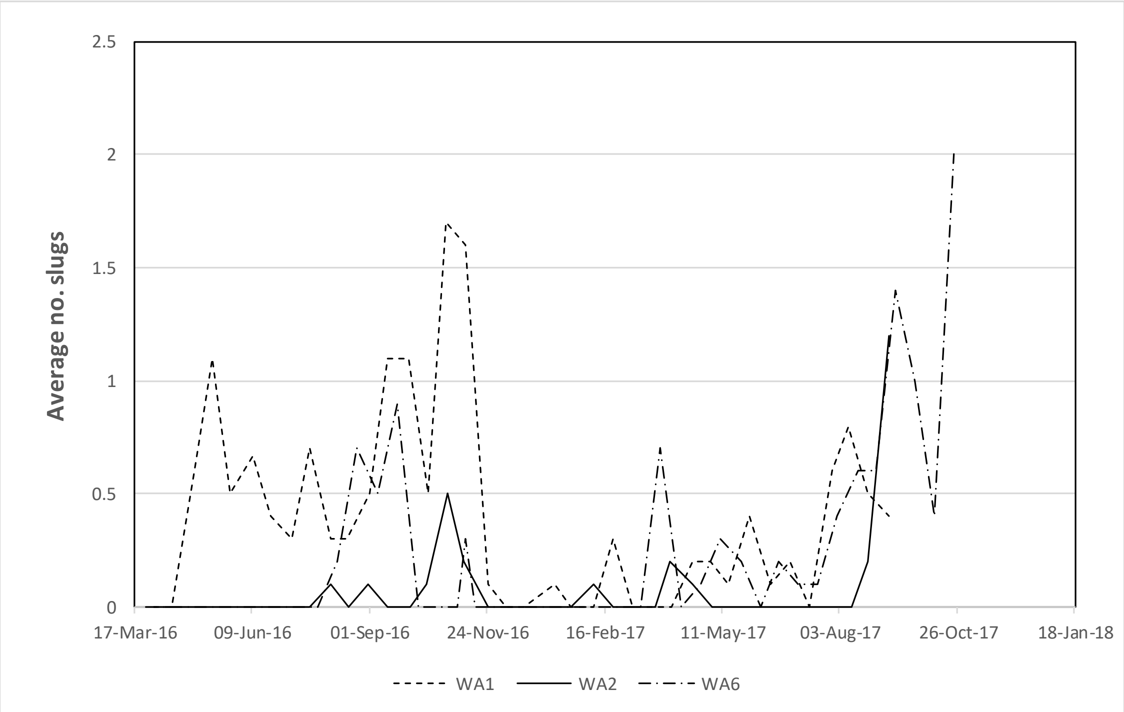


***Fig. 1.2.17*.** The abundance of slugs in pitfall traps in two orchards in NSW (top graph) and three orchards in WA.

Slaters were recorded at moderate to high numbers in all but one orchard where intensive monitoring was conducted (Fig. 1.2.18). Their potential to reach high populations in truffle orchards is indicated by orchards NSW1 and WA 3 where maximum average number of slaters in pitfall traps reached just over 900 and around 390 respectively. It was only shown later that slaters could in fact damage truffles. Before the project commenced, slaters were regarded as non-pests, but this assumption was seen to be incorrect.

Black truffles start to form and grow during December and commence ripening late May to early June. This monitoring shows that slaters are present in significant numbers during this interval and possibly inflicting damage to truffles at this time.

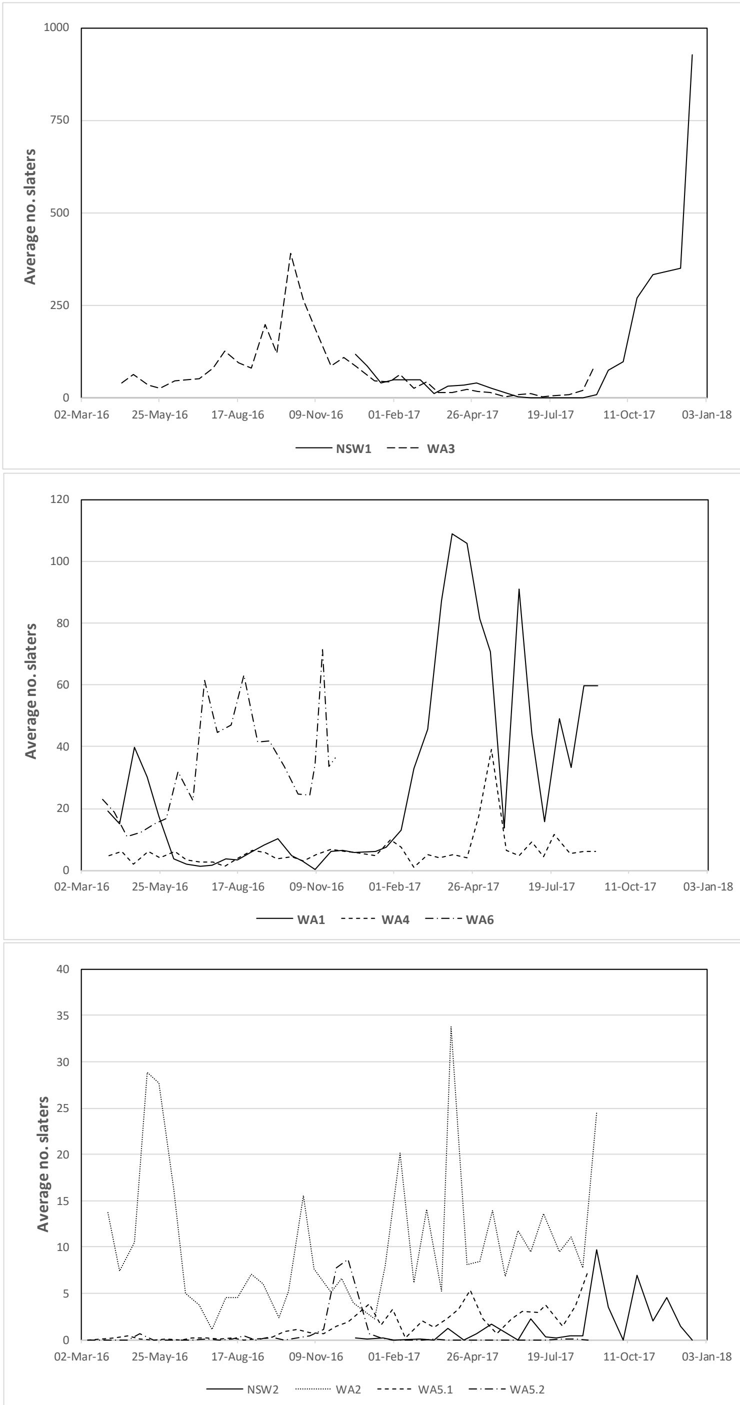


Fig. 1.2.18. The abundance of slaters in pitfall traps on two NSW and six WA truffle orchards, including one orchard where two blocks were monitored.

The most common millipede found in truffle orchards in NSW and WA was the ‘brown millipede”, *Cylindroiulus latestriatus*. The most abundant millipedes observed in truffle orchards during this project are included in Fig. 1.2.19

Fig. 1.2.19. The most abundant millipedes found in Australian truffle orchards during the project were, from top left and then clockwise: ‘brown millipede, ‘hairy’ millipede, ‘pale’ millipede, Portuguese millipede, ‘striped’ millipede and ‘spiral’ millipede. For Species name, see Table 1.2.5.

The seasonal abundance of 'brown' millipede is presented in Fig. 1.2.20.

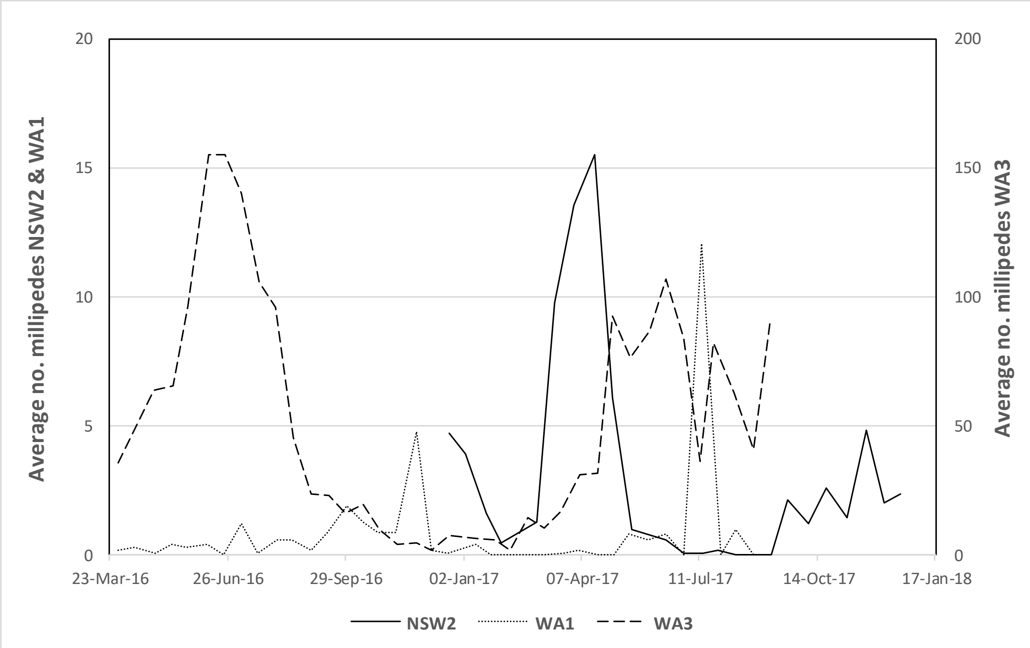


Fig. 1.2.20. The abundance of 'brown millipede' in pitfall traps on one NSW and two WA truffle orchards.

The millipede was most abundant in the cooler times of the year and therefore may pose a threat to causing damage to truffles. In comparison, Portuguese which was thought to be more likely to be an important pest of truffles, was much less abundant.

The abundance of springtails is shown in Fig 1.2.21, all in WA truffle orchards.

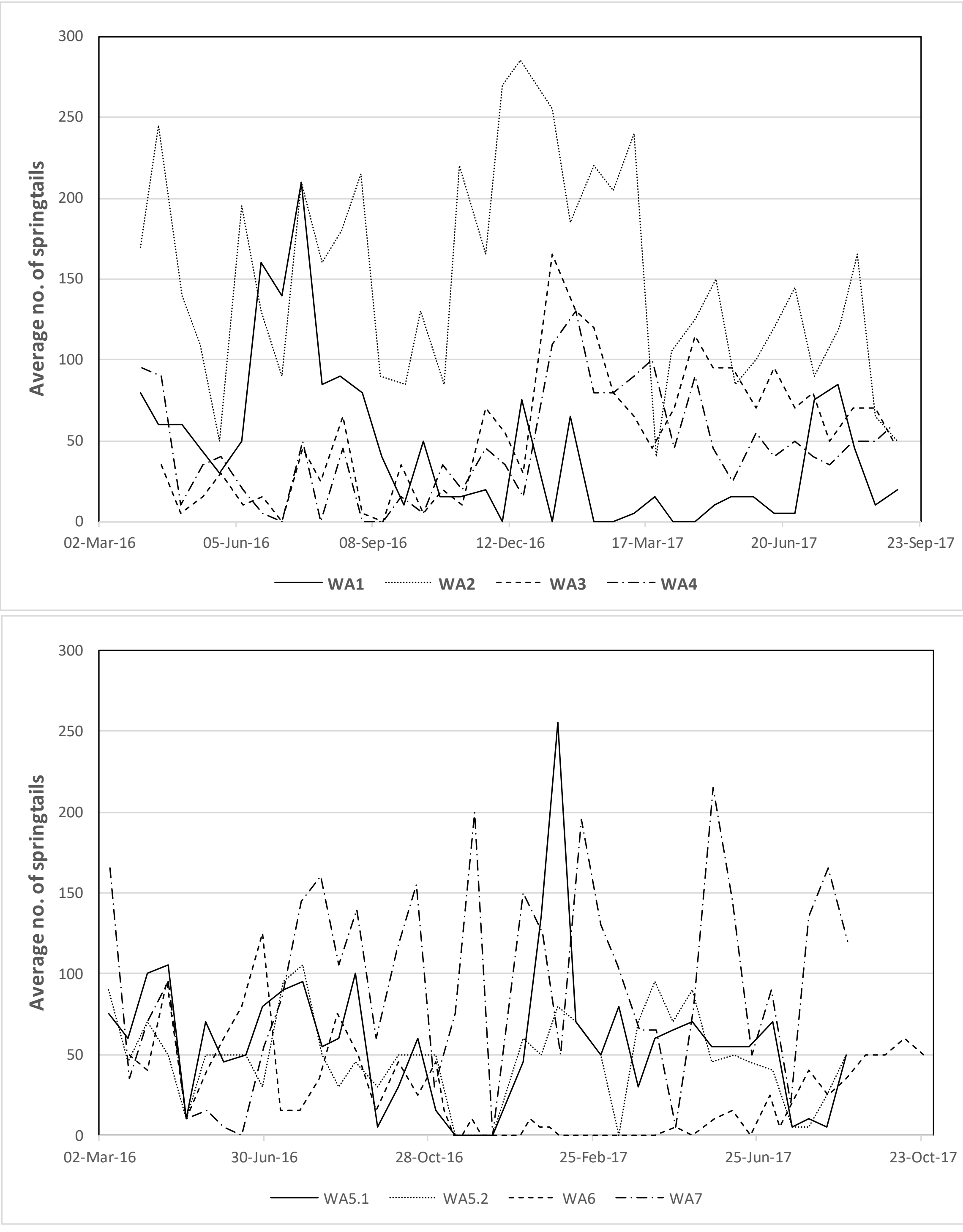


Fig. 1.2.21. The abundance of springtails in pitfall traps on seven WA truffle orchards, including one orchard where two blocks were monitored.

There was no clear seasonal trend in the abundance of springtails. They appeared to be most abundant in winter in one orchard and summer in others. The results showed that they may be present in high numbers at any time of the year.

Invertebrates observed in monitoring traps

From the pitfall trap and tile monitoring, many different ground dwelling invertebrates were collected. Specimens of slugs, slaters, millipedes, earwigs and springtails as well as truffle related beetles and other beetles commonly seen in truffle orchards were sent to taxonomists for clarification of their identification. The following tables lists the main species for each group.

The species of slugs encountered during the study are listed in Table 1.2.3.

Table 1.2.3 The species of slugs most commonly observed in pitfall traps and under tiles during the study of pests and disease in Australian truffle orchards

Common name	Scientific name	Comments
Black-keeled slug	*Milax gagates*	Widespread distribution
Brown field slug	*Deroceras panormitanum*	Widespread distribution
Hedgehog slug	*Arion intermedius*	New record for WA and restricted distribution in WA – *Arion* spp. in two orchards only. In eastern Australia, not yet recorded damaging truffles but widespread occurrence there (Anon. 2019a).
Reticulated or grey field slug	*Deroceras reticulatum*	Widespread distribution
Striped field slug	*Lehmannia nyctelia*	Widespread distribution

Table 1.2.4 The species of slaters most commonly observed in pitfall traps and under tiles during the study of pests and disease in Australian truffle orchards

Common name	Scientific name	Comments
common pillbug	*Armadillidium vulgare*	Widespread distribution
common rough woodlouse	*Porcellio scaber*	Widespread distribution

The species of millipedes encountered during the study are listed in Table 1.2.5. Other common millipedes encountered require further examination before they can be named (J. Waldock, pers. comm.).

Table 1.2.5 The species of millipedes most commonly observed in pitfall traps and under tiles during the study of pests and disease in Australian truffle orchards

Common name	Scientific name	Comments
“brown” millipede	*Cylindroiulus latestriatus*	Widespread distribution
‘hairy” millipede	*Brachyiulus* sp.	Widespread distribution
“pale” millipede	*Brachydesmus superus*	Not widely distributed
Portuguese millipede	*Ommatoiulus moreleti*	Widespread distribution
“spiral” millipede	*Ophyiulus pilosus*	Not widely distributed
“striped” millipede	*Solaenodolichopus* sp.	Not widely distributed

The species of earwigs encountered during the study are listed in Table 1.2.6. A species of a black earwig was observed feeding on truffles in the field, but it has yet to be identified (M. Binns, pers. comm.)

Table 1.2.6 The species of earwigs most commonly observed in pitfall traps and under tiles during the study of pests and disease in Australian truffle orchards

Common name	Scientific name	Comments
Common brown earwig	*Labidura truncata*	Widespread distribution
European earwig	*Forficula auricularia*	Widespread distribution
-	*Anisolabis* sp	Moderate distribution
-	*Gonolabis*	Moderate distribution

Table 1.2.7 The species of springtails most commonly observed in pitfall traps and under tiles during the study of pests and disease in Australian truffle orchards

Common name	Scientific name	Comments
Purple scum springtail	*Hypogastrura vernalis*	Widespread distribution
-	*Ceratophysella* sp. cf. *gibbosa*	Widespread distribution
-	*Hemisotoma thermophila*	Widespread distribution
-	*Parisotoma notabilis*	Widespread distribution
-	*Entomobrya unostrigata*	Moderate distribution
-	*Heteromurus* sp. cf. *nitidus*	Moderate distribution

Invertebrates of host trees from observations in truffle orchards and grower enquiries

There was an emphasis on gathering information on ground dwelling invertebrates primarily because they are the most likely agents to damage truffles. However, the project also sought to collate a check list of invertebrates that feed on truffle host trees.

A list of the more common agents is given in Table 1.2.8.

Table 1.2.8 The species of invertebrates associated with feeding on truffle host trees. This includes agents recorded prior to this project, those observed by the project team and responses from Australian truffle growers both through the national survey of truffle growers and direct enquiries

Common name, important stage	**Scientific name**	**Comments**
African black beetle, adult	*Heteronychus arator*	Ringbarks trunks of young trees killing them
Apple weevil, adult	*Otiorhynchus cribricollis*	Leaf scalloping; ringbark below growing tip killing them
Fullers rose weevil, adult	*Naupactus cervinus*	Leaf feeding; egg masses block mini-sprinklers
Garden weevil, adult	*Phlyctinus callosus*	Leaf scalloping
Hypsomus weevil, adult	*Hypsomus* sp.	Small adults disrupt mini-sprinklers
Red legged weevil, adult	*Catasarcus* spp.	Leaf feeding
Whitefringed weevil, adult	*Naupactus leucoloma*	Leaf damage
Spring beetle, adult	*Colymbomorpha vittata*	Leaf damage
Stinking longicorn, larva	*Stenoderus suturalis*	Stem boring larvae; branch weakness, entry point for disease; hazelnut trees affected
Cockchafers, adult	Melolonthinae spp.	Leaf damage
Wingless grasshopper	*Phaulacridium vittatum*	Leaf damage
Oak leaf miner, larva	*Phyllonorycter messaniella*	Leaf blotches, mainly aesthetic
Fruit tree borer, larva	*Maroga melanostigma*	Stem weakening; death in advanced stages; disease entry point; hazelnut trees affected
Painted apple moth & western tussock moth, larva	*Teia anartoides* & *T. anthlophora*	Leaf damage
Lightbrown apple moth, larva	*Epiphyas postvittana*	Leaf and shoot damage
Aphids	Unident. spp.	Damage shoots and new leaves
Heliothis budworm, larva	*Helicoverpa* sp.	Leaf and shoot damage; may chew green hazelnut tree nuts and acorns
Soft scale	Coccidae spp.	Stem/branch damage; only observed on hazelnut trees
Greenhouse thrips	*Heliothrips haemorrhoidalis*	Leaf feeding; minor occurrence; only observed on oak trees
Mites	Unident. spp.	Leaf feeding
"Native ant", adult	*Cardiocondyla nuda* near *atalanta*	Blocks mini-sprinklers
Small pointed snail	*Prietocella barbara*	Disrupt mini-sprinklers
Garden snail	*Cornu aspersum*	Leaf damage

The source of this information included results from previous studies, the national grower questionnaire as well as observations made during the regular visits to orchards for the pitfall trap and tile monitoring. Information on pests of the host trees was provided by growers through their enquiries to the projected team on pest identification. More information on these is available in the IPDM Manual

Invertebrates from truffle damage assessments

Harvest assessments were conducted over three harvest seasons – 2016, 2017 and 2018. The number of orchards where truffle damage assessments were conducted is provided in Chapter 1.4.

The invertebrates found either in the orchard during harvest or in the grading room are listed in Table. 1.2.9.

Table 1.2.9 The species of invertebrates associated with damaged truffles

Common name	Scientific name	Comments
'Australian truffle beetle', adult, larva	*Thalycrodes* sp. nr *australe*	"Honeycomb' of tunnels through truffles; obligate truffle feeding beetle
Slugs	[1] Several species	Damage truffles; may introduce rot
Slaters	[2] Several species	Damage truffles; may introduce rot
Earwigs	[3] Several species	Damage truffles; may introduce rot
Millipedes	[4] Several species	Damage truffles; may introduce rot
Springtails	[5] Several species	"Honeycomb' of narrow tunnels under the peridium; may be a secondary pest
Fungus gnat, larva	Family Sciaridae	May be a primary pest of truffles; often associated with rotten truffles
African black beetle, adult, Apple weevil, larva Fuller's rose weevil, larva garden weevil, larva whitefringed weevil, larva	*Heteronychus arator, Otiorhynchus cribricollis, Naupactus cervinus, Phlyctinus callosus, Naupactus leucoloma*	Opportunistic feeding on truffles
Click beetle/true wireworm, larva	Family Elateridae	Opportunistic feeding on truffles
Flies, not otherwise classified, larva	-	Most common in rotten truffles
Potworm	Family Enchytraeidae	Only present in rotten truffles
Garden centipede	Class Symphyla	Not a primary pest

[1, 2, 3, 4, 5] for species in these groups, see Tables 1.2.3, 4, 5, 6, 7.

Of the invertebrates listed in Table 1.2 9, Australian truffle beetle *Thalycrodes* sp. nr. *australe*, was not detected until truffle harvesting commenced in 2016. This beetle is an obligate truffle feeder where it must feed on truffle in order to complete its life cycle. It is the first species of beetle to be observed with this association with cultivated truffles in Australia, no doubt existing on the suite of native truffles that occur in Australia (Bougher & Lebel, 2001). In Europe, there is also an obligate truffle feeding beetle, European truffle beetle, *Leiodes cinnamomea*. This beetle is an important pest of truffles in Europe where there are few management options available (Morcillo et al, 2015). This beetle is not closely related to Australian truffle beetle which is in the beetle family Nitidulidae, whereas European truffle beetle is in family Leiodidae.

After Australian truffle beetle was detected, a number of monitoring methods was compared so that the abundance of the beetle could be assessed. A description of activities involved with this are presented in Chapter 2.2.

Discussion

A wide range of invertebrates was recorded during the yearlong monitoring in nine orchards across Australia which included pests, beneficial agents and suspected benign species that were considered to be neither pest nor beneficial. After comparing their abundance in pitfall traps to tiles, in all cases more invertebrates were recorded for pitfall traps and comments regarding their abundance and seasonality was based primarily on the pitfall trap data.

The identification of those invertebrates not known to the project team and that were considered as potential pests of truffles and/or their host trees was obtained from specialists.

The most abundant invertebrates implicated as pests of trees in truffle orchards were African black beetle and snails in WA. The most abundant invertebrates implicated in damaging truffles, were slaters, slugs and millipedes in both states, and springtails in WA. Predatory beetles were most abundant in all WA truffle orchards. Rather than this indicating exclusivity in the location of these invertebrates as pests or beneficial agents, this monitoring is regarded as highlighting differences likely to be seen among truffle orchards across Australia. Their presence in individual truffle orchardists will only be clarified by monitoring.

The yearlong monitoring clarified the species of slugs associated with truffle orchards, with all of them being introduced species. Some of these are the same species that are important pests in broadacre cropping in Australia (Anon., 2019b). The monitoring indicated somewhat contradictory seasonality. In NSW, slugs were more abundant in summer, whereas in WA, slugs were more abundant in the cool, moist period of winter to spring. Differences in rainfall patterns may account for this variation.

It was not known at the time the project commenced that slaters would be found to be capable of damaging truffles, so the preliminary information gathered here is relevant for developing sustainable management practices for this group.

Since the conclusion of yearlong truffle orchard monitoring, it has been observed that springtails produce a honeycomb of galleries within truffles. Feeding on fungus such as mushrooms, is common for this group and their feeding on truffles is considered to be secondary attack, predisposed by a number of factors. These include growth or physiologically derived cracks in the peridium, damage from other invertebrates such as slugs and slaters, the commencement of breakdown of truffles either due to primary infection or environmental conditions that induce rots or over-ripe truffles that become soft and susceptible to being attacked.

Further information on the implications of the seasonality of the pest and beneficial agents described here is presented in Chapter 2 on management of pests and diseases.

References

Anon. 2019a. Atlas of living Australia website: http://www.ala.org.au/

Anon. 2019b. Slugs and snails, Dairy Australia fact sheet. 2pp. file:///C:/Users/stewartl/Downloads/Slugs%20and%20Snails%20FP%20190201.pdf

Bougher, N.L., Lebel, T. 2001. Sequestrate (truffle-like) fungi of Australia and New Zealand. *Australian Systematic Botany* **14**, 439–484.

Morcillo, M., Sanchez, M., Vilanova, X. (2015). Truffle Farming Today, a Comprehensive World Guide. Publisher: Micologia Forestal & Aplicada. ISBN 978-84-617-1307-3.

Seago, A. (2014). Australian truffle beetles and the insect fauna of truffières. http://www.trufflegrowers.com.au/wp-continuedent/uploads/2014/10/Ainslie_Seago_truffles_2014.pdf

1.3 Observations and pest status of diseases of host trees and truffles

Objectives

Diseases of truffle host trees potentially pose a serious threat to truffle production in Australia through their adverse effect on tree health. The aim of this section of the project was to investigate whether truffle host trees exhibit disease symptoms, as well as the cause of those symptoms. Diseases of only *Corylus avellana* in Australia are documented, with only bacterial wilt of hazel recorded as important (Snare, 2006). Because *Quercus ilex* and *Q. robur* are not commercially important trees in Australia (apart from recent truffle production), their diseases of are poorly studied, but see Moricca et al, 2016.

The syndrome termed truffle rot has emerged as a significant issue for truffle growers across Australia. Depending on the orchard, soil and rainfall, truffle rot can be as high as 70%. For this report, we have not specifically set out to study truffle rot, because an in depth study has already been conducted on this issue in WA (Eslick, 2012, 2013). However, we show the main symptoms from observations in Australia, as well as highlighting the findings from the Eslick report.

Methodology

Disease survey and isolations

In total, 30 truffle orchards were investigated for diseased trees - oaks (*Q. robur* and *Q. ilex*) and hazelnut. Some orchards were visited and sampled two to three times during the five year period. Orchards investigated spanned all areas in Australia where truffles are grown, except Queensland. On average, seven trees selected based on ill health were sampled per orchard, and subjected to pathogen isolation.

Isolations were conducted within 7 days after samples were collected from the field. Diseased twigs, branches and cankers were surface sterilised with 70% ethanol to remove surface contaminants before isolation. The bark was cut away with a sterile scalpel blade and isolation of small segments of cambium and/or xylem were made from diseased margins, plated onto potato dextrose agar (PDA) and incubated at 25 °C.

Bacterial identification: *Xanthomonas arboricola* pv *corylina* causing bacterial blight of hazel was isolated and identified following methods in Lamichhane & Varavro, 2014.

Fungal identification: Fungal isolates were identified by categorising them in groups based on growth rate and morphology. For representative samples of each morphological group, DNA was extracted and sequenced for the ITS (nuclear ribosomal internal transcribed spacer region) locus, using primers ITS1 and ITS4 (Gardes & Bruns, 1993). PCR products were sequenced bi-directionally with an ABI PRISM Big Dye Terminator v3.1 sequencing kit (Applied Biosystems) on an ABI-3100 automated sequencer. Sequences were edited using the program Sequencher v4.7 (GeneCodes, Ann Arbor, Michigan, USA), aligned in Geneious v 10 (Biomatters). GenBank BLAST searches were conducted (http://www.ncbi.nlm.nih.gov/) and sequences of the top BLAST hits were used to assign names to fungal isolates. In some cases, sequences of the top BLAST hits were downloaded and aligned with our sequences in Geneious. Bayesian inference using the program MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003) was used for phylogenetic inference.

Pathogenicity studies – tree diseases

A number of fungi isolated are not known as pathogens on oak or hazel, but are pathogens on other hosts. Therefore, fungal isolates representing genera and species that are known, or potential pathogens of truffle hosts, were selected for pathogenicity testing in the field. Initially, an inoculation trial on 15-year-old hazel was conducted in an abandoned truffle orchard near Canberra in October 2017. In October 2018, the inoculation trial was repeated in a truffle orchard near Tumbarumba, on all three truffle hosts. Trees were approximately 20 years old. The level of pathogenicity of each isolate was assessed through inoculation onto the truffle hosts. For inoculations, isolates were grown on PDA for two weeks at 25°C. Mycelial discs were used to inoculate wounds on the stem of each tree, after the bark had been removed with a 10-mm diameter cork borer. In hazel, suckers 2 to 3 years old were inoculated, whereas individual branches (20 to 40 mm in diameter) were inoculated on the oaks. Each tree was inoculated with up to 10 isolates on separate branches. In each trial, each isolate was used to inoculate 10 trees in a randomised block design. For control inoculations, 10 trees in each trial were inoculated with a sterile disc of PDA. Wounds were sealed with masking tape to prevent desiccation. Lesion lengths in the secondary phloem were measured two months after inoculation. Re-isolations onto PDA were made from control and inoculated trees to satisfy Koch’s postulates.

Results

Disease survey and isolations

The number of diseased trees varied among orchards, from very few (less than 1%) to up to 45%. Unhealthy trees typically had twig and branch dieback, distorted or under-developed leaves, or poor development compared to the general orchard. Some of the “unhealthy” symptoms are likely due to genetic variability in the seed source used to establish the trees - these trees typically display as “runts”. However, many of the symptoms may have been produced by pathogenic fungi. The purpose of this study was, therefore, to identify the agents that cause disease in truffle hosts.

The majority of isolated fungi encountered in diseased material were *Diaporthe* spp., followed by *Fusarium* spp, *Discula* and *Paraconiothyrium* (Table 1.3.1). In total, 73% of diseased trees analysed yielded *Diaporthe*. Many trees were also infected with wood/white rot fungi, as observed from fungal fruiting bodies.

Bacterial identification: Bacterial blight of hazelnut is caused by a bacterium, *Xanthomonas arboricola* pv *corylina* (Lamichhane & Varvaro, 2014). This is a serious disease of hazelnut, but fortunately was identified in one orchard only, near Canberra. Unfortunately, in that case, at least 10% of hazelnut trees were severely infected with recurring shoot dieback every year.

***Phytophthora cinnamomi*:** Two cases of *Phytophthora cinnamomi* infection were confirmed in this study. In Western Australia, a few (6) trees of *Q. robur* was infected and died, whereas a *Q. ilex* orchard in NSW suffered 1.6% (12 trees) mortality. Both orchards were characterised by a shallow slope with mortality experienced at the bottom of the slope in high rainfall years, where waterlogging was most pronounced.

Fungal identification

Neofusicoccum australe

Only approximately 5% of examined diseased trees showed infection with *N. australe.* However, *N. australe* is a serious pathogen of many woody species and causes dieback (also known as Botryosphaeria dieback).

Table 1.3.1 Bacteria and fungi isolated or observed from diseased *Quercus ilex, Q. robur* and hazelnut trees

	Disease	**Host**$^{\$}$
Bacteria		
Xanthomonas arboricola pv *corylina**	Bacterial wilt of hazel	Hazel
Fungi		
Cytospora eucalypticola	Weak stem canker pathogen	Hazel
Cytospora sp.	Weak stem canker pathogen	*Q. robur, Q. ilex*
Diaporthe spp. (*Phomopsis*)	Stem, branch cankers	All
*Discula quercina**	Oak dieback	*Q. robur*
Dothierella sp.	Wilt	*Q. robur*
Didymella coffea-arabica (*Phoma*)	? Endophyte	Hazel
Entoleuca sp.	Poplar canker	*Q. ilex*
Fomitoparia australiensis	Bracket fungus – wood rot	Hazel
Fusarium spp.	Wilt	All
Ilyonectria sp. (*Cylindrocarpon*)	Wilt	*Q. ilex*
Neofusicoccum australe (*Botryosphaeria*)*	Dieback	All
Nigrospora sphaerica	Leaf pathogen	*Q. ilex*
Paraconiothyrium spp.	? Endophyte	All
Paraphaeosphaeria sp.	? Endophyte	Hazel
Peniophora lycii	Wood rot	*Q. robur*
Quambularia sp.	? Canker	*Q. robur*
*Phytophthora cinnamomi**	Root and collar rot	*Q. robur, Q. ilex*
Observed only:		
*Erysiphe alphitoides**	Powdery mildew	All
Pycnoporus sp.	Wood rot	All
Trametes sp.	Wood rot	All
Armillaria sp.	Root rot	All

* Indicates causal organisms reported as a pathogen of either *Quercus robur*, *Q. ilex* or hazel.

? Pathogen status on any tree host unknown

$^{\$}$All = *Quercus ilex, Q. robur* and hazel.

Powdery mildew *Erysiphe alphitoides*

All orchards examined showed some level of powdery mildew infection, mostly on *Q. robur,* but also on *Q. ilex*. In drier areas, powdery mildew infection may only be visible at the end of the growing season and is not of much concern for truffle production. However, powdery mildew incidence and severity was high during the growing season in orchards which experienced many rainy days (eg. 13 rainy days in Manjimup area during October 2018) providing ideal conditions for infection and an epidemic.

Discula quercina

Discula quercina was found in Tasmania, as well as in three orchards in Western Australia, always on *Q. robur*. However, sporulation of *D. quercina* was found on dead tissue in a stem crack in *Q. ilex*. It was always associated with trees in poor health and even with dying trees. In all cases where *D. quercina* was found, sites were characterised as moist and in high rainfall areas. Two sites also had a closed canopy with dense planting, limiting air movement, thereby increasing humidity within the orchard.

Diaporthe

The majority of isolates which are potentially pathogenic to oaks and hazelnut trees were identified as *Diaporthe* species. Four previously described *Diaporthe* species (*D. australafricana, D. rudis, D. amygdali* and *D. foeniculina*) and an isolate representing an unknown/undescribed species of *Diaporthe* were found (Fig 1.3.1). The two most common species, *D. australafricana* and *D. foeniculina* were found on both oak species as well as hazelnut, and is geographically widespread in Australia.

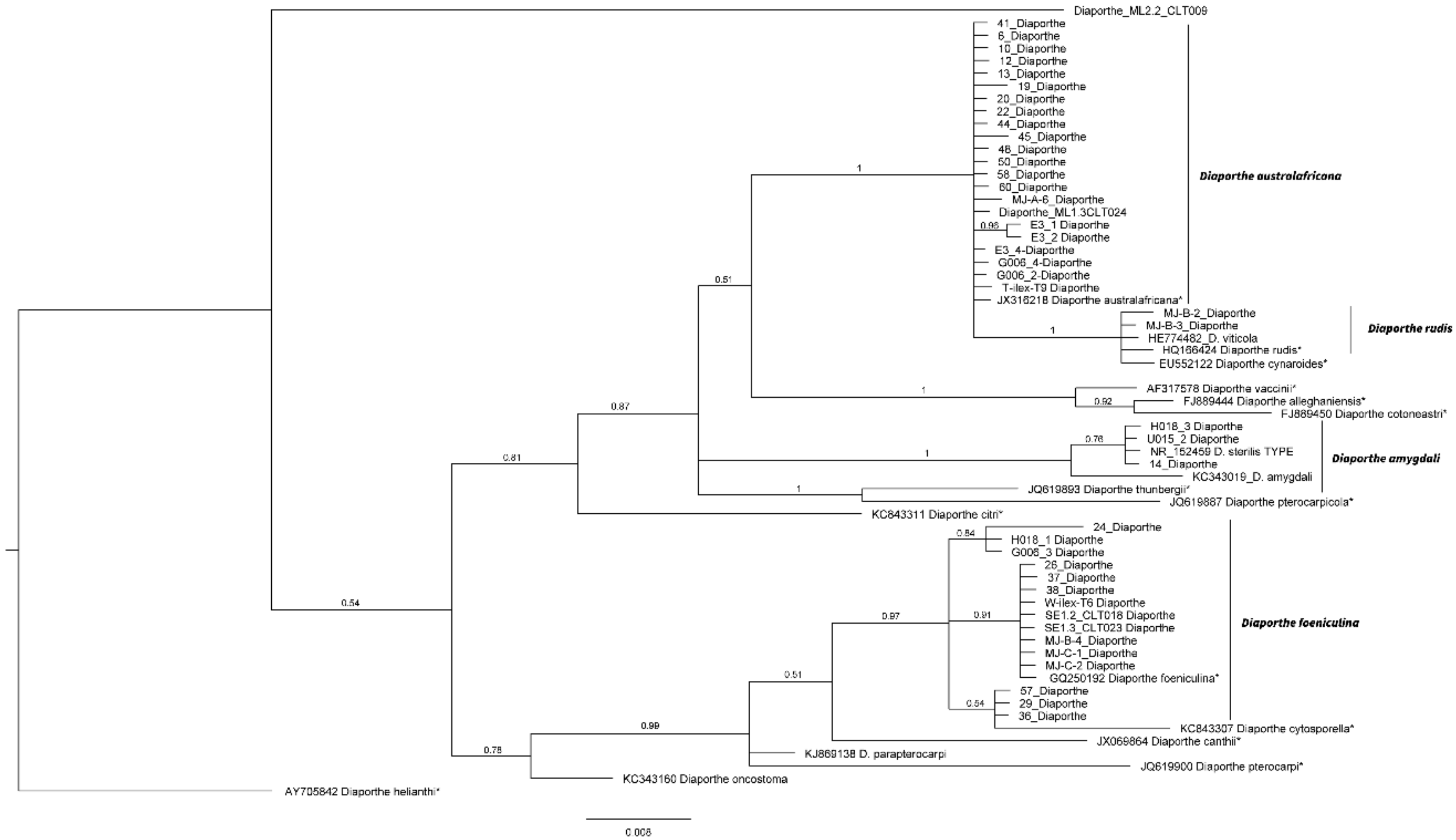


Fig 1.3.1. A MrBayes phylogenetic tree of Diaporthe isolates from this study, based on the ITS locus. Reference sequences from GenBank were included.

Diaporthe australafricana

We found *D. australafricana* most frequently on diseased hazelnut, but also on *Q. robur* and *Q. ilex.* Disease symptoms include twig and branch dieback on *Q. robur*, frequently associated with powdery mildew and/or *Discula quercina* infection (Fig 1.3.2), suggesting high humidity conditions.

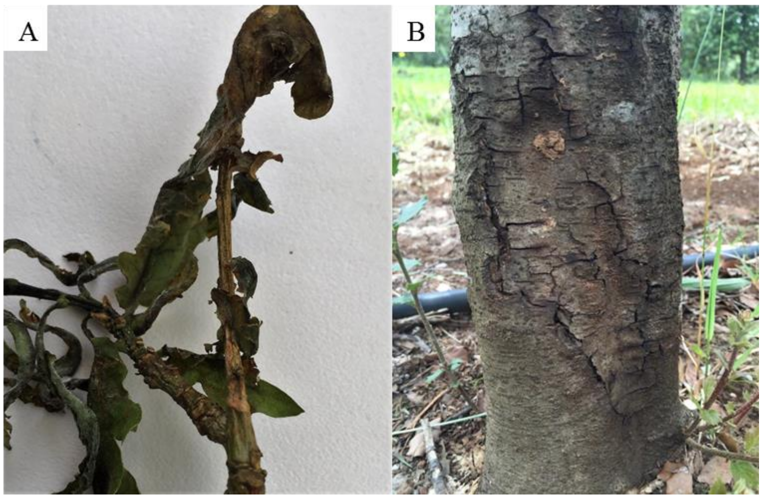


Fig 1.3.2. A) New growth dieback of *Quercus robur* associated with powdery mildew, *Diaporthe australafricana* and *Discula quercina* infection. B) *Quercus ilex* canker associated with *D. australafricana*.

On hazelnut, it is associated with dieback of branches 1-2.5 cm thick. Dieback is characterised by discoloured wood (Fig 1.3.3).

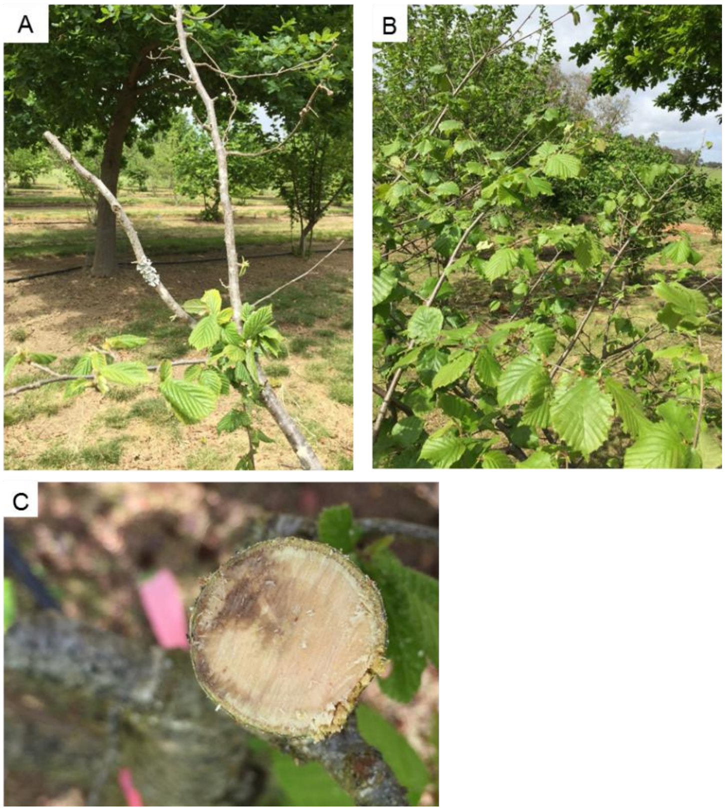


Fig 1.3.3. A-B) Dieback and C) wood discolouration on hazelnut caused by *Diaporthe australafricana*.

Diaporthe foeniculina

D. foeniculina was isolated from all three truffle host trees. Symptoms included dieback and twig cankers (Fig 1.3.4). Typically, *D. foeniculina* was isolated from diseased branches ranging in size from 5mm to 2 cm in diameter, but also from *Q. robur* main trunks of trees displaying wilting symptoms.

Fig 1.3.4. Twig canker on *Quercus ilex*.

Diaporthe rudis

Diaporthe rudis was previously known as *D. viticola.* We found *D. rudis* only twice in this study, from the same orchard in Tasmania, associated with stunted *Q. ilex* trees.

Diaporthe amygdali

We found *D. amygdali* associated with only *Q. robur* from an orchard in Denmark, WA. Powdery mildew, *Discula quercina* and *Neofusicoccum australe* could also be observed in *D. amygdali* infected trees. In one case, *D. amygdali* and *N. australe* were associated with infection through a pruning wound, with subsequent wood discoloration (Fig 1.3.5).

Fig 1.3.5. Wood discoloration associated with *Diaporthe amygdali* and *Diaporthe australafricana* as a result of a pruning wound.

Unidentified *Diaporthe* sp.

One isolate, ML2.2_CLT009, could not be identified to species level. This likely represents a new species as recent studies in *Diaporthe* revealed numerous undescribed species. Phylogenetic relationships of new species with other *Diaporthe* species, should however, also utilise DNA sequence analyses based loci such translation elongation factor 1-alpha (TEF), partial regions of the β-tubulin (BT) and calmodulin (CAL). In this study, isolates were sequenced using the ITS primers only.

Wood rot fungi

Wood rot fungi such as *Trametes, Pycnoporus* and *Fomitoparia* were observed frequently on dead or dying trees. In almost all cases, the affected trees either had herbicide damage, severe pruning damage or were waterlogged for long periods of time.

Pathogenicity studies – tree diseases

The fungi causing the longest lesions on all three hosts were *P. cinnamomi* followed by *N. australe* and *D. quercina* (Figs 1.3.6-1.3.7). The rest of the fungi produced lesions of similar length but extended the wound size on average by 20-40%, indicating weak pathogenicity. Lesions caused by *Cytospora* either did not extend beyond the 10 mm cork borer hole, or callused over, suggesting it is not a pathogen of truffle hosts tested. Some fungi showed host specific interactions, e.g. one *N. australe* isolate caused longer lesions on hazel than on oaks (Fig 1.3.7).

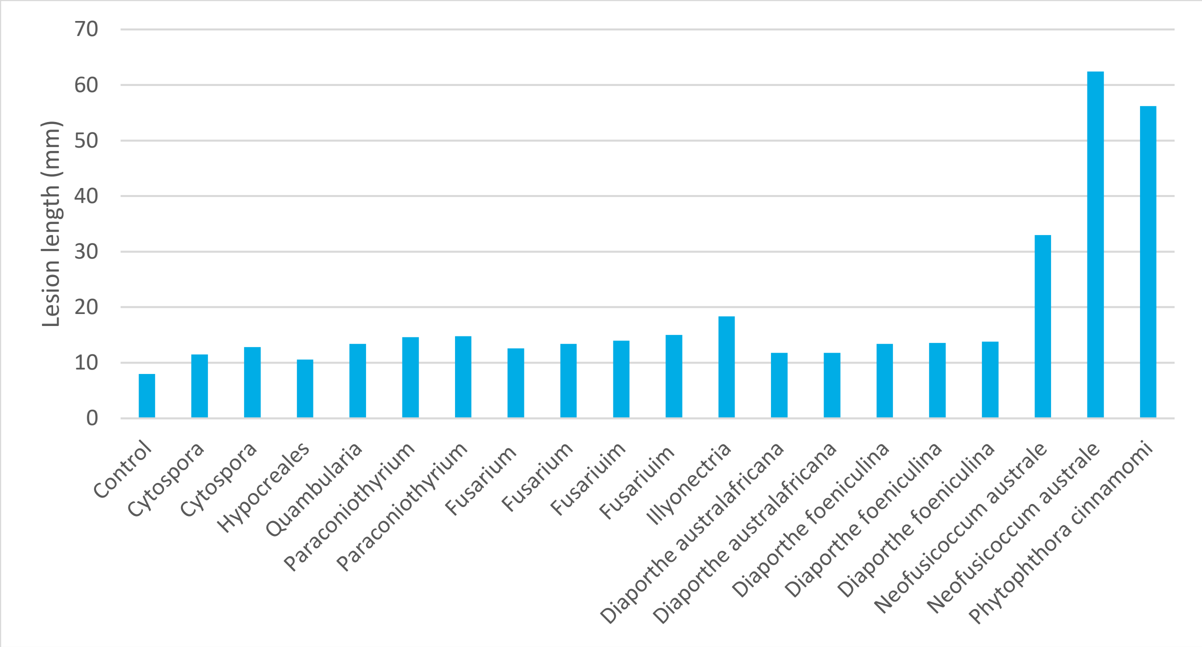


Fig. 1.3.6. Average length of lesions on *Corylus avellana* trees inoculated with 19 isolates representing fungi isolated from truffle hosts. Data presented are averages of lesion length data obtained 2 months after inoculations at the Canberra, ACT site.

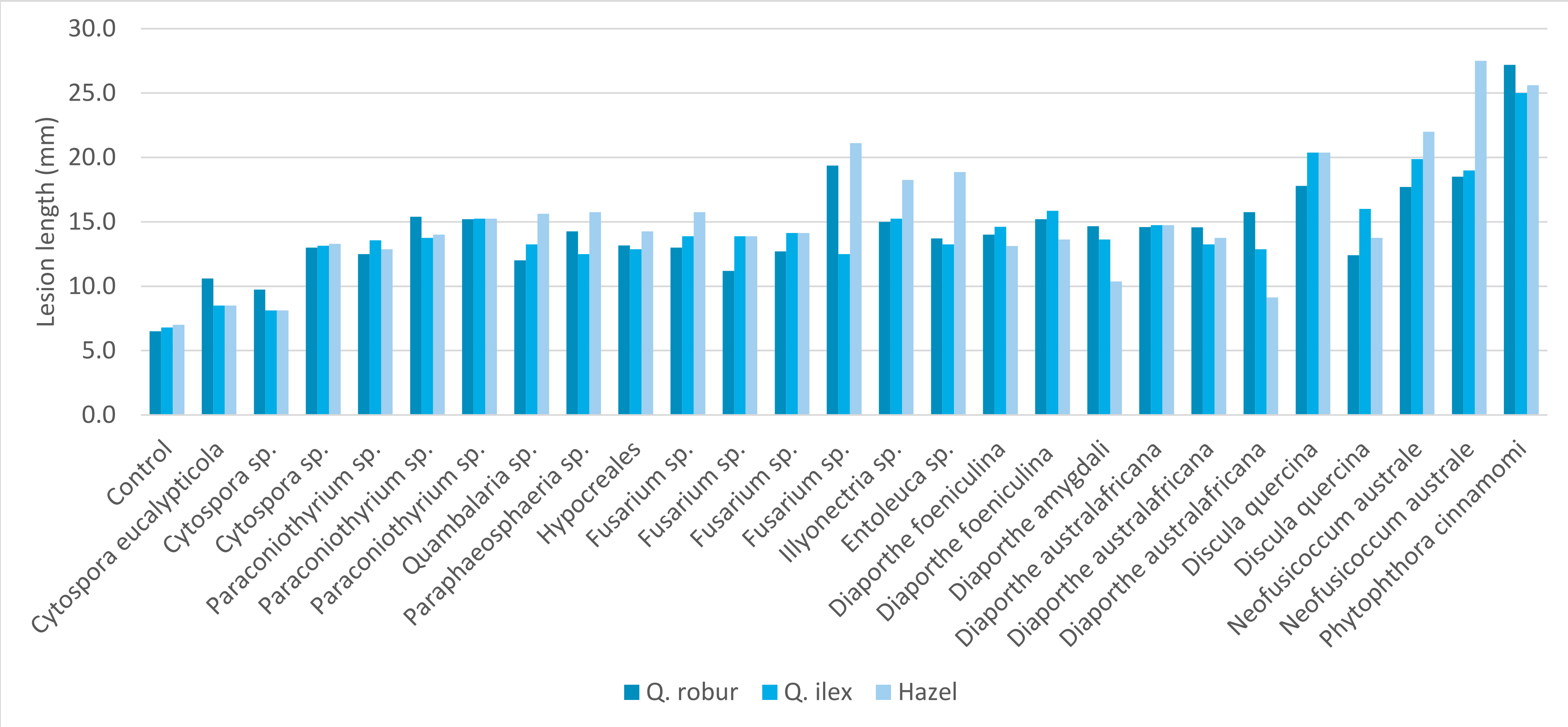


Fig. 1.3.7. Average length of lesions on *Quercus robur*, *Q. ilex* and *Corylus avellana* trees inoculated with 26 isolates representing fungi isolated from truffle hosts. Data represented are averages of lesion length data obtained two months after inoculations at the Tumbarumba, NSW site.

Truffle rot:

Truffle decay in the orchard often initiates at the site of invertebrate damage (Fig. 1.3.8), or on the upper side of the truffle when it has breached the soil surface (Fig. 1.3.9). Many different symptoms of truffle rot could be observed (Fig. 1.3.10). Attempts to isolate fungi from a small subset of those consistently yielded *Fusarium* spp. See below for a discussion on the implications of this finding.

Fig. 1.3.8. Truffle rot initiating at the site of invertebrate damage.

***Fig. 1.3.9*.** Truffle rot initiating on the part of the truffle that has breached the soil surface

Fig. 1.3.10. A diversity of truffle rot symptoms.

Fig. 1.3.10. (continued)   A diversity of truffle rot symptoms.

Discussion

Diseases of truffle host trees

Although tree mortality in a truffle orchard is relatively low (usually less than 10%, mostly less than 2%), some trees displayed disease symptoms which impacted on tree vigour. This in turn, may also impact on truffle production. A number of fungi were isolated from diseased trees. Some are well-known as plant or specifically tree pathogens, while others are reported as endophytic or saprophytes. From our pathogenicity trials, it is clear that the most pathogenic fungi are *P. cinnamomi* (not actually a fungus, but belonging to the Kingdom Straminopila), *N. australe* and *D. quercina*. The most common fungi isolated were *Diaporthe* species. Many trees were infected with white/wood rot fungi such as *Pycnoporus* and *Fomitopora*.

Root and collar rot caused by *P. cinnamomi* is a serious disease of many host species (> 800) and iconic for causing jarrah (*Eucalyptus marginata*) dieback in Western Australia and general dieback of Myrtaceae and Proteaceae in Australia (Weste, 2003). In both orchards where *P. cinnamomi* was detected, mortality occurred, highlighting the devastation this pathogen can cause. Management of *P. cinnamomi* is therefore important, starting with site selection to have sufficient slope and soil with good drainage to help prevent waterlogging.

Neofusicoccum australe is a pathogen of a diversity of host species, causing dieback, which may lead to mortality. The genus *Neofusicoccum* is a member of the Botryosphaeriaceae (Botryosphaeriales, Dothideomycetes) comprising numerous species found on a wide range of plant hosts of agricultural, forestry, ecological and economic importance (Slippers & Wingfield, 2007). A related fungus, *Diplodia corticola* causes a significant disease in cork oak (*Quercus suber*) in Spain (Sanchez et al., 2003, Serrano et al., 2015) and *Quercus virginiana* in Florida, USA (Mullerin & Smith, 2015). It is an endophyte, thus has the ability to colonize without producing any external symptoms (Slippers & Wingfield, 2007). Being an endophyte and having a broad host range, means there is always a source of inoculum for new infections. The change from the endophytic to pathogenic phase is often related to stress such as drought, extreme temperature fluctuations, nutrient deficiencies and mechanical injuries (Slippers & Wingfield, 2007).

Discula quercina (previously identified as *Gloeosprorium;* teleomorph *Apiognomonia quercina*) has a worldwide distribution, found on many oak species (including *Q. robur* but not *Q. ilex*), hazel, maples, beech and dogwood. It is also considered an endophyte of oaks (Moricca et al., 2016, Moricca & Ragazzi, 2011, Ragazzi et al., 2003, Ragazzi et al., 1999). In Europe, it causes a disease known as oak anthracnose (Moricca & Ragazzi, 2008) where infected leaves results in premature leaf fall in winter, with infection often spreading to twigs causing cankers and dieback. It is considered one of the most important pathogens involved in the aetiology of oak decline in Mediterranean countries (Moricca & Ragazzi, 2011, Sanchez et al., 2003), likely because of phytotoxins produced (Maddau et al., 2011). Similar to other endophytes, pathogenicity in *D. quercina* is triggered when the plant is subjected to physiological stress such as drought and/or senescence (reviewed in Moricca & Ragazzi, 2008). Although high humidity and moisture favours new leaf infections of *D. quercina*, it is ultimately plant stress such as drought that triggers phytotoxin production and hence pathogenicity (Moricca & Ragazzi, 2008). The level of disease it is able to cause when subjected to artificial inoculations, suggests it may be an underrated pathogen in cultivation of *Q. robur* in Australia.

In Europe, the distribution of the *D. quercina* is not explained by site, soil, altitude or exposure to the sun, but disease incidence, severity and spread are closely linked to the weather (Moricca, 2011). In Australia, it appears that *D. quercina* is more prevalent on high rainfall sites and/or sites with closed canopies, because its presence often coincided with powdery mildew infection. Symptoms vary depending on climate, with humid conditions required for infection while extended summer drought promotes the disease in Europe. Thus, in Europe it is recommended to maintain adequate spacing between trees and maintain relative humidity below 40-50% to reduce inoculum production (Moricca & Ragazzi, 2011). Relative humidity may be lowered by regular pruning to improve airflow, and by an appropriate irrigation regime.

Success of isolation of this fungus in Europe is highest during June (summer), then declining gradually in the next four months (Sahashi et al., 1999), suggesting with more early summer sampling the disease incidence in Australia is likely higher than currently recorded. Host specificity of strains was shown with artificial inoculations in Europe (Ragazzi et al., 2000), which might explain the difference in lesion lengths of the two isolates tested in the pathogenicity trials.

The genus *Diaporthe* consists of many species (anamorph *Phomopsis*) which are economically important as plant pathogens causing diseases on a wide range of crops, ornamentals and forest trees. *Diaporthe* species are present worldwide as endophytes in healthy leaves, stems, seeds and roots, or as saprobes on decaying tissues of a wide range of hosts. Multiple species of *Diaporthe* can often be found on the same host species, and any one *Diaporthe* species can infect many hosts (Udayanga et al., 2014). For example, *Diaporthe rudis* comprises isolates derived from 18 different hosts from 13 countries representing the geographic regions of Canada, Europe, New Zealand, South America and South Africa, including the epitype culture of *D. viticola* (Udayanga et al., 2014).

Although *Diaporthe* are well-known pathogens of trees and other crops, they have not been reported as pathogens of oaks and hazelnut (except *D. australafricana* on hazelnut, see below). On hazel, *Diaporthe* spp. are associated with kernel moulds (Battilani et al., 2018, Snare, 2006), but they have not been identified to species level. The occurrence of *Diaporthe* spp. is noted infrequently as endophytes of truffle tree hosts, although our results suggest it is a common endophyte in Australian truffle hosts. A known endophyte of *Quercus* in Europe*,* *D. quercina* (Gonthier et al., 2006, Ragazzi et al., 2003), was not identified in this study.

Diaporthe australafricana was previously only known from Africa and Australia on *Vitis* but have been found on *Corylus* in Chile (Guerrero & Pérez, 2013). It is the only *Diaporthe* species known to cause disease on a truffle host species. In Chile, it is reported to cause a stem canker and dieback of twigs on six-year-old hazel trees, with an incidence of 15% in high humidity conditions. Hazelnut plants between 1 and 3 years old developed a basal stem canker, especially under conditions of high humidity and weed infestation, with subsequent possible mortality (Guerrero & Pérez, 2013).

Diaporthe foeniculina includes the synonym *Diaporthe neotheicola* (Udayanga et al., 2014). It is associated with decline and mortality of *Eucalyptus camaldulensis* (Deidda et al. 2016), stem and shoot cankers on *Castanea sativa* (Annesi et al. 2016) as well as branch cankers and stem-end rot of *Persea americana* (Guarnaccia et al. 2016). Although *D. foeniculina* was reported on oak from New Zealand, its pathogenicity on truffle host trees is unknown.

D. amygdali is reported as causing a twig canker in walnut (Meng et al., 2018), twig canker and blight of almonds (*Prunus dulcis*) and peach (*Prunus persica*) wherever these hosts are grown (Diogo et al., 2010). It is known as an endophyte of hazel (Akay et al., 2014).

The pathogenicity study showed that *Diaporthe* species are pathogenic, but only weak pathogens of truffle hosts. Similar to *N. australe*, it is also known as an endophyte and thus switching from being endophytic to a pathogen is likely related to stress such as water stress (either drought or waterlogging), predisposition by other pathogens, extreme temperature fluctuations, nutrient deficiencies and mechanical injuries.

The majority of fungi able to cause lesions in artificial inoculations of truffle hosts are likely endophytes, which switch to being weak pathogens when trees are stressed. These include fungi such as *Paraconiothyrium, Paraphaeosphaeria* and *Quambularia.* Others might be considered as opportunistic pathogens, such as the *Fusarium* spp., requiring suitable conditions for disease expression. An example is a *Fusarium* sp., which was isolated from wilting *Q. robur* in WA, causing high levels mortality. However, this orchard was characterised by poor drainage, increasing the plant's susceptibility to a pathogen, which under normal circumstances might not be able to cause disease.

Truffle rot

Damage of truffles resulting in truffle rot, is a significant problem in Australia with previous studies showing up to 70% of truffles discarded in some cases, due to rot. Much lower averages were recorded during this study (see Chapter 1.4). It was shown in Western Australia, that erumpent (surface-breaching) truffles are four times more likely to show truffle rot symptoms than hypogeous (sub-surface) ones (Eslick, 2012, 2013). The main factors associated with truffle rot, were shown to be soil management, irrigation and fungal infection. Soil tillage significantly reduced the proportion of truffles affected by rot, presumably by promoting fewer erumpent truffles, although it also reduced rot in hypogeous truffles. High rates and frequency of irrigation increased the frequency of erumpent truffles. This suggests that soil hypoxia may be a primary driver of truffle rot, exacerbated when winter temperatures and rainfall are high. Lastly, the Eslick study showed the occurrence of many different fungi associated with truffles showing rot symptoms. Two of these fungi, *Trichothecium crotocinigenum* and *Acrostalagmus luteoalbus* were inoculated into healthy truffles to test their pathogenicity. Only *T. crotocinigenum* could cause rot symptoms in healthy truffles, suggesting it is a primary cause of truffle rot in erumpent truffles, which are exposed to environmental stressors (Eslick, 2012, 2013). Unfortunately, none of the *Fusarium* spp. isolates was tested for pathogenicity. *Fusarium* are common soil fungi but also important plant pathogens, therefore, they may play a role in development of truffle rot symptoms.

References

Akay Ş., Ekiz G., Kocabaş F., Hameş-Kocabaş E.E., Korkmaz K.S., Bedir E. 2014. A new 5,6-dihydro-2-pyrone derivative from *Phomopsis amygdali,* an endophytic fungus isolated from hazelnut (*Corylus avellana*). *Phytochemistry Letters* **7**:, 93-6.

Battilani, P., Chiusa, G., Arciuolo, R., Somenzi, M., Fontana, M., Castello, G., Spigolon N. 2018. *Diaporthe* as the main cause of hazelnut defects in the Caucasus region. *Phytopathologia Mediterranea* **57**: 320-33.

Diogo E.L.F., Santos J.M., Phillips A.J.L. 2010. Phylogeny, morphology and pathogenicity of *Diaporthe* and *Phomopsis* species on almond in Portugal. *Fungal Diversity* **44**: 107-15.

Eslick, H. 2012. Identifying the Cause of Rot in Black Truffles and Management Control Options. RIRDC Publication No. 12/005.

Eslick, H. 2013. Identification and management of the agent causing rot in black truffles - Part 2 Identifying the Cause of Rot in Black Truffles and Management Control Options. RIRDC Publication No. 12/005.

Gardes, M., Bruns, T.D. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**:, 113-8.

Gonthier, P., Gennaro, M., Nicolotti, G. 2006. Effects of water stress on the endophytic mycota of *Quercus robur*. *Fungal Diversity* **21**: 69-80.

Guerrero, J., Pérez, S. 2013. First report of *Diaporthe australafricana*-caused stem canker and dieback in european hazelnut (*Corylus avellana* L.) in Chile. *Plant Disease* **97**: 1657.

Lamichhane, J.R., Varvaro, L. 2014. *Xanthomonas arboricola* disease of hazelnut: current status and future perspectives for its management. *Plant Pathology* **63**: 243-54.

Maddau, L., Perrone, C., Andolfi, A., Spanu, E., Linaldeddu, B.T., Evidente, A. 2011. Phytotoxins produced by the oak pathogen *Discula quercina*. *Forest Pathology* **41**: 85-9.

Meng, L., Yu C., Wang, C., Li, G. 2018. First Report of *Diaporthe amygdali* causing walnut twig canker in Shandong Province of China. *Plant Disease* **102**: 1859.
Moricca, S., Linaldeddu, B.T., Ginetti, B., Scanu, B., Franceschini, A., Ragazzi, A. 2016. Endemic and Emerging Pathogens Threatening Cork Oak Trees: Management Options for Conserving a Unique Forest Ecosystem. *Plant Disease* **10**:, 2184-93.
Moricca, S., Ragazzi, A. 2008. Fungal endophytes in Mediterranean oak forests: A lesson from *Discula quercina*. *Phytopathology* **98**: 380-6.
Moricca, S., Ragazzi, A. 2011. The Holomorph *Apiognomonia quercina/Discula quercina* as a Pathogen/Endophyte in Oak. In: Pirttilä A, Frank A, eds. *Endophytes of Forest Trees.* Forestry Sciences, vol 80. Springer, Dordrecht.
Mullerin, S., Smith, J.A. 2015. Bot canker of oak in Florida caused by *Diplodia corticola* and *D. quercivora*. *FOR318. School of Forest Resources and Conservation, UF/IFAS Extension*.
Ragazzi, A., Moricca, S., Capretti, P., Dellavalle, I. 2000. Analysis of *Discula quercina* isolates from *Quercus* spp. *Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant Diseases and Protection* **107**: 170-5.
Ragazzi, A., Moricca, S., Capretti, P., Dellavalle, I., Turco, E. 2003. Differences in composition of endophytic mycobiota in twigs and leaves of healthy and declining *Quercus* species in Italy. *Forest Pathology* **33**: 31-8.
Ragazzi, A., Moricca, S., Dellavalle, I. 1999. Epidemiological aspects of *Discula quercina* on oak: inoculum density and conidia production. *Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant Diseases and Protection* **106**: 501-6.
Ronquist, F., Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572-4.
Sahashi, N., Kubono, T., Miyasawa, Y., Ito, S. 1999. Temporal variations in isolation frequency of endophytic fungi of Japanese beech. *Canadian Journal of Botany-Revue Canadienne De Botanique* **77**: 197-202.
Sanchez, M.E., Venegas, J., Romero, M.A., Phillips, A.J.L., Trapero, A. 2003. *Botryosphaeria* and related taxa causing oak canker in southwestern Spain. *Plant Disease* **87**: 1515-21.
Serrano, M.S., Romero, M.A., Jimenez, J.J., De Vita, P., Ávila, A., Trapero, A., Sánchez, M.E. 2015. Preventive control of Botryosphaeria canker affecting *Quercus suber* in southern Spain. *Forestry.* **88**, 500-7.Slippers B., Wingfield M.J., 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews* **21**: 90-106.
Snare, L. 2006. Pest and Disease Analysis in Hazelnuts. Project NT05002, NSW Department of Primary Industries. In. (HA Ltd, ed.).
Udayanga, D., Castlebury, L.A., Rossman, A.Y., Hyde, K.D. 2014. Species limits in *Diaporthe*: molecular re-assessment of *D. citri, D. cytosporella, D. foeniculina* and *D. rudis*. *Persoonia* **32**: 83-101.
Weste, G. 2003. The dieback cycle in Victorian forests: a 30-year study of changes caused by *Phytophthora cinnamomi* in Victorian open forests, woodlands and heathlands. *Australasian Plant Pathology* **32**: 247-56.

1.4 Assessment of pest damage and disease in truffles

Objectives

Truffle harvest assessments were undertaken to quantify the levels of damage and therefore financial loss as a result of feeding by invertebrates and truffle rots. This information was not readily available from earlier studies. It was recognised that such data would be useful to monitor the importance of these agents. Also, it was planned to determine whether there was an association between pest occurrence from previous orchard monitoring of invertebrates and the subsequent level of invertebrate damage and rot and reduced value of the truffles after grading.

Methodology

Due to personnel and geographic constraints within this project, gathering regular and detailed information associated with this study was restricted to truffle orchards in Western Australia. Limited observations were made in truffle orchards in eastern Australia during study tours there.

In the south western truffle growing region of Australia, truffle harvesting occurs on a weekly basis, from the start of the season as early as May and concludes as late as September. The main harvest months are June, July and August. During the 2016, 2017 and 2018 seasons, truffles were harvested for assessment across a number of truffle orchards but not always the same orchards each season.

In 2016 harvest assessments took place on four properties, with four to six assessments conducted per property. The first harvest was on 21 June and the last on 23 August.

In 2017, harvest assessments took place on five properties, with five to seven assessments conducted per property. The first harvest was on 2 June and the last on 4 September.

In 2018, harvest assessments took place on eleven properties. Five of the properties had three harvests assessed, one property six harvests, one property two harvests and the remaining four properties had only one harvest assessed. The first harvest was on 12 June and the last on 23 August.

Truffles were assessed for levels of rot and levels and types of invertebrate damage. Because this type of assessment had not been conducted before, the method was modified based on experience with assessments each season. There were field and laboratory components to each harvest assessment. Lab assessments took place after truffles were washed and dried.

<u>Equipment - Field</u>

- Collection bag
- Two plastic bags, for partially and fully rotten truffles
- Field observation sheet
- Collecting vials, tweezers & hand lens
- Truffle spade
- Disposable gloves, over-boots and/or footbath.

Equipment - Lab

- Grading room scoresheet
- Collecting vials, ‘seekers’, tweezers & hand lens
- Truffle knife.

Field collection and information, all years
In all years and harvests, a member of the project team accompanied the truffle hunters and harvesters and these steps followed:

- Collect 50 truffles, preferably non-consecutive
- Where there are many truffles in one hole include no more than two of average size
- Include partly rotten truffles; if asked, collect them separately into a plastic bag
- Include fully rotten truffles on the scoresheet. If asked, collect them in a separate plastic bag or leave in the orchard
- If pests are present in the field, record pest details and depth of the top of the truffle on the field observation sheet
- If the pest is unknown, collect and record details on vial.

Field collection and Field and Lab information, 2016

- In the field, record the depth that each of the 50 truffles was growing according to the depth of the top of the truffle:
    - ‘exposed’ (E) if the top of the truffle had breached the soil surface
    - ‘shallow’ (S) if the top of the truffle was below the soil surface but no deeper than 2.5cm and
    - ‘deep’ (D) if the top of the truffle was greater than 2.5cm below the soil surface.
- Record in-field assessment of the estimated percentage of each truffle that had been damaged with pinholes or gouges by slugs and slaters
- After field assessment, place all truffles in a single collection bag
- In the lab weigh truffles and again assess for estimated percentage of each truffle that had been damaged with pinholes or gouges by slugs and slaters.

Field collection and Field and Lab information, 2017

In an analysis of the 2016 data it was found that estimating damage in the field was not accurate and varied from that recorded in cleaned truffles in the lab. The method was changed the following years to reflect this.

The following equipment was added for the field component for 2017 and 2018:

- Three coloured collection bags, one for each depth
- One plastic bag for partly rotten truffles, per collection bag
- One plastic bag for fully rotten truffles. Or these may be left in orchard as requested by the grower.

No assessments of damage were done in the field in 2017 or 2018. The following steps were added to the field and lab methods:

- In the field collect truffles according to the depth of the top of the truffle as described above, place truffles in separate coloured bags
- In the lab, record truffle harvest depth, weigh and assess for estimated percentage of each truffle that had been damaged with pinholes or gouges by slugs and slaters.

Field collection and Field and Lab information, 2018

In 2018 more detailed information was gathered in the lab assessments to provide more insight into the impact of invertebrate damage and rot on truffle grades and monetary value. Pre and post trim truffles classes were recorded to determine the effect of adverse factors on downgrading the class to which a damaged truffle would have otherwise been assigned. Project team members worked alongside professional truffle graders in the lab to ensure market standards in truffle grading were adhered to.

The following steps were added to the field and lab methods:

- In the field, if possible, only collect truffles that are estimated to be 15g or greater to avoid truffles that are automatically classed as trim/peel
- In the lab, record weight of un-trimmed truffle
- Assess and record damage type – slug/slater, rot, pinholes or neat holes
- Work with the truffle grader to record pre-trim truffle market class for each truffle, this class is determined by truffle attributes such as size, shape and colour, overlooking any invertebrate damage
- Record a post-trim class for each truffle
- Re-weigh truffle after trimming and record the post-trimmed weight
- When truffle grading is completed for each depth, record the total weights of the trim classes.

Results

In the figures below, some data are labelled as “all properties”. For 2016 this is data that were combined from all four properties. For 2017 data from all five properties are used for the “all properties” averages. In the 2018 figures, data from the all eleven properties are included in the “all properties” average yet only the five properties that were assessed three or more times is shown separately.

Properties were allocated the same identification letter over the course of the project. Property A was assessed as one property in 2016; in 2017 and 2018 it was monitored as two separate sections of the one property because each received different management strategies leading to differences in the abundance of ground dwelling invertebrates. This suggested there may be differences in the level of damage to truffles.

Truffle Depth

There was no apparent major variation in the average proportion of truffles harvested from each depth from all properties across the three years of harvest (Figs. 1.4.1, 2 and 3). Truffles in the shallow depth range accounted for around 50% of truffles harvested in each year and for the other depth categories, exposed and deep truffles were of roughly equal proportions.

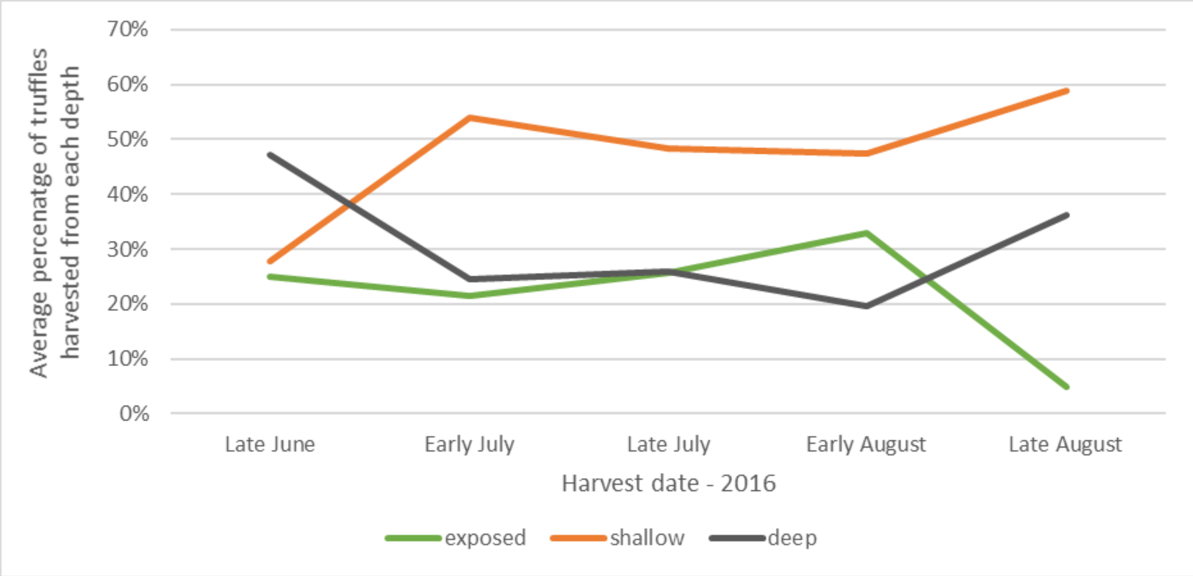


Fig. 1.4.1. Percentage of truffles harvested from each depth category from properties A, B, C and D for the 2016 season. Harvest dates varied among properties and so have been categorised as either early harvest, first half of the month or late harvest, last half of the month, for each month.

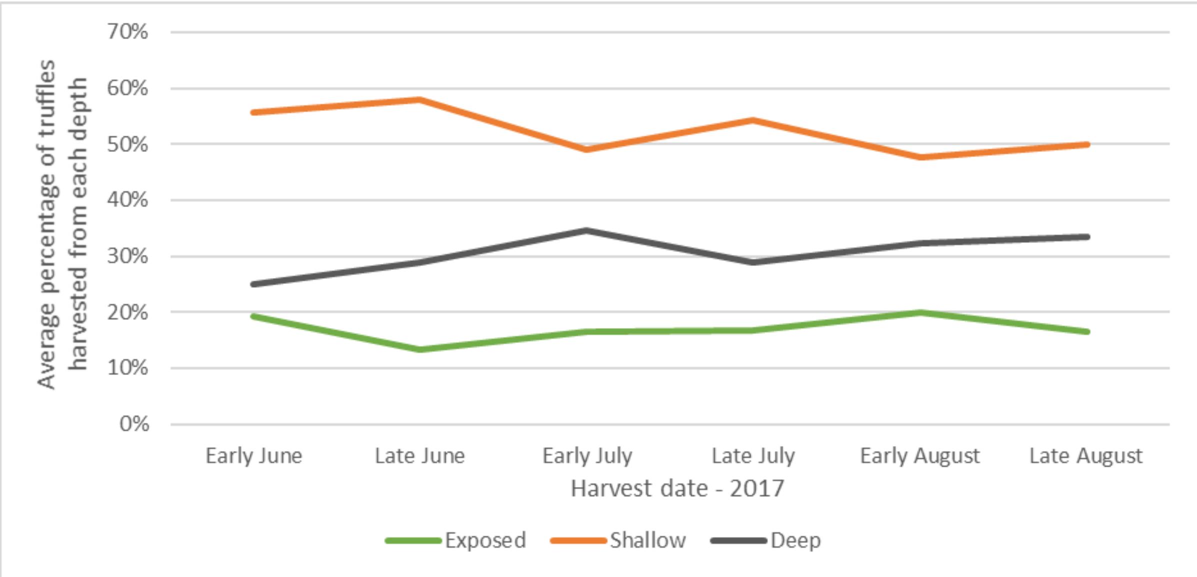


Fig. 1.4.2**.** Percentage of truffles harvested from each depth category from properties A1, A2, B, C, E and F for the 2017 season. Harvest dates varied among properties and so have been categorised as either early harvest, first half of the month or late harvest, last half of the month, for each month.

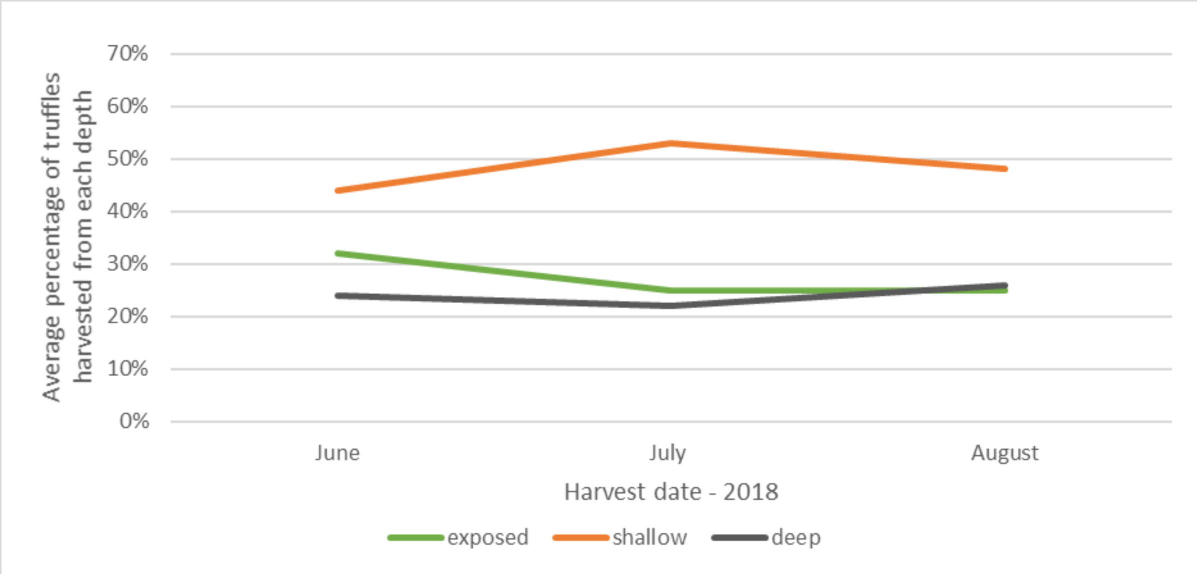


Fig. 1.4.3. Percentage of truffles harvested from each depth category from properties A, B, E, F, G, H, I, J, K, L and M for the 2018 season. Harvest dates varied among properties and so have been categorised as either early harvest, first half of the month or late harvest, last half of the month, for each month.

When the harvests for each entire season are averaged individually for each property, there is considerable variation in the percentage of truffles harvested from each depth among properties (Figs. 1.4.4, 5 and 6). The proportion of harvested truffles at the shallow depth category were consistently high across properties, ranging from the lowest season average of 38% at property H in 2018 to a high of 60% at property A in 2016. There was greater variation in the other two depth categories, with the proportion of truffles in the depth category of exposed ranging from a season average of 3% at property A2 in 2017 to 52% for property D in 2016. Likewise there was a considerable range in the proportion of truffles in the depth category of deep, ranging from a season average of 8% for property G in 2018 to 54% for property A2 in 2018.

For most properties, the proportion of truffles harvested from each depth remained very similar among all years.

Fig. 1.4.4. Average percentage of truffles harvested from every harvest, from each depth category for each property assessed in 2016.

Fig. 1.4.5. Average percentage of truffles harvested from every harvest, from each depth category for each property assessed in 2017.

Fig. 1.4.6. Average percentage of truffles harvested from every harvest, from each depth category for each property that had three of more harvests assessed in 2018, and combined average from all properties assessed in 2018.

Truffle Weight

In 2016 there was a slight trend to higher individual truffle weights for truffles harvested in early to mid July that tapered off as the season progressed (Fig. 1.4.7). This was not repeated in 2017, where truffles weights as an average across all properties for each depth category remained consistent over the course of the season between 35 and 45g (Fig. 1.4.8). In 2018, average truffle weights declined slightly as the season progressed, but again remained between 35 and 45g (Fig. 1.4.9). There was no difference in weights among truffles harvested from different depths (Figs. 1.1.8 and 1.4.9).

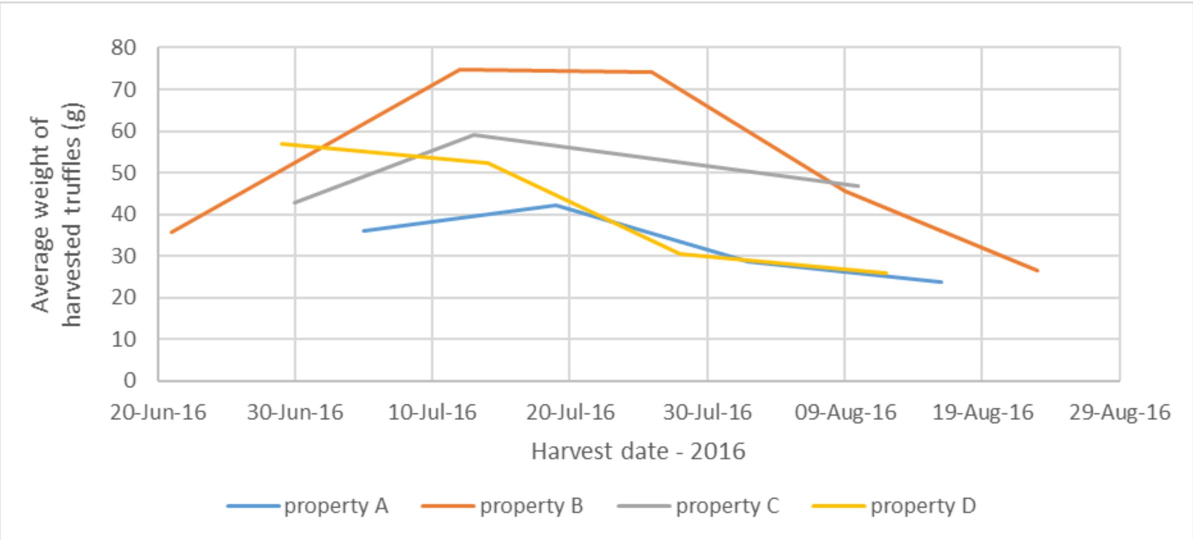


Fig. 1.4.7. Average weight of all truffles from each harvest for each of the four properties assessed in 2016.

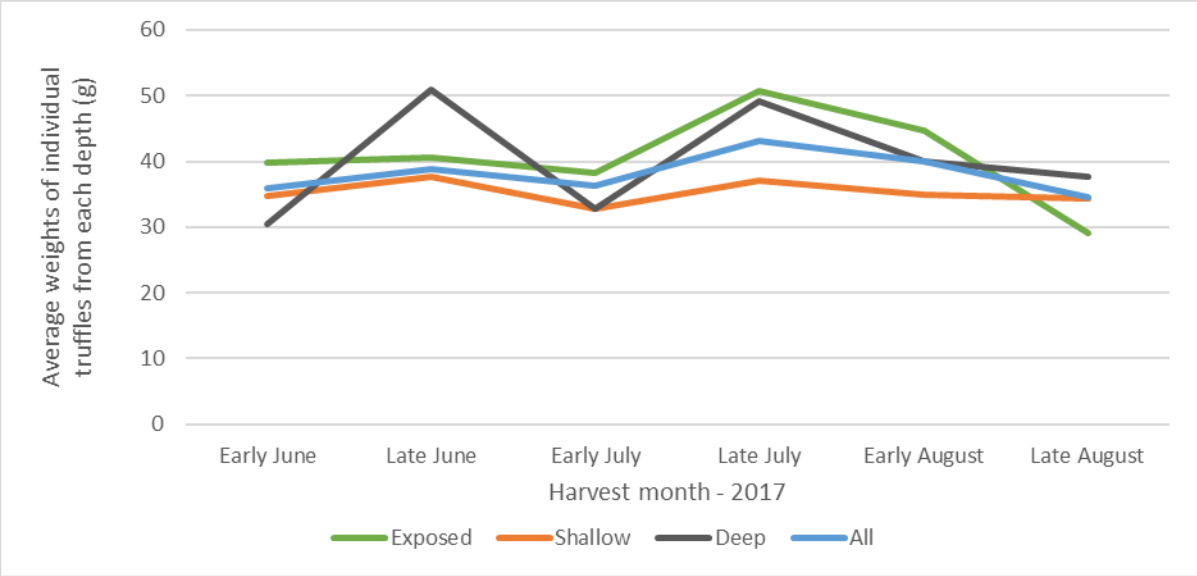


Fig. 1.4.8. Average weights of truffles harvested from each depth category from properties A, B, C, E and F for the 2017 season. Harvest dates varied among properties and so have been categorised as either early harvest, first half of the month or late harvest, last half of the month, for each month.

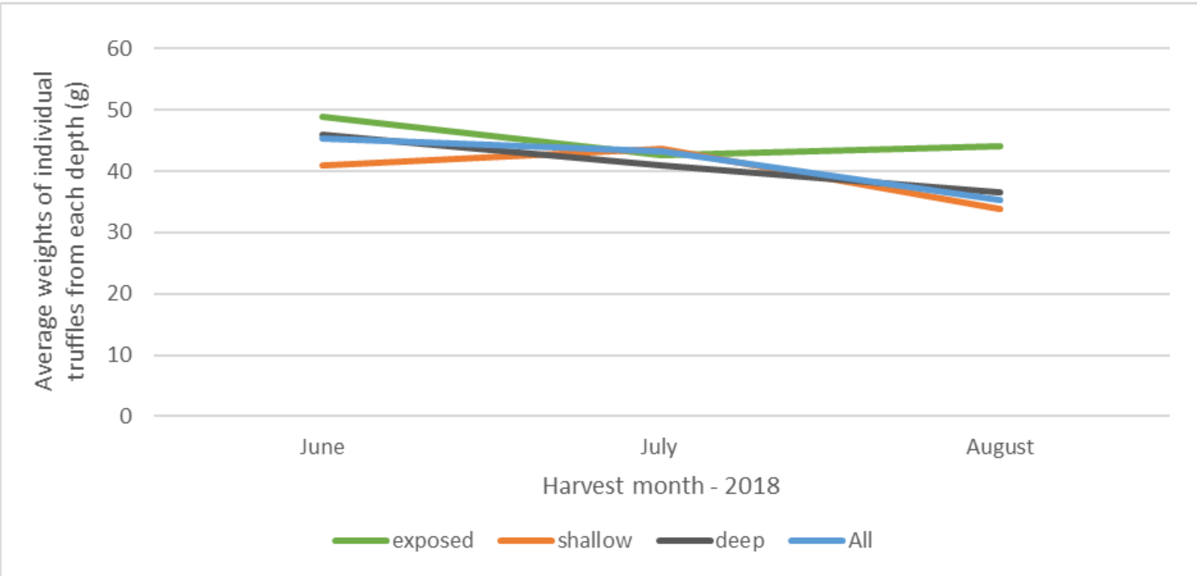


Fig. 1.4.9. Average weights of truffles harvested from each depth category from properties A, B, E, F, G, H, I, J, K, L and M for the 2018 season. Harvest dates varied among properties and so have been categorised as either early harvest, first half of the month or late harvest, last half of the month, for each month.

There was some variation in average truffles weights among properties and also for the same property among seasons (Figs. 1.4.10 and 11). For example the average harvested weight across all properties and depths increased only marginally between 2017 and 2018 from 39g to 42g, yet the average weight of truffles harvested from property E dropped from 42g to 35g, while the average for property F increased from 44g to 62g. Properties B and F were above average for both years, while property A1 and A2 were below average in both years.

Fig. 1.4.10. Average weight of individual truffles harvested from each depth category for each property assessed in 2017.

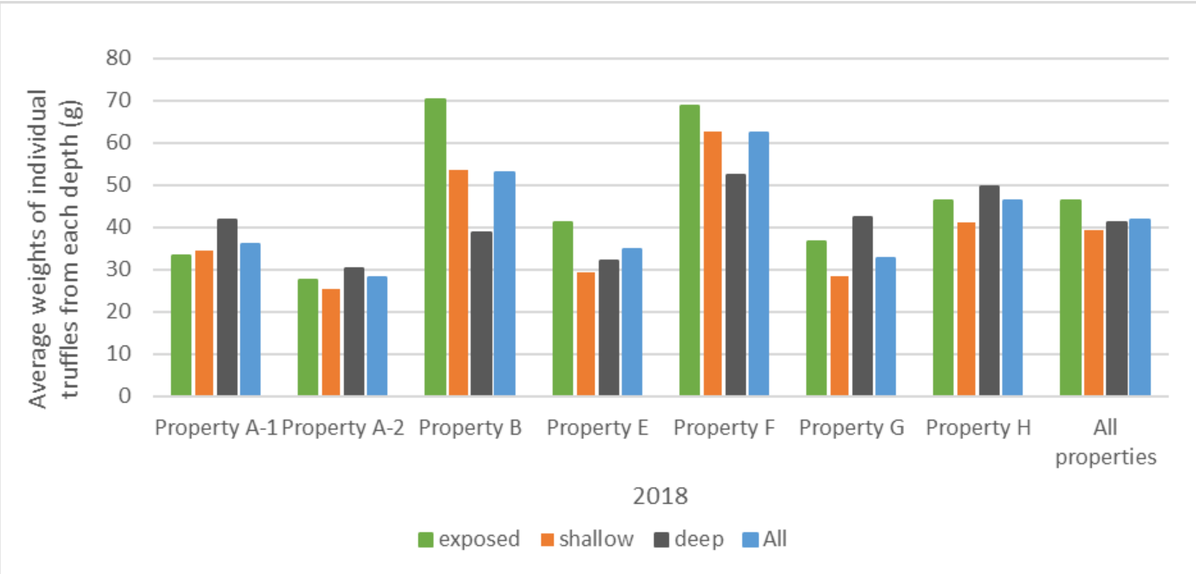


Fig. 1.4.11. Average weight of individual truffles harvested from each depth category for each property that had three of more harvests assessed in 2018, and combined average from all properties assessed in 2018.

<u>Truffle Damage – Rot</u>

While the percentage of truffles with rot in individual orchards varied greatly over the course of the season in 2016 (Fig. 12), when the seasonal data was averaged for all properties in 2017 and 2018 there were more consistent trends (Figs. 13 and 14) - the trends for these two years were opposite. In 2017 the proportion of truffles with rot was highest at the start of the season at over 15%, dropping quickly to an average of less than 5%, then rising slightly again at the end of the season (Fig. 13). In 2018 the percentage of truffles with rot was lower for the early harvests at 10%, increasing slightly before dropping slightly again at the end of the season (Fig. 14). Although with fewer harvests assessed per property in 2018, a seasonal trend is less likely to be demonstrated.

Fig. 1.4.12. Percentage of truffles with any sign of rot from every harvest for each property assessed in 2016.

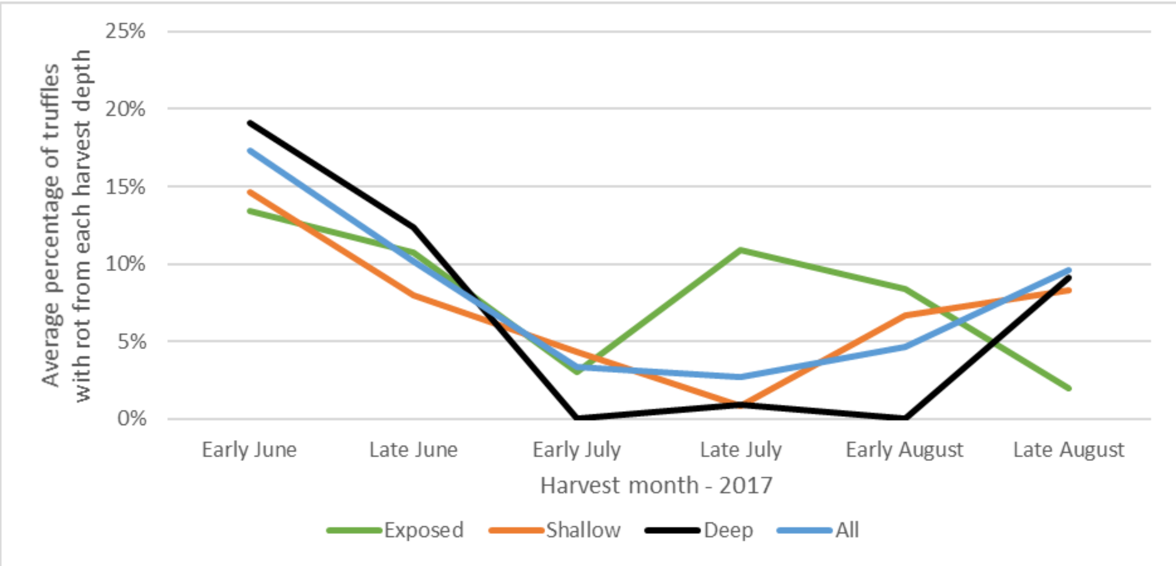


Fig. 1.4.13. Average percentage of truffles with any sign of rot from each depth expressed as a combination across all properties assessed in 2017. Harvest dates varied between properties and so have been categorised as either early harvest, first half of the month or late harvest, last half of the month, for each month.

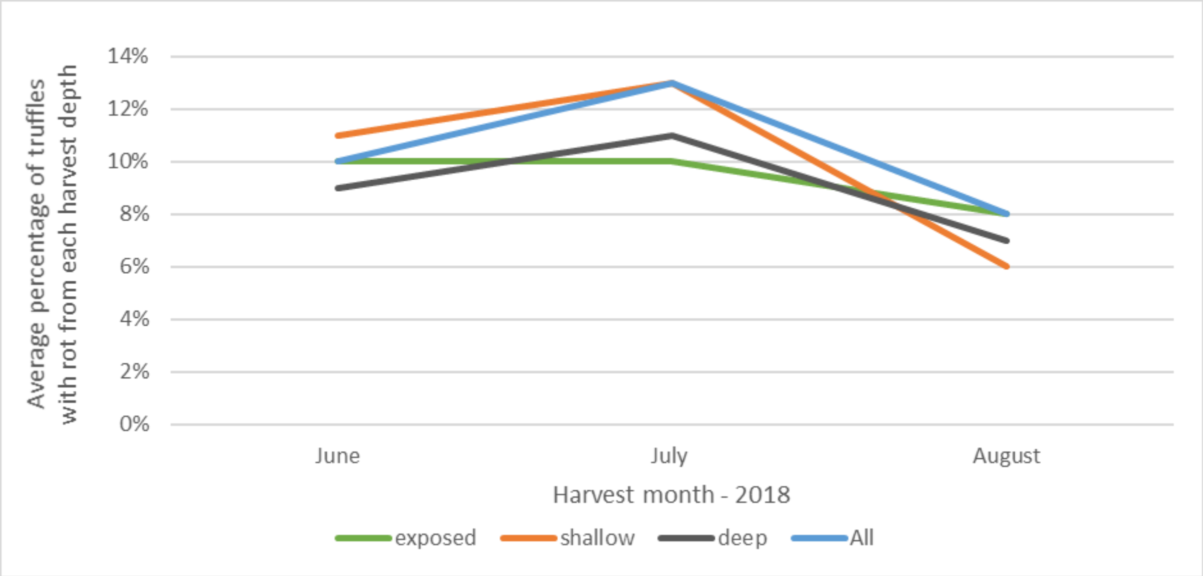


Fig. 1.4.14. Average percentage of truffles with any sign of rot from each depth and expressed as a combination across all properties assessed in 2018. Harvest dates varied among properties and so have been categorised as either early harvest, first half of the month or late harvest, last half of the month, for each month.

When the season averages for each property are compared, there was much higher variation among properties in 2017 compared to 2018 (Figs. 1.4.15 and 16).

Between the 2017 and 2018 seasons, the average percentage of truffles with any sign of rot across all properties and depths increased from 9% to 12%. Each of the properties that were assessed in both season experienced an increase in rot levels, most notably properties A1, A2, and E where the average percentage of truffles with rot across all depths doubled between 2017 and 2018.

The pattern of rot with depth also changed across seasons. In the average of all properties in 2017 the proportion of truffles with rot decreased with depth. Properties A2, E and F had relatively high levels of rot in their exposed truffles and very little in the deep truffles. Properties B and C showed the opposite trend - the level of rot increased with depth. In 2018 the average for all properties had a trend of increasing rot with depth. Properties A2 and G had very low rot levels in the exposed truffles and property H also had low levels when compared with their deep truffles. Property A2 went from having no rot recorded in deep truffles in 2017 to above average levels in 2018. However, it must be noted that most harvests from A2 in 2017 did not reach 50 truffles and so there is a smaller sample size from this property.

Fig. 1.4.15. Average percentage of truffles with any degree of rot from each depth category for each property assessed in 2017.

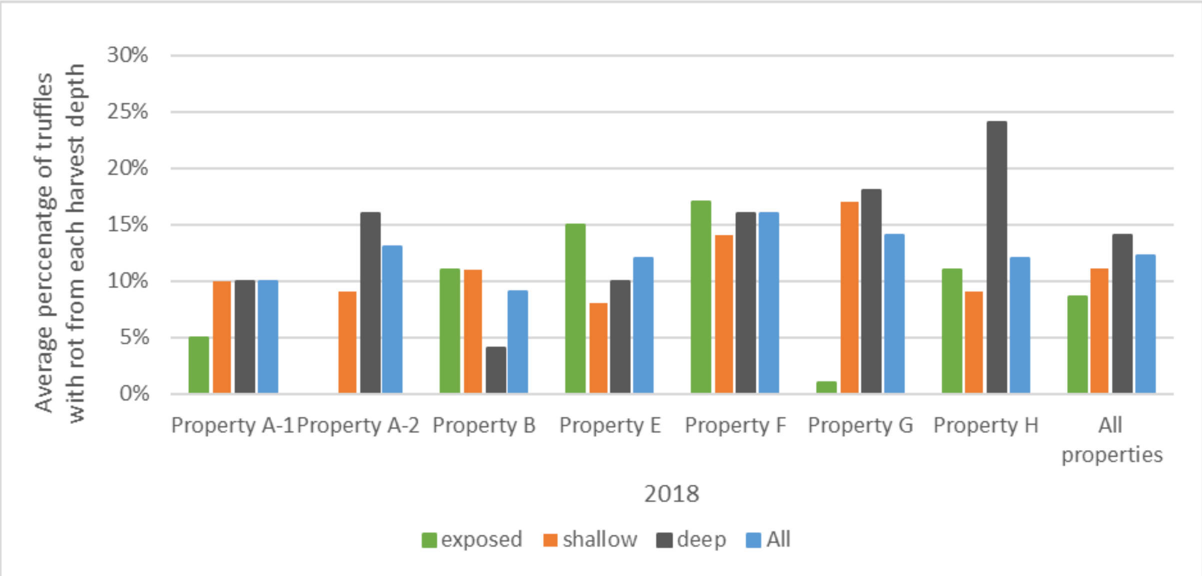


Fig. 1.4.16. Average percentage of truffles with any degree of rot from each depth category for each property that had three of more harvests assessed in 2018.

The seasonal trends for the average percentage of rot per truffle, that is, the percentage of each truffle that is rotten, was very similar to the average percentage of all truffles with any rot. In 2017, the average percentage of rot per truffle was highest at the start of the season, with the average for all depths being 6.6%. This proportion gradually decreased over June and July and remained relatively low, at around 1% for the last month of the season (Fig. 1.4.17). In 2018, the percentage of rot per truffle, across all harvest depths, was consistent for the whole season at, around 5% (Fig. 1.4.18). Although, with fewer harvests per property in 2018 the data are less likely to show seasonal trends.

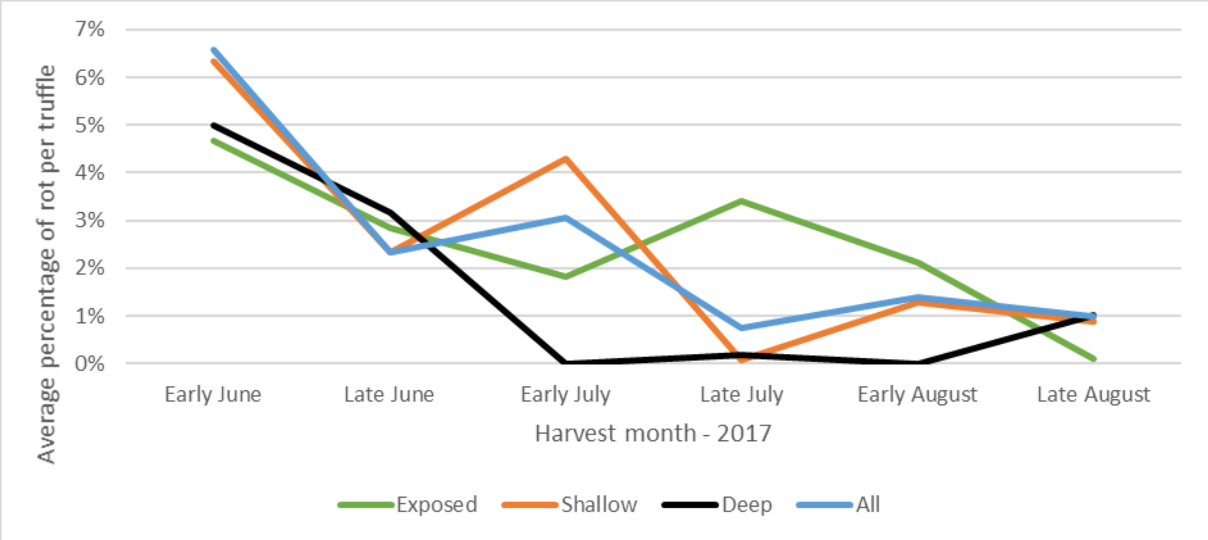


Fig. 1.4.17. Average percentage of each truffle that is rotten from each depth and all properties assessed in 2017. Harvest dates varied between properties and so have been categorised into either early harvest, first half of the month or late harvest, last half of the month, for each month.

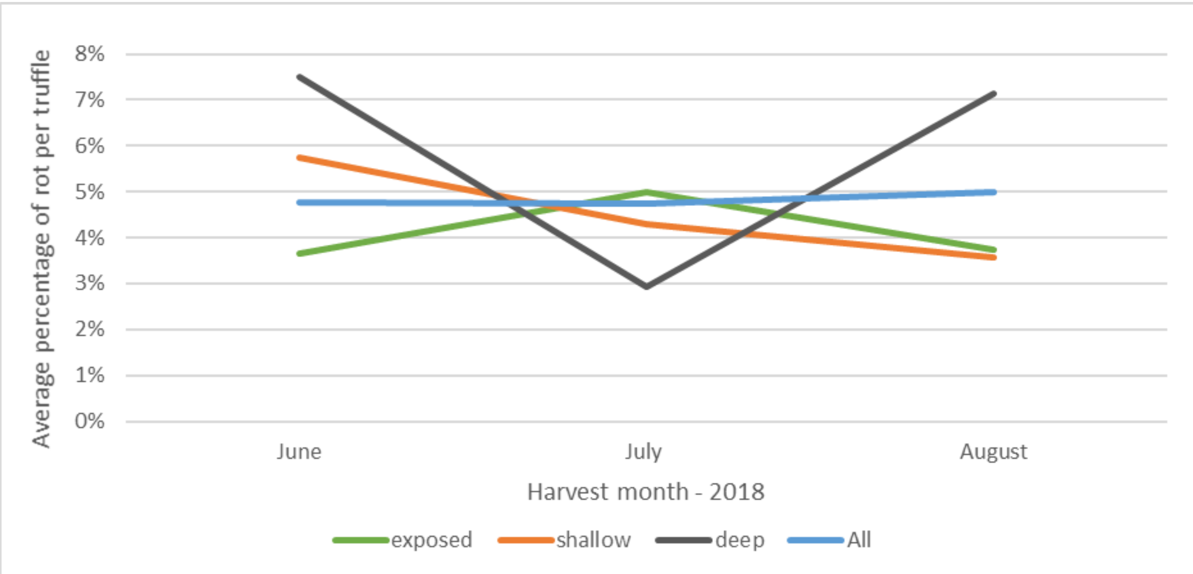


Fig. 1.4.18. Average percentage of each truffle that is rotten from each depth and all properties assessed in 2018. Harvest dates varied between properties and so have been categorised into either early harvest, first half of the month or late harvest, last half of the month, for each month.

Similar comparisons for the proportion of individual truffles with rot between the 2017 and 2018 seasons were evident when examined on an individual property basis. The seasonal averages showed a decreasing trend with depth in 2017 and increasing in 2018 (Figs. 1.4.19 and 20). Not only did more truffles have signs of rot in 2018 compared to 2017 but the amount of rot per truffle was also greater for many properties and the average for all properties for all depths was 2.7% in 2017 and 4.2% in 2018.

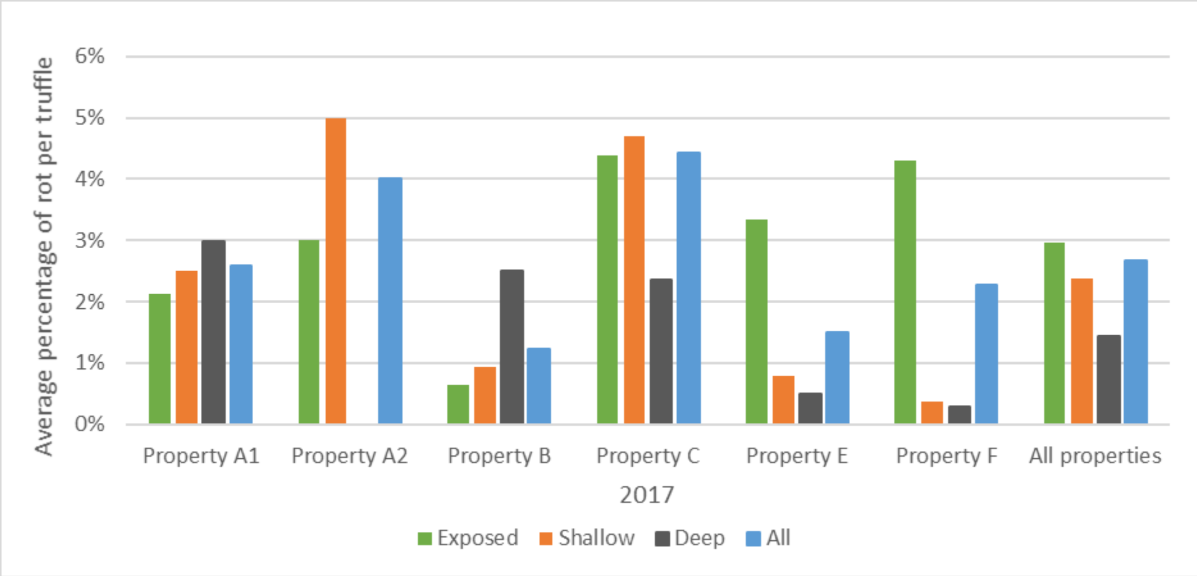


Fig. 1.4.19. Average percentage of each truffle that is rotten from each depth for each property assessed in 2017.

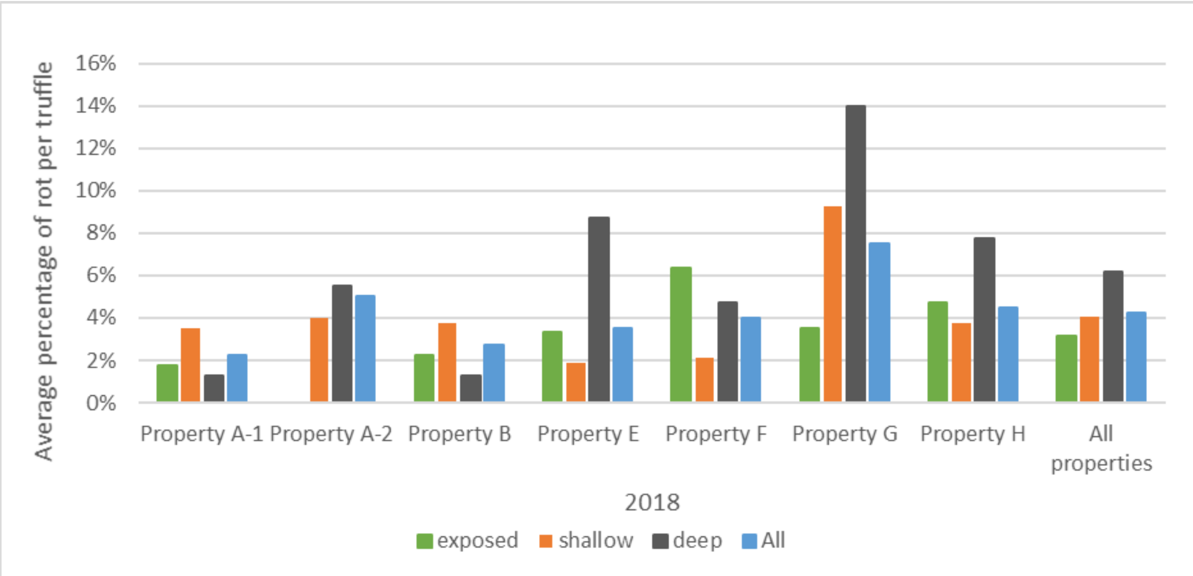


Fig. 1.4.20. Average percentage of each truffle that was rotten for each depth for each property that had three of more harvests assessed in 2018, and combined average from all properties assessed in 2018.

<u>Truffle Damage – Invertebrates</u>

Although the method of data collection changed from season to season, the seasonal trends for average level of invertebrate damage from all properties remains relatively consistent within any of the seasons (Figs. 1.4.21, 22 and 23). There were no obvious trends across each harvest season for the magnitude of invertebrate damage.

The percentage of truffles with any sign of invertebrate damage was very similar for 2016 and 2017, at around 55-65%. The average was lower in 2018, at around 30-35% for most of the season. In all years, the exposed truffles had a higher percentage that were damaged compared to shallow or deep truffles.

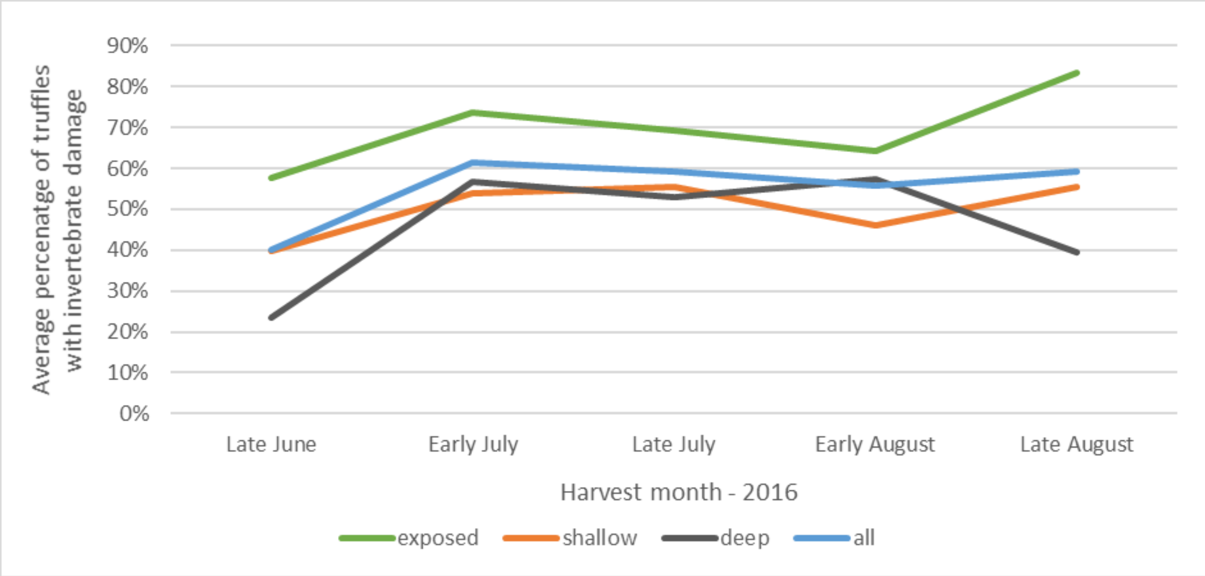


Fig. 1.4.21. Average percentage of truffles with any sign of invertebrate damage for all properties assessed in 2016. Harvest dates varied among properties and so have been categorised as either early harvest, first half of the month or late harvest, last half of the month, for each month.

Fig. 1.4.22 shows both the total level of truffles with any damage from invertebrates and damage attributed to slugs and slaters. It is evident that the majority of truffles with invertebrate damage have damage attributed to slugs and slaters. Some truffles may have both slug and slater damage as well as damage from other invertebrates, however slugs and slaters accounted for most of the invertebrate damage.

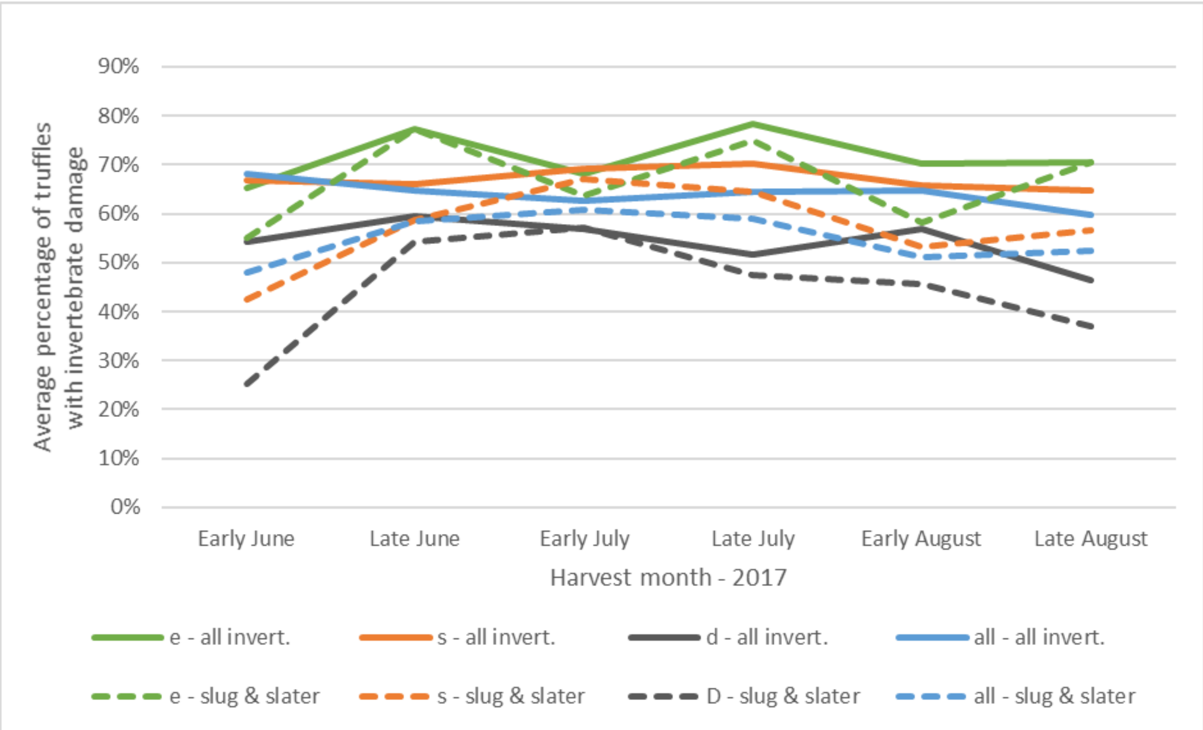


Fig. 1.4.22. Average percentage of truffles with any sign of invertebrate damage (solid line) and slug/slater damage (dashed line) for all properties assessed in 2017. Harvest dates varied among properties and so have been categorised as either early harvest, first half of the month or late harvest, last half of the month, for each month. Depths at which truffles were harvested: e=exposed, s=shallow, d=deep (see text for details).

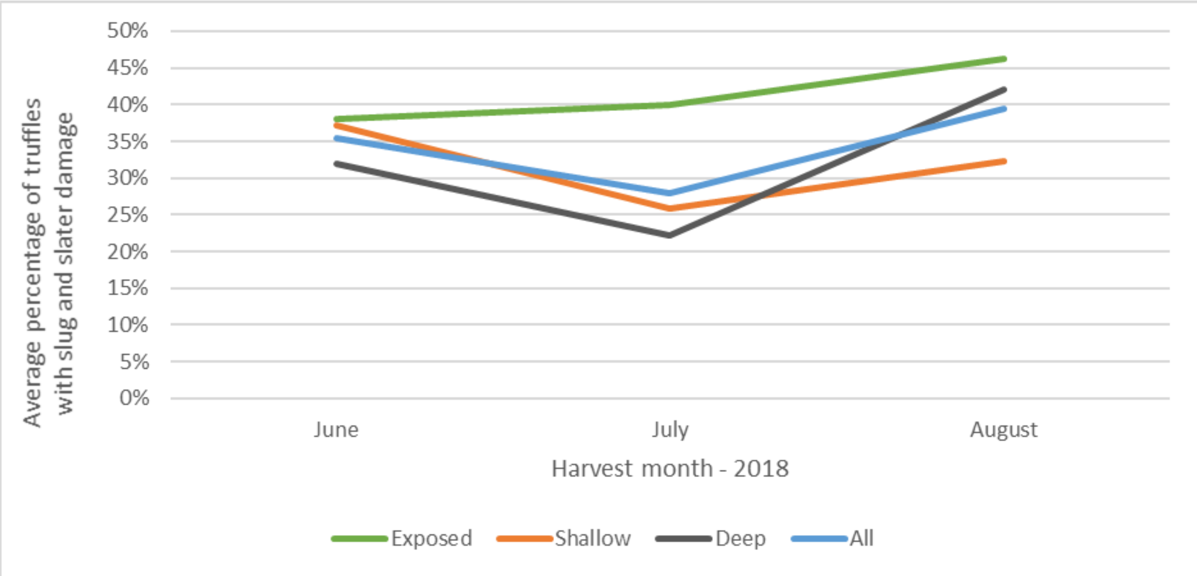


Fig. 1.4.23. Average percentage of truffles with any sign of slug/slater damage for all properties assessed in 2018. Harvest dates varied among properties and so have been categorised as either early harvest, first half of the month or late harvest, last half of the month, for each month.

For each property in 2016 the exposed truffles had the greatest percentage with invertebrate damage (Fig. 1.4.24). Average percentage of truffles with damage was 68% for those exposed compared to 50% for shallow and 48% for deep. Property A had the highest rate of damage at 81% in the exposed truffles and 65% over all depths.

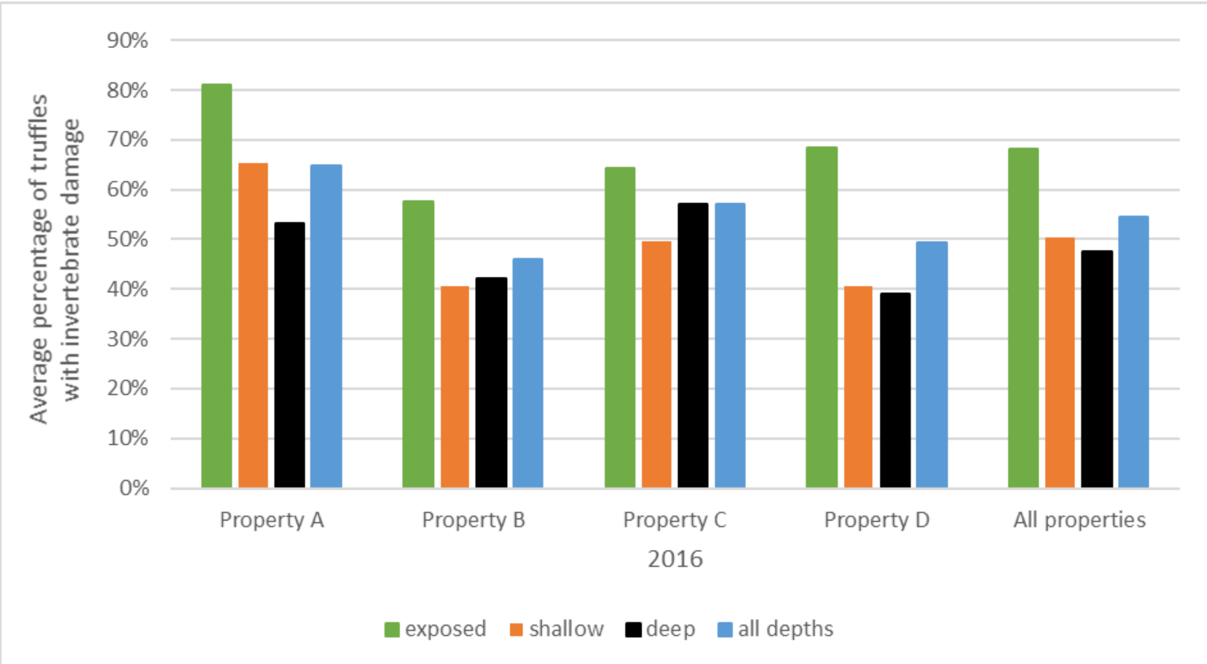


Fig. 1.4.24. Average percentage of truffles with any sign of invertebrate damage from each depth category for each property assessed in 2016.

This trend of less damage with depth was evident again for the 2017 season with only properties B and C experiencing more damage in the shallow and deep truffles, respectively. For properties B and C, the higher levels of damage at depth were attributed to invertebrates other than slugs and slaters, namely 'Australian truffle beetle' and springtails. There was greater variation among properties in 2017 - property B had the least percentage of invertebrate-damaged truffles at 24%, compared to property A2 with an average of 85% of truffles damaged across all depths. This compares to 2016 when the high and low were 46% and 65%.

For 2018 Fig. 1.4.26 shows only slug and slater damage and again the exposed truffles show the greatest percentage damage, with the exception of property F.

For most properties the majority of damage can be attributed to slugs and slaters. However, despite properties B and C having the lowest levels of damage, the percentage of truffles with damage caused by invertebrates other than slugs and slaters is highest in these two orchards (Fig. 1.4.25). These two properties had the lowest numbers of slugs and slaters recorded as part of regular monitoring.

Fig. 1.4.25. Green, orange, grey and blue bars show average percentage of truffles with any sign of slug and slater damage from each depth category (E=exposed, S=shallow, D=deep, see text for details) and property average across depths for each property assessed in 2017. Red bars show damage from all other invertebrates at each depth and average for all depths.

The average percentage of truffles across all depth with any sign of invertebrate damage was very similar for 2016 and 2017, at around 55%. There was a marked reduction in slug and slater damage between 2017 and 2018 (Figs. 1.4.25 and 26) for all properties, the average declined from 71% for exposed and 57% for all depths to 41% for exposed and 34% for all depths. Properties E and F had particularly large reductions between 2017 and 2018 for slug and slater damage at all depths. In property E, damage in exposed truffles declined from 87% to 48%, while in property F the damage level declined from 96% to 36%. Slug and slater damage across all depths declined from 82% to 38% and 79% to 35% for properties E and F respectively. This is consistent with lower abundance of slugs and slaters recorded in those orchards. Property A2 had very high slug and slaters numbers and although only a small number of exposed truffles were harvested, all of them had some level of slug and slater damage. In 2018, the variation in the percentage of damaged truffles among orchards was greater than previous years.

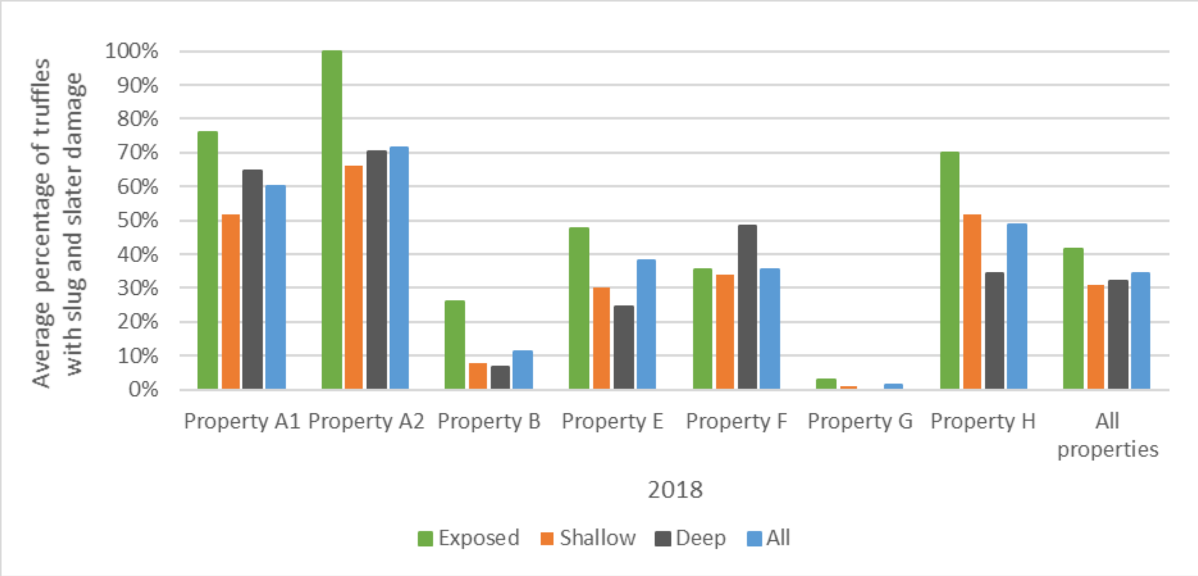


Fig. 1.4.26. Average percentage of truffles with any sign of slug/slater damage from each depth category and each property that had three of more harvests assessed in 2018, and combined average from all properties assessed in 2018

Slugs and slaters may not always be the main cause of invertebrate damage to truffles. Properties B and G had some of the lowest incidences of slug and slater damage in 2018, but had some of the highest incidences of damage from other invertebrates (Fig. 1.4.27). Property J also had a very high percentage of truffles damaged by other invertebrates and a low percentage damaged by slugs and slaters. Most of the other invertebrate damage at property B was attributed to 'Australian truffle beetle", at property G springtails and at property J millipedes.

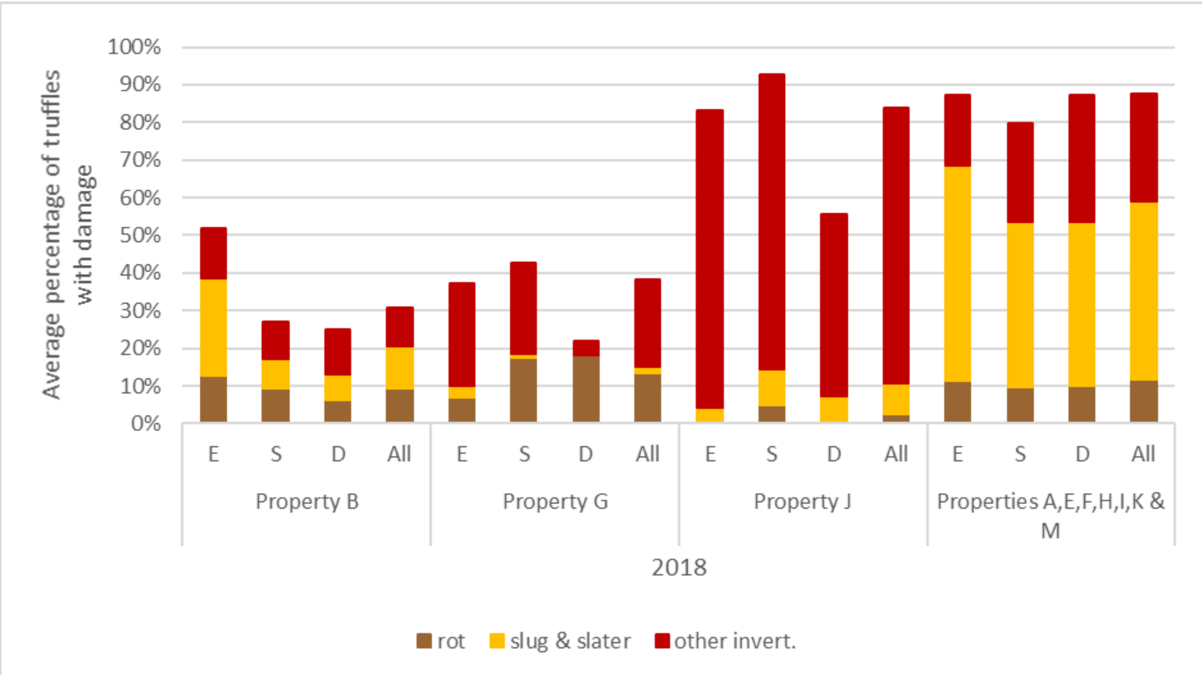


Fig. 1.4.27. Average percentage of truffles with damage attributed to rot, slugs/slaters or all other invertebrates for properties B, G, and J and a combined average for properties A, E, F, H, I, K and M, for the 2018 harvest

The percentage of each individual truffle damaged by invertebrates, across all orchards assessed for invertebrate damage in the 2017 harvest season, was highest for exposed truffles with decreasing proportions of truffles damaged with depth (Fig. 1.4.28). In addition, slugs and slaters contributed the majority of this damage.

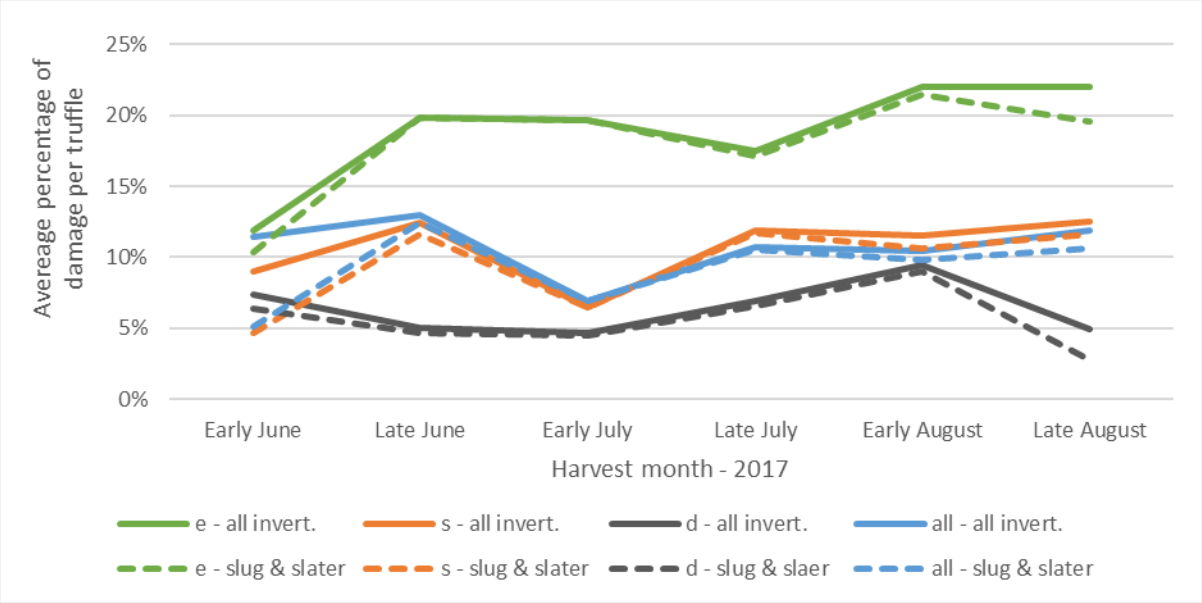


Fig. 1.4.28. Average percentage of each truffle damaged by invertebrates (solid line) and slugs and slaters (dashed line) for all properties assessed in 2017. Harvest dates varied between properties and so have been categorised into either early harvest, first half of the month or late harvest, last half of the month, for each month; e=exposed, s=shallow, d=deep.

The same trend of slug and slater damage with depth was recorded for truffles among orchards where assessments were made in the 2018 season (Fig. 1.4.29).

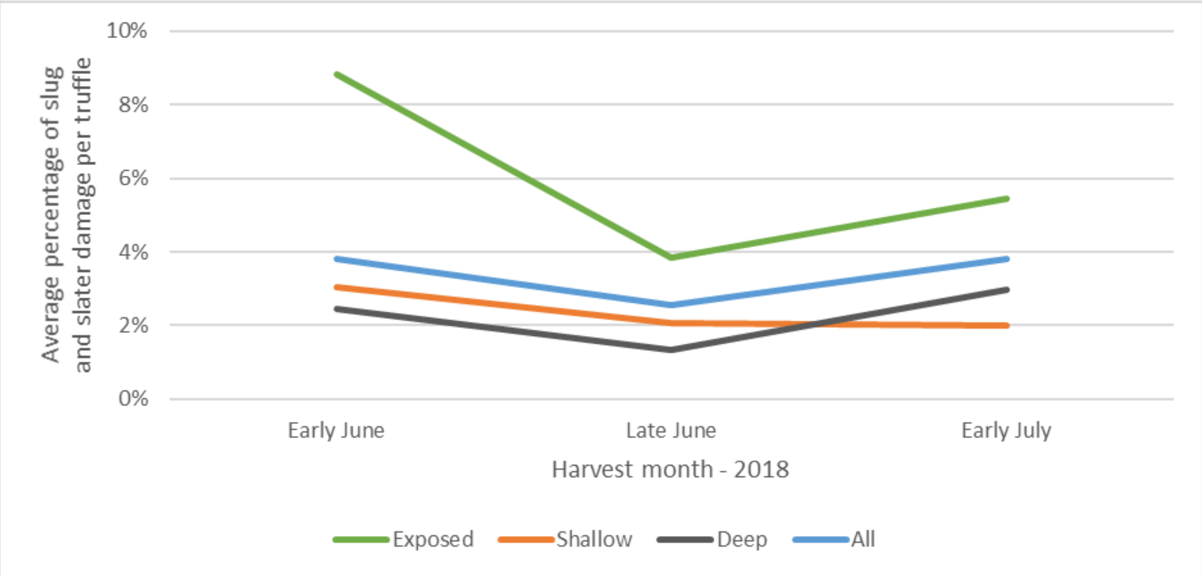


Fig. 1.4.29. Average percentage of each truffle damaged by slugs and slaters for all properties assessed in 2018.

Those orchards that had lower proportions of truffles with any damage also had a low average percentage of damage to individual truffles (Fig. 1.4.30). The percentage of damage to individual truffles caused by other invertebrates was very low across most orchards. However, the method of recording this data provides a good indicator of truffle loss for most invertebrates; particularly slugs and slaters where generally only a relatively small amount of undamaged truffle needs to be trimmed prior to sale, however this is not the case for tunnelling pests such as 'Australian truffle beetle'. The beetles only eat a small percentage of the truffle yet this tunnelling may result in considerably more being trimmed to ensure a clean, pest free truffle.

Fig. 1.4.30. Green, orange, grey and blue bars show average percentage of each truffle damaged by slugs/slaters from each depth category for each property assessed in 2017. Red bars show damage from all other invertebrates. E=exposed, S=shallow, D=deep.

There were generally lower slug and slater numbers recorded in orchards in 2018, compared to 2017. The percentage of damage to individual truffles was also lower in 2018, with the exception of property A2 where slug and slater numbers remained high. The greatest reduction in truffle damage was for property F that averaged 24% damage to each truffle in 2017, with over 40% of exposed truffles damaged, compared to much lower averages in 2018 of 2.3% damage to each truffle across all depths and 3.5% for exposed truffles (Fig. 1.4.31).

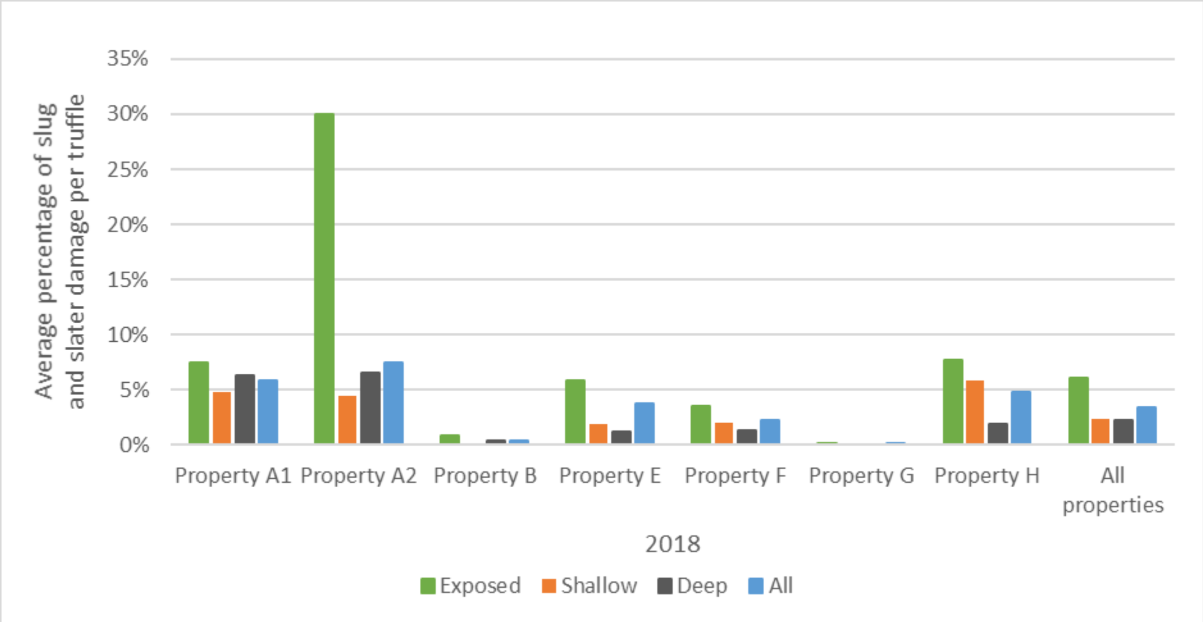


Fig. 1.4.31. Average percentage of each truffle damaged by slugs/slaters from each depth category and average across depths for each property that had three of more harvests assessed in 2018, and the combined average from all properties assessed in 2018.

Truffle Damage – Grading

The grading results are from 2018 and only include properties A1, A2, B, E, F, G and H. Grading information was not collected in assessments from the other properties.

Due to the assessment process and the constraints of being in commercial grading rooms each piece of trim could not be attributed to a single cause. All trim from truffles with any sign of invertebrate damage has been attributed to invertebrates in these results. All trim from truffles without any sign of invertebrate damage has been attributed to “other”. Other reasons for trimming include rot, cracks and poor colour.

Among all properties, on average, 21% of all exposed truffles had to be trimmed off due to invertebrate damage (Fig. 1.4.32). Property A2 had extreme levels of damage to exposed truffles, when this property is not included, the average decreases from 21% to 13.9% of each truffle trimmed due to invertebrates. The amount trimmed in shallow and deep truffles is less at 13% and 10% respectively.

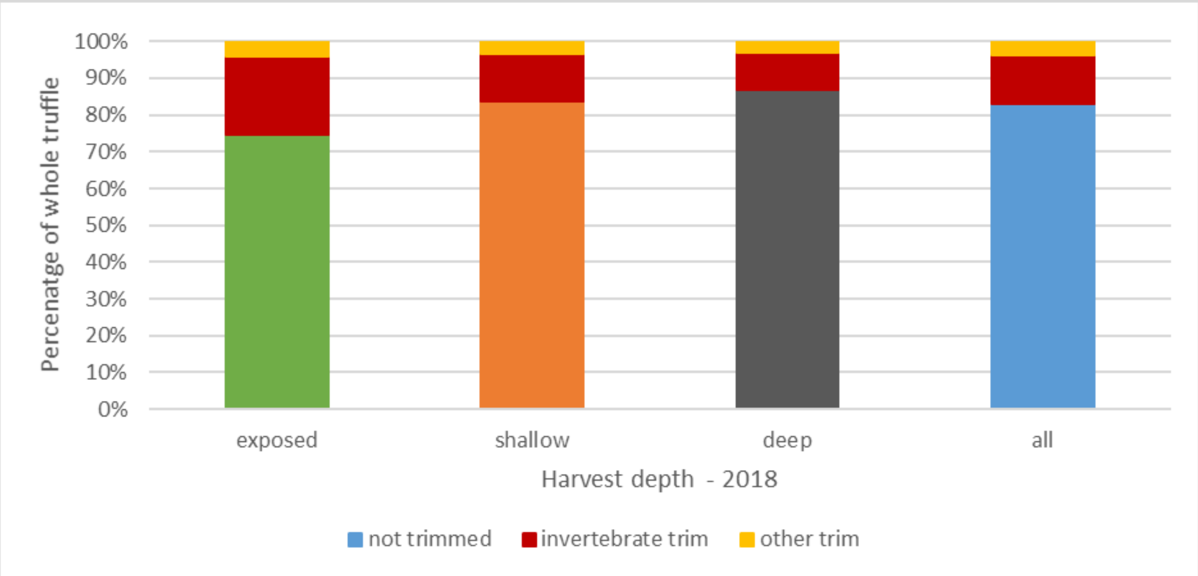


Fig. 1.4.32. Green, orange, grey and blue bars show average percentage of each truffle that was not trimmed from each depth category and the average across all depths for properties A1, A2, B, E, F, G and H. Red bars show the average percentage of each truffle trimmed when invertebrate damage was present. Yellow bars show the average percentage of each truffle trimmed for other issues when no invertebrate damage was present.

At individual properties, the average proportion of exposed truffle that was trimmed due to invertebrate damage ranged from 8.5% for property F to 100% for property A2. For shallow truffles, it ranged from 4.7% for property B to 23.4% for property A2 and for deep truffles, 6.5% for property B to 28% for property A2.

A season average wholesale price was obtained for each grade and a value in $/kg for each truffle determined based of their pre and post trim grades. Pre-trim grades were determined based on all other attributes other than invertebrate damage so any downgrade is due to that damage only. The largest drop in value was for exposed truffles from property A2 that fell from $1250/kg to $428/kg (Fig.1.4.33). Property A1 had the largest decrease in value over all depths from $1130/kg to $900/kg. For all properties the average value of exposed truffle dropped, on average, by $151/kg after trimming. Shallow truffles reduced by $63/kg and deep truffles $71/kg.

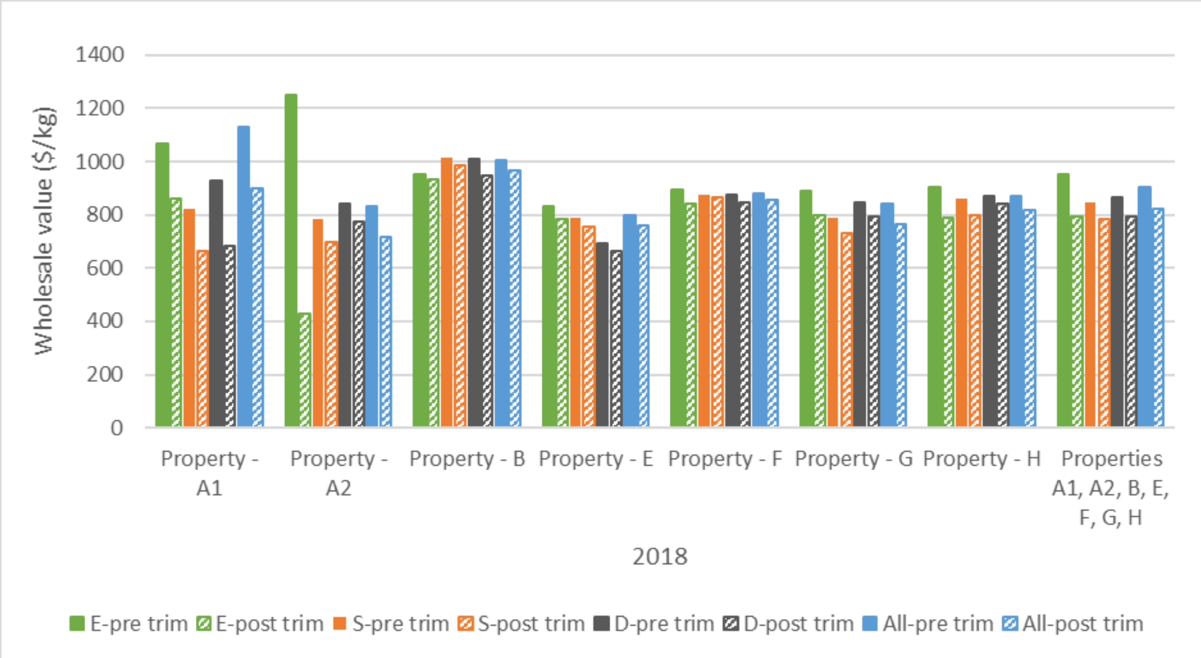


Fig. 1.4.33. 2018 season average wholesale value ($/kg) of pre and post-trimmed truffle harvested from each depth category for properties A1, A2, B, E, F, G and H. E=exposed, S=shallow, D=deep.

Property A2 had high slug and slater numbers observed in the orchard. It is an outlier in terms of the drop in truffle value. When A2 is included in the average for all properties, invertebrate damage reduces the value of exposed truffles by almost 17% (Fig. 1.4.34). When A2 is not included, the reduction in value is closer to 10%. With or without property A2 included, the overall value of shallow and deep truffles is reduced by around 7-9% due to invertebrate damage.

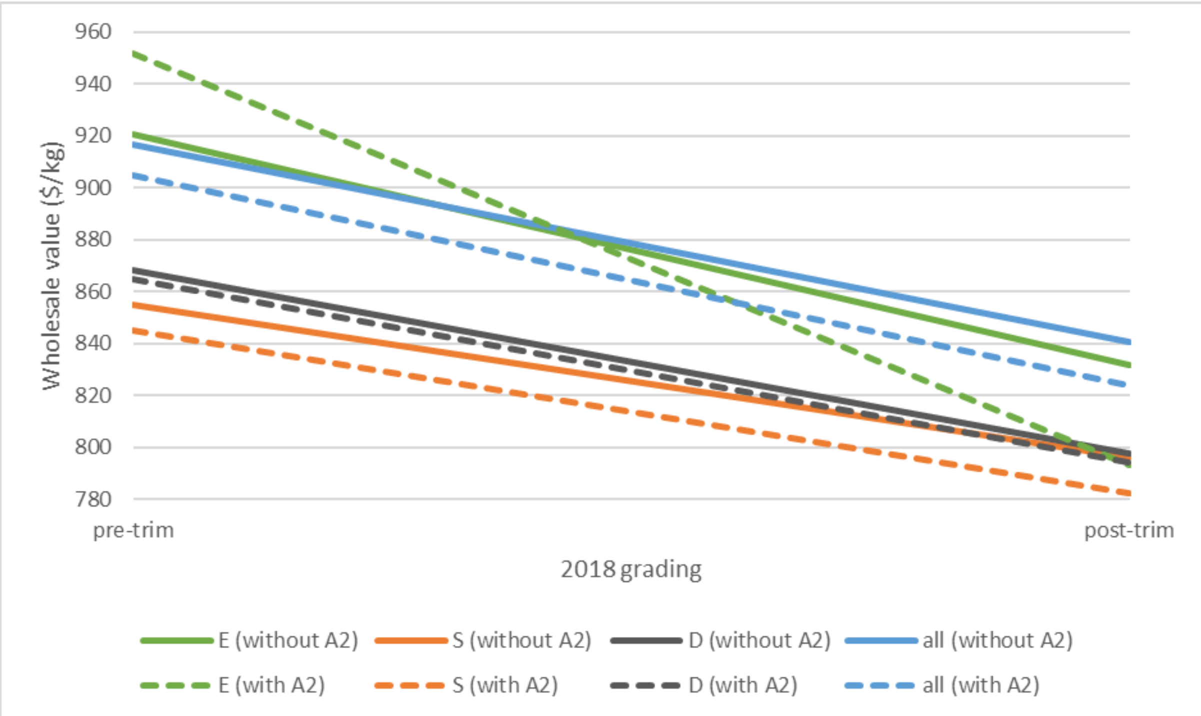


Fig 1.4.34. Reduction in 2018 season average wholesale value ($/kg) from pre to post-trimmed truffle harvested from each depth category. Figures are the average of properties A1, A2, B, E, F, G and H for the dashed lines and average excluding property A2 for the solid lines. E=exposed, S=shallow, D=deep.

Conclusions

Slugs and slaters are the most significant pest of truffles. In addition to their generally high abundance leading to high levels of damage, they are also considerably widespread in their distribution.

The shallower a truffle forms and grows, the more likely it is to be fed upon by most invertebrate pests, including slugs and slaters. Management strategies such as cultivation and irrigation, which encourage deeper forming truffles would assist in reducing the amount and severity of invertebrate feeding damage in most orchards. Covering truffles would also assist in management as it reduces the likelihood of slugs and slaters encountering them.

Differences in the proportion of truffles that formed at different depths were found among orchards. Some of the reasons for this could be intrinsic to the site, e.g. soil type, however, the reasons for these differences could be looked at in more detail to determine if there are any other management strategies that could increase the depth at which truffles form.

Slugs and slaters are not the primary cause of invertebrate damage in all orchards. ‘Australian truffle beetle’, springtails and millipedes can potentially cause high levels of damage to truffles.

Truffle beetles are native to large portions of Australia, in which truffles are cultivated, where they feed on numerous and diverse native truffle species that are associated with our diverse native flora. However, in this project only one property was identified as having significant amounts of feeding damage from native truffle beetles. This study has shown that, when native truffle beetles do feed on commercial truffle, their impact can be significant. Because of their tunnelling nature, larger amounts of truffle need to be trimmed to achieve a saleable piece.

There is also no correlation between the amount or level of truffle beetle damage and the depth of truffle growth. Given its large native range across Australia, its devastating impact when in high number in an orchard and the difficulty in managing an insect that is an obligate truffle feeder that spends a large portion of its lifecycle underground, the ‘Australian truffle beetle’ warrants further study.

Springtails were observed in several orchards, however, were only considered a major pest in one. The reasons for this are not known. The property at which springtails caused significant damage was only included in the final year of the project so no research was conducted on springtail management. Because springtails are present across Australian truffle producing regions, their potential to become a more significant pest is high. Orchardists should be aware of this potential and check for damage during grading. This is an area where future research may be warranted.

Rot did not cause as much damage to truffles as invertebrate damage. After the work of Eslick (2012, 2013) covering of truffle has become common practice and has likely reduced levels of rot both within the year of covering and possibly has resulted in lower inoculum to further reduce the level of rot in subsequent years. The results for rot assessments that show decreasing incidence with increasing harvest depth (as reported by Eslick, 2012, 2013) were not consistently repeated across properties and seasons in this study, and the opposite was also found in some cases. In this project there was variability into the pattern of rot with depth and harvest month, and the amount of rot between properties and also between seasons. Seasonal weather conditions and orchard attributes and management likely played a part in this variability but the contributing factors are not known.

This study has emphatically demonstrated that every orchard is different. Accordingly, a major recommendation from this study is that all truffle growers undertake regular monitoring.

Only in this way can pests be identified in the orchard and confirmed from observations at harvest and grading. This will assist growers in determining what pests they have, and whether their pest and damage levels are high enough to warrant action. Further monitoring is recommended to determine the impact of management interventions used. Guidelines for monitoring and further information on integrated pest management is included in the IPDM Manual for Australian truffle orchards.

References

Eslick, H. 2012. Identifying the Cause of Rot in Black Truffles and Management Control Options. RIRDC Publication No. 12/005.

Eslick, H. 2013. Identification and management of the agent causing rot in black truffles - Part 2 Identifying the Cause of Rot in Black Truffles and Management Control Options. RIRDC Publication No. 12/005.

Hall, I.R. Brown, G.T., Zambonelli, A. 2007. Taming the Truffle, The History, Lore, and Science of the Ultimate Mushroom. Timber Press. 304pp.

Morcillo, M., Sanchez, M., Vilanova, X. 2015. Truffle Farming Today, a Comprehensive World Guide. Publisher: Micologia Forestal & Aplicada. ISBN 978-84-617-1307-3.

Chapter 2: Managing pests and diseases of the host trees and truffles

With knowledge on the priority pests and diseases in Australian truffle orchards detailed in Chapter 1, the following sections report on considerations with respect to their management.

Some of the management practises mentioned below can of course be considered when orchards are being established or existing ones are being expanded. The implementation of these will improve tree health and at the same time improve their ability to resist or be less susceptible to diseases infection and attack by invertebrates. The same applies to managing the ground floor of truffle orchards through management aspects such as cultivation and irrigation so truffles will be forming under the most suitable conditions. More detail on these aspects are discussed below.

2.1 Managing invertebrate pests of truffle trees

Introduction

The range of the more important invertebrate pests of the truffle host trees was outlined in Chapter 1.2 (Table 1.2.8). This includes ones that are primarily important for tree establishment, ongoing potential direct feeding on mature trees and ones that interfere with the mini-sprinkler irrigation system.

Objectives

Members of the project team sought to define strategies that would minimise the damage these pests inflict on the truffle tree hosts.

Methodology

Resources within the current project were insufficient to conduct detailed studies to devise sustainable management practices on the extensive list of pests. From experience within the project team and reviewing recommendations and research conducted by others, preliminary management practices at the very least could be suggested as a means of protecting the host trees from damage by these agents.

Results

One of the major outputs of this project is the publication of an IPDM Manual for truffles and their host trees. This includes information on suggested management methods which have been summarised in Table 2.1.1.

Table 2.1.1 A summary of suggested management practices for the more important species of invertebrates associated with feeding on truffle host trees

Common name, important stage	**Management suggestions**
ESTABLISHMENT PESTS	
African black beetle, adult	Land preparation for long term fallow to remove habitat to reduce pest load in soil; bury corflute guards around each tree to prevent beetles feeding on the trunk near ground level.
Apple weevil, adult	Land preparation for long term fallow; wrap trunks with crafter's batting to prevent weevil access to the tree canopy.
Garden snail	Land preparation for long term fallow.
Whitefringed weevil, adult	Land preparation for long term fallow.
Wingless grasshopper	Locate source outside the orchard, i.e. egg beds, and treat preventatively in spring.
Lightbrown apple moth, larva	Monitor with pheromone traps for moths; apply a bacterium against young larvae.
MATURE TREE PESTS	
Fullers rose weevil, adult	Leaf feeding unlikely to be economically important.
Garden weevil, adult	Leaf feeding unlikely to be economically important.
Red legged weevil, adult	Leaf feeding unlikely to be economically important.

Common name, important stage	Management suggestions
Whitefringed weevil, adult	Leaf feeding unlikely to be economically important.
Spring beetle, adult	Leaf feeding unlikely to be economically important.
Stinking longicorn, larva	Ensure trees are not stressed by over- or under-watering.
Cockchafers, adult	Leaf feeding unlikely to be economically important.
Oak leaf miner, larva	Leaf mining unlikely to be economically important.
Fruit tree borer, larva	Prune and destroy infested branches/suckers; locate holes by checking for webbed frass and spike larvae in borer holes with wire.
Painted apple moth & western tussock moth, larva	Leaf feeding unlikely to be economically important.
Lightbrown apple moth, larva	Leaf feeding unlikely to be economically important.
Heliothis budworm, larva	Leaf, shoot and nut feeding unlikely to be economically important.
Wingless grasshopper	Leaf feeding unlikely to be economically important.
Aphids	Leaf feeding unlikely to be economically important; aphids are often controlled by a range of natural control agents.
Soft scale	Starts as a hot spot usually; monitor regularly; apply localised oil spray against crawler stage.
Greenhouse thrips	Leaf feeding unlikely to be economically important.
Mites	Leaf feeding unlikely to be economically important; predatory mites have been observed in orchards.
PESTS THAT INTEFERE WITH MIMI-SPRINKLER IRRIGATION	
Fullers rose weevil, adult	Some sprinkler types are less likely to be blocked.
“Native ant”, adult	Increase water pressure; run the system occasionally through winter.
Small pointed snail	Monitor for build-up and remove by hand if minor infestation; introduce ducks for short periods.
Hypsomus weevil, adult	Some sprinkler types may be less likely to be blocked.

Discussion

The key to successful protection of truffle host trees is correct and timely identification of the occurrence of invertebrate pests. While the suggested management methods which were summarised in this section are very broad, they indicate likely areas for further study. Over time other methods may become available for more reliable protection.

For the correct identification of any pest or disease in the truffle orchard, use the Truffle P&D Identification guide. If the agent cannot be identified, contact others to assist.

For the latest in management options, consult the IPDM Manual regularly.

2.2 Managing diseases of truffle trees

Introduction

Managing diseases of truffle trees, as with any disease in any host, start with general orchard hygiene. Orchard hygiene includes:

- Remove dead or dying plant material from the orchard, as it will be a source of fungal pathogen inoculum
- Only prune when at least 24 hours of dry weather is expected. Rain on open wounds encourage germination of pathogen spores, which may lead to infection
- Maintain air flow in the orchard by pruning lower branches in particular, adequate site selection (e.g. no wind breaks), and adequate orchard layout which promotes air flow. Airflow reduces humidity within the orchard, thereby minimising favourable conditions for pathogen infections
- Use footbaths at the entrance of orchards to prevent the spread of soil borne pathogens such as *Phytophthora*
- Source disease free nursery stock
- Avoid having irrigation sprinklers close to tree stems
- Choose truffle host species carefully according to climatic conditions of the orchard. For instance, *Quercus ilex* will present with more stem diseases and diebacks in high rainfall areas than under drier conditions.

It is recommended that the general orchard hygiene principles be followed, regardless of whether a disease is present in a particular orchard or not. This is because prevention is better than subsequent disease management. Also, diseases are mobile, they have propagules that are transmitted by wind, rain, soil and humans. Furthermore, many diseases are not host specific and may be present in alternative hosts such as occur in native forest and fruit orchards in the vicinity of truffle orchards, providing inoculum pressure. Thus, when conditions in the truffle orchard favours infection by pathogens, it may only be a matter of time before diseases arrive.

Management of specific diseases

Phytophthora root and root collar rot

- Install drainage if the slope or other aspects of the orchard are insufficient to prevent waterlogging.
- Because infection usually occurs at the bottom of a slope, do not cultivate soil up the slope, as it will spread the pathogen.

Diebacks and stem cankers

- If tree trunks are wet through irrigation, modify to avoid this
- Remove and burn dead trees and diseased branches to reduce the spread of inoculum
- Sterilise pruning shears between cuts on infected trees
- Reduce tree stress as these can trigger disease infection.

Discula quercina

A case study on managing this disease is presented in the boxed text below. A summary of the most important key aspects are:

- Improve airflow in the orchard, as high humidity, especially during spring and summer increases disease incidence
- Do not overwater as it will increase humidity, facilitating infection
- Minimise tree stress (e.g. over or under watering, other pathogens).

Wood rots

- Remove dead or dying trees from the orchard and burn to prevent fruiting of fungi and release of inoculum
- Minimise large open wounds
- Only plant host species suited to the site.

Powdery mildew

- Airflow, airflow, airflow
- If in a high rainfall/humidity area, insist on nursery stock with some level of powdery mildew resistance
- Hazels and *Q. ilex* are less susceptible to powdery mildew than *Q. robur*.

Case study: *Discula quercina* in WA

During spring 2017 routine invertebrate monitoring, a high number *Quercus robur* deaths were observed in an orchard in Western Australia. A count was performed on a portion of the orchard for the number of trees affected. Subsequently, samples of diseased trees were collected for possible pathogen identification. Mortality in *Q. robur* was estimated at 20% and further trees displayed dieback and defoliation symptoms. *Q. ilex* from the same orchard, did not suffer from the same symptoms. Samples were sent to ANU for pathogen identification. A well-known dieback-associated fungal pathogen, *Neofusicoccum* (also known as *Botryosphaeria*), and *Diaporthe* species could be isolated from diseased material. *Neofusicoccum* is a well-known fungal pathogen causing dieback in many hosts. However, another pathogen, *Discula quercina*, was isolated from diseased twigs. This pathogen may pose a quarantine risk as it has not previously been found in WA, but is known to occur in Eastern Australia. Symptoms of *Discula quercina* include defoliation, twig and leaf dieback, leaf distortion and angular necrotic spots on mature leaves. Symptoms are usually most severe on the lower branches where moisture tends to remain for longer periods of time. Severe outbreaks have been known to kill nearly all the foliage on highly susceptible trees. Outbreaks usually subside by mid-summer, as the leaves mature and become more resistant to pathogen. Succulent growth, however, can still be attacked at any time of the growing season when wet conditions are prevalent.

Discula quercina is an endophyte of oaks where pathogenicity is triggered by drought stress. In contrast to reports from Europe where switching to pathogenicity is triggered by drought stress, the orchard in WA expressed disease symptoms even when drought conditions were not present. We hypothesise that other stressors may trigger progressive dieback in WA. Conditions that may enable other known endophytes to become pathogenic include a variety of stressors such as any physiological stress or other pathogen infection. Because *Neofusicoccum* and *Diaporthe* pathogens were also present in the diseased material, it is likely that they provided the tree stress necessary for *Discula* disease progression.

This particular orchard had a tendency to ‘over water’, thereby maintaining near perfect conditions for *Discula* infection and spread. Furthermore, the tree canopy was dense, enclosed and continue to ground level. A dense windbreak also reduced airflow, providing ideal, humid conditions for fungal sporulation and infection. This is also evident in the high incidence of powdery mildew observed at this orchard. It is anticipated that new infections can be minimised with a reduced watering regime. Furthermore, opening up the tree canopy and increasing airflow through the orchard, will also reduce powdery mildew infection, which may have contributed to the initial tree stress. Reducing tree stress should minimise disease expression in trees already infected with *Discula*.

2.3 Managing invertebrate pests of truffles

Introduction

This project is the first dedicated study of invertebrates associated with damage to truffles in Australia. The range of the more important invertebrate pests of truffles was discussed in Chapter 1.2. and listed in Table 1.2.9. This chapter discusses monitoring and management of pests with a focus on ground dwelling invertebrates including slugs, slaters and the 'Australian truffle beetle'.

Objectives

The results of the grower survey and the yearlong monitoring in truffle orchards enabled the prioritisation of the pest invertebrates associated with damage to truffles. Most of the ground-dwelling invertebrates that have been associated with damage to truffles are regarded as opportunistic feeders, consuming truffles as part of their diet. This group includes invertebrates that cause the primary damage and others that may rely on this damage or breakdown of truffles if they develop cracks or become rotten, in order to be able to attack truffles. The primary feeders include slugs, slaters, a range of the soil borne stages of beetles and others (Table 1.2.9). Examples of secondary pests include springtails, some fly larvae and potworms (Table 1.2.9).

The other important agent which is an obligate truffle feeding insect is Australian truffle beetle (ATB). The existence of this insect revolves around having access to truffles – initially native truffle and now introduced culinary truffle produced in a veritable monoculture in truffle orchards.

The next phase of the project involved studies on their management and the two feeding groups of primary/secondary opportunistic feeders and the obligate truffle feeding beetle are considered separately.

Methodology

Monitoring methods

Ground-dwelling invertebrates

The use of pitfall traps and tiles to monitor the invertebrates associated with truffles was reviewed to determine whether more efficient means could be employed. Pitfall traps, while more efficient than tile traps for collecting invertebrates, have some disadvantages. Pitfall traps are more labour intensive to deploy and are not easily moved to other locations. While this was not an issue for the yearlong monitoring, the lack of flexibility in moving them made pitfall traps undesirable for more intensive monitoring, which is required for assessing population levels of pests over small areas. Also pitfall traps require a higher level of maintenance to keep them free of leaf litter and the collection container kept clean.

Field trials were conducted with tile traps looking to optimise their use as a monitoring method for the early detection of pests. These trials looked at the use of attractants, tile placement and type of tile. While the use of ceramic tiles was shown to be less efficient than pitfall traps in the earlier study (see Chapter 1.2), it may be possible to improve their monitoring efficiency by placing an attractant under them (Glen, 2006, Nash, pers. comm.). Tests were run in a truffle orchard to determine if this were possible.

In two truffle orchards infested with slugs, a range of attractants was compared to determine whether the efficiency of using tiles to monitor for them could be improved. Four attractants were selected for placement under tiles:

- Flaky bran
- Poultry layer pellets
- Trout pellets (based on a mixture of grains and fish meal) and
- A mixture of yeast and sugar at 1:3 by weight.

There were five replications for each set of attractants. Tiles were checked for slugs at three, five and ten days after placement.

In one commercial orchard, tiles were placed sequentially along transects to indicate the abundance of slugs across the orchard. The effect of placing flaky bran under them on slug abundance was compared. Tiles were deployed without flaky bran and checked for slugs. As they were checked, a teaspoon of flaky bran was placed under each tile and they were assessed two days later for slugs.

Also, an assessment was made in relation to the location of the monitoring tile and the truffle host tree. Two locations were compared - the base of a hazelnut tree around which leaf litter had accumulated in suckers that had not been removed and halfway across a bare interrow. In addition to using a ceramic tile, a wooden sheet also 20 cm square with spacers 2 cm square at each corner to allow room under it for invertebrates to shelter (see Fig. 2.4.1), was paced at the same two locations within the orchard. Flaky bran was placed under half the ceramic tiles and wooden sheets. The number of slugs under the tiles and sheets was recorded one, two and three days after placement.

Fig. 2.3.1. Testing tile type and placement to monitor for ground dwelling invertebrates in truffle orchards – a wooden sheet 20cm square with 2 cm square spacers on the underside at each corner placed over flaky bran and next to the base of a hazelnut tree that had not been desuckered.

In another truffle orchard infested with slaters, a range of attractants was compared to determine whether the efficiency of using tiles for monitoring could be improved. The attractants were:

- No attractant
- Dog pellets
- Flaky bran
- Cracked wheat
- A mix of dog pellets and flaky bran.

There were five replications for each set of attractants. Ceramic tiles were placed in the orchard adjacent to the base of trees. Two days after the attractants were placed, the tiles were checked for slaters. Attractants were removed at that time. The procedure was repeated six weeks later.

Australian truffle beetle

Australian truffle beetle (ATB) is a ground dwelling insect that uses flight to disperse and find food. It is an obligate truffle feeding insect and would have a strong olfactory response to find fungus associated volatiles; this appears to be the case for other fungus feeding beetles, all of which must locate a transient food source. The options that were compared for monitoring ATB to clarify its seasonality and abundance were based on these attributes. The following methods were compared:

- Dry pitfall traps as described in Chapter 1.2 (see Fig. 1.2.1) without and with pieces of ripe truffle placed in the 300 mL collection container
- Flight intercept traps which consisted of fibreglass flywire gauze, 2800mm long by 915mm high, held over a 200mm long, 140mm diameter half pipe filled with water/detergent mixture (Fig. 2.3.2). Five such traps were constructed and spaced along a tree row either side of which were located the pitfall traps in the ATB infested orchard. Flight intercept traps were placed centrally between adjacent the hazelnut trees and about 30 m apart
- ‘Wet traps’ with 100mL of a liquid bait, a standard for beetles of family Nitidulidae: 50 mL molasses: 50 mL beer:1 teaspoon yeast in the collection container which had:
    - The same set up as the dry pitfall trap (see Fig. 2.3.3)
    - A pipe trap, consisting of a 160 mm length of 110 mm outer diameter PVC pipe with twelve 5mm diameter holes 25mm below the top and equidistant around it. The pipe was buried in soil so that the holes were 5mm above the soil surface. A pitfall trap base containing 100mL liquid bait and attached to a funnel is placed within the pipe such that the top of the funnel is just below the holes on the pipe. The open end of the pipe is covered with a PVC lid (Fig. 2.3.4).

Fig. 2.3.2. The flight intercept trap used to monitor Australian truffle beetle.

***Fig. 2.3.3*.** The ‘wet pitfall trap’ used to monitor Australian truffle beetle. The red funnel is placed above the collection container holding a beetle attractant solution indicated by the yellow arrow.

***Fig. 2.3.4*.** The ‘wet pipe trap’ used to monitor Australian truffle beetle. The funnel is placed in a pipe that extends above the soil surface with 5mm holes to allow beetle entry and a lid is placed over the exposed pipe to prevent debris falling into the trap.

Management methods

Effect of orchard management practices on ground dwelling invertebrates

Management practices that reduce truffle rot (Eslick 2012, 2013) may also protect truffles from attack by ground dwelling invertebrates. It was beyond the scope of this project to study the effect of these management practices on pests. Even so, observations were made on the potential impact these practices would have on invertebrates.

Cultivation for instance that encourages truffles to form deeper in the soil, will provide a physical barrier to ground invertebrates accessing the tops of truffles. Practices that modify the conditions of the orchard floor to reduce incidence of truffle rot, for instance regulating the amount and frequency of irrigation, covering truffles to minimise their exposure to the extremes of temperature, sunlight and moisture/humidity, may also reduce the favourability of the orchard floor as a suitable habitat for invertebrates.

Other management practices that would reduce the favourability of truffle orchards for invertebrates include desuckering hazelnut trees to reduce the build-up of decaying organic matter that collects between them, pruning truffle trees to increase the amount of light in the orchard, removing any prunings from the orchard which might otherwise act as refuge for ground dwelling invertebrates and removing leaf litter from the orchard floor which also acts as food and shelter for invertebrates. Whether the orchard floor between the rows of truffle host trees is covered with vegetation may also influence the occurrence of invertebrates.

Some preliminary studies on the effects of desuckering hazelnut trees and removing leaf litter (Fig. 2.3.5) and cultivation (see Fig. 2.3.6) were made.

Fig. 2.3.5. Investigating the effect of desuckering hazelnut trees (tree to the left) and litter removal on slug abundance and distribution in a truffle orchard.

Fig. 2.3.6. The implement used to encourage deeper formation of truffles. The spikes are vibrated and when they penetrate the soil, help to loosen and aerate the soil in the interrow.

Effect of seasonal conditions on ground dwelling invertebrates

Attempts to study the seasonal activity of ground-dwelling invertebrates with an emphasis on slugs, in relation to weather parameters in truffle orchards was done by placing a weather station with a digital camera in an orchard. The camera was attached to car battery that was re-charged using a solar panel. The camera was set to take footage every 3 minutes. A daylight sensitive night light activated on sunset to enable the camera to record slug movement. The weather station recorded relative humidity, air and soil temperature (Fig. 2.3.7). The digital camera and weather station did not record continuously and results are not presented. One outcome of the use of this equipment was the observation that slaters and millipedes are attracted to light (Mathews 2018). Implications of this for management of invertebrates are discussed below.

***Fig. 2.3.7*.** Equipment consisting of a video camera in the horizontal silver pipe to monitor the activity of slugs and probes to record temperature and humidity in a truffle orchard.

Effect of predators on ground dwelling invertebrates

The other aspect of managing ground-dwelling invertebrates is the possibility of biological control. The abundance and seasonality of the ground dwelling beetle predators from the yearlong study was examined more closely to determine whether any possible links exist that might indicate they play a role in reducing the abundance of any of the invertebrate pests of truffles.

Parasitic nematodes have been considered for control of a range of invertebrate pests including slugs with varying degrees of success (Georgis, 2006). With encouragement from Dr Jenna Ross, a slug specialist from Institute of Biological and Environmental Sciences, UK, a survey of slugs for the presence of nematodes by dissecting larger sized slugs was conducted during field monitoring in truffle orchards from late winter 2018.

Understanding the lifecycle of slugs

During the field monitoring, all slugs of all species or a representative proportion of slugs based on size, were retrieved from field monitoring and weighed in the laboratory. Heavier slugs are older and more likely to be sexually mature than lighter slugs (Barker 1991). The record of slug weights from the field studies in Western Australia provided some indications of their seasonal activity in relation to timing of reproduction and therefore breeding cycles and abundance.

Australian truffle beetle

Management of ATB is a significant challenge. In truffle orchards in Europe a beetle with similar pest status is the European truffle beetle. Management approaches for the control of ATB is discussed in light of research in Europe.

Results and Discussion

Monitoring methods

Ground-dwelling invertebrates in truffle orchards

Using an attractant improved the efficiency of tiles as a monitoring method for slugs. The number of slugs varied between the truffle orchards (Fig. 2.3.8). After ten days in one orchard tiles with trout pellets attracted the most slugs while at the other site, tiles with layer pellets were the most attractive (Fig. 2.3.8). However, the most slugs were found consistently under tiles with flaky bran (Table 2.3.1).

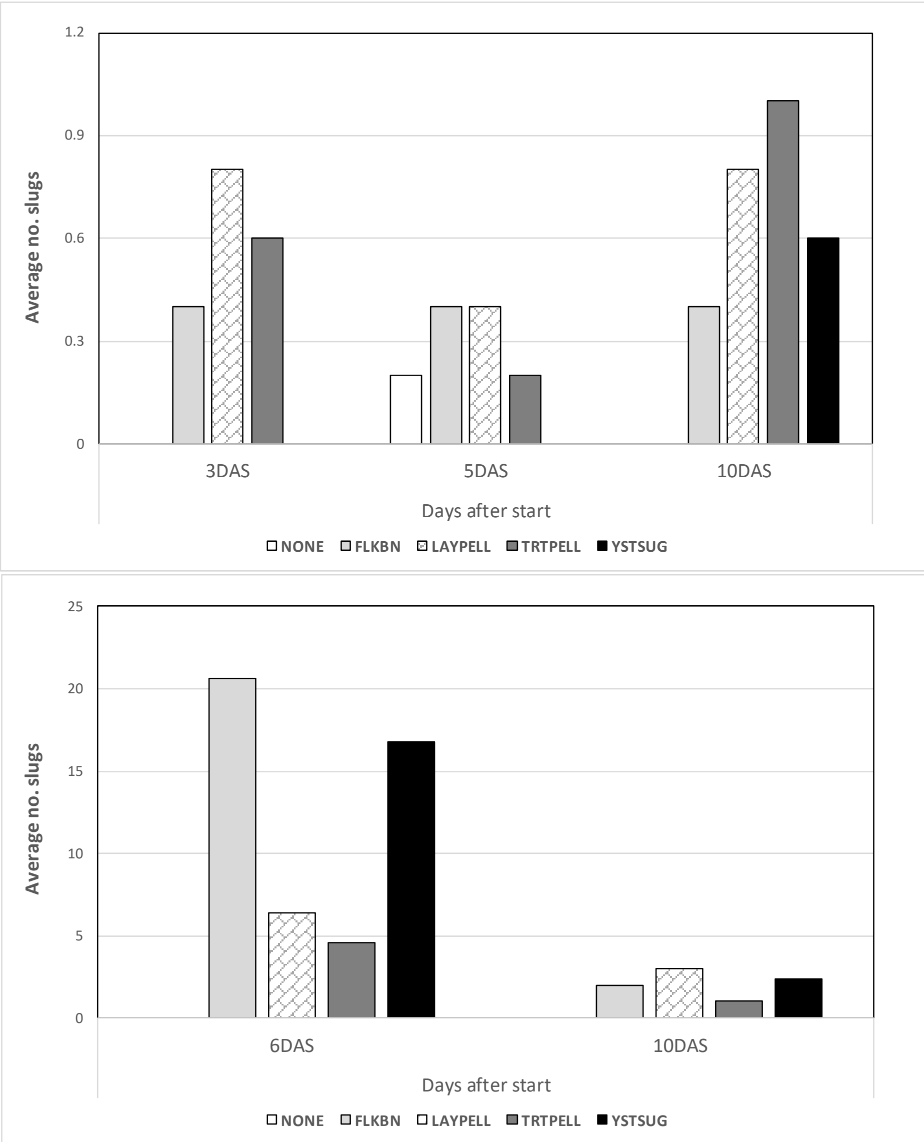


Fig. 2.3.8. The average number of slugs under tiles with different attractants under each on two truffle orchards – after 3, 5, and 10 days and 6 and 10 days after placement. The attractants compared: None, FLKBN = flaky bran; LAYPELL = poultry layer pellets; TRT PELL = trout pellets; YSTSUG = mixture of yeast and sugar.

Table.2.3.1 Statistical analysis of the average number of slugs under tiles with different attractants placed under each tile on two truffle orchards. Means followed by the same letter were not significantly different at P<0.1; SE is standard error

	Site 1*		Site 2**	
Treatment	**Average**	**SE**	**6 DAS**	**SE**
Untreated	0.1b	0.1	0c	0
Flaky bran	0.4ab	0.2	21a	6
Laying pellets	0.7ab	0.3	6b	1
Trout pellets	0.6a	0.2	5b	2
Yeast: Sugar=1:3	0.2ab	0.1	17ab	6

* Average based on all dates after set-up.
**Average based on data from six days after set-up. Means followed by the same letter are not significantly different at P<0.1; SE is standard error

Some of the attractants had inherent disadvantages:

- Ants were attracted to the yeast/sugar mix and appeared to interfere with invertebrates sheltering there
- Trout pellets had a fish meal base with an associated unattractive aroma
- Poultry pellets under dry conditions remain hard, dry and unattractive to invertebrates; also different batches of pellets, even if obtained from the same manufacturer can vary in their composition.

Flaky bran is the exterior coating of cereal grains and is of reasonably consistent composition. Because it has a large surface area, it would readily absorb any moisture from the soil and dew in the orchard making it more likely to attract and hold the invertebrates under the tile until they are scored. It is lightweight and easy to apply. Ants do harvest flaky bran, but it was not as attractive to ants as the yeast/sugar mixture. Therefore, flaky bran was chosen as the attractant to use under tiles (Fig. 2.3.9).

***Fig. 2.3.9*.** Flaky bran was chosen as the most appropriate attractant to place under tiles for monitoring ground dwelling invertebrates, especially slugs, in truffle orchards.

Flaky bran was observed to be attractive also to the other invertebrates associated with damage to truffles - slaters, millipedes and to some extent earwigs.

In order to obtain a consistent result for a given area it was further decided that tiles would not be checked until two days after tiles had been deployed. In this way invertebrates had two nights to locate the tile and attractant, and remain there to be monitored. When checking baited tiles, any bran remaining was retrieved to be removed from the orchard so as not to interfere in the usual dispersal of invertebrates on the orchard floor.

Placing flaky bran under tiles improved efficiency of the tiles to attract slugs. On average four times as many slugs were found under tiles where flaky bran had been placed (Fig. 2.3.10). Similarly, flaky bran improved the attractiveness of tiles or wood sheets to slugs (Fig. 2.3.11).

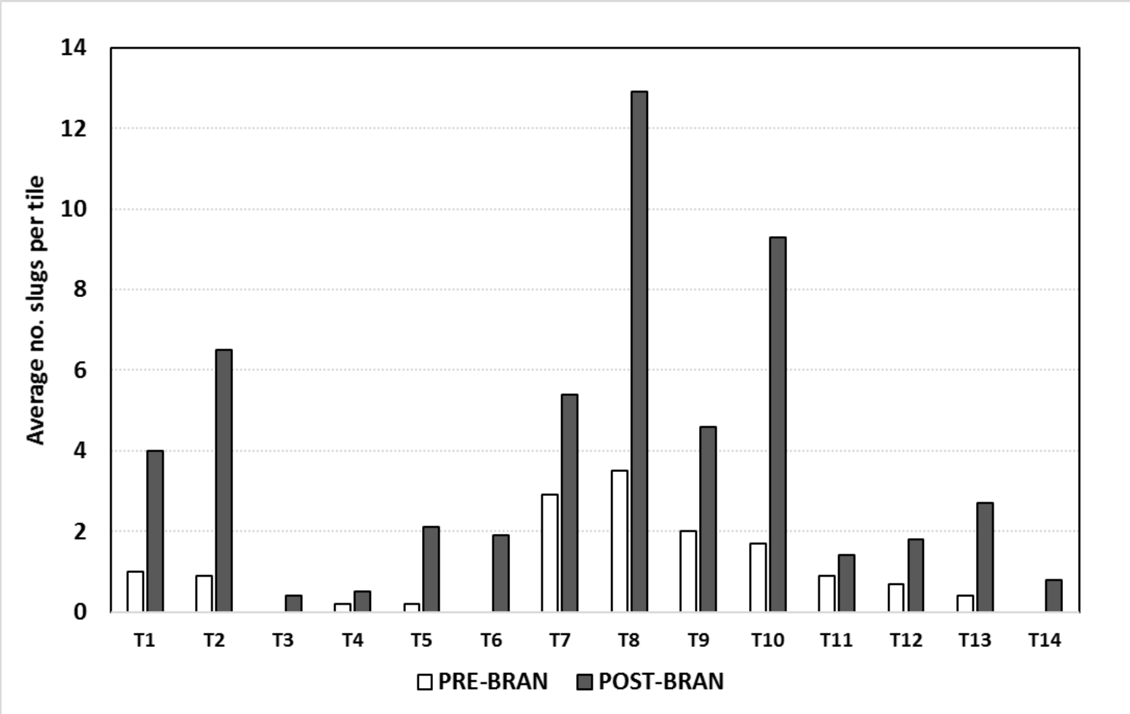


Fig. 2.3.10. The average number of slugs under tiles without flaky bran and, at the same location, the number of slugs after flaky bran had been placed under them.

The placement of tiles as well as the use of an attractant is important to attract slugs. The average number of slugs under the tiles or wood sheets was greatest when they were placed adjacent to the hazelnut tree (Fig. 2.3.11). The suckers and associated accumulated leaf litter acted as both a suitable shelter and source of food. This moist, cool shaded habitat was no doubt contributing to the survival and reproduction of slugs in the truffle orchard. However, 20% more slugs were found under tiles rather than under wood (Table 2.3.2) suggesting tiles are more efficient.

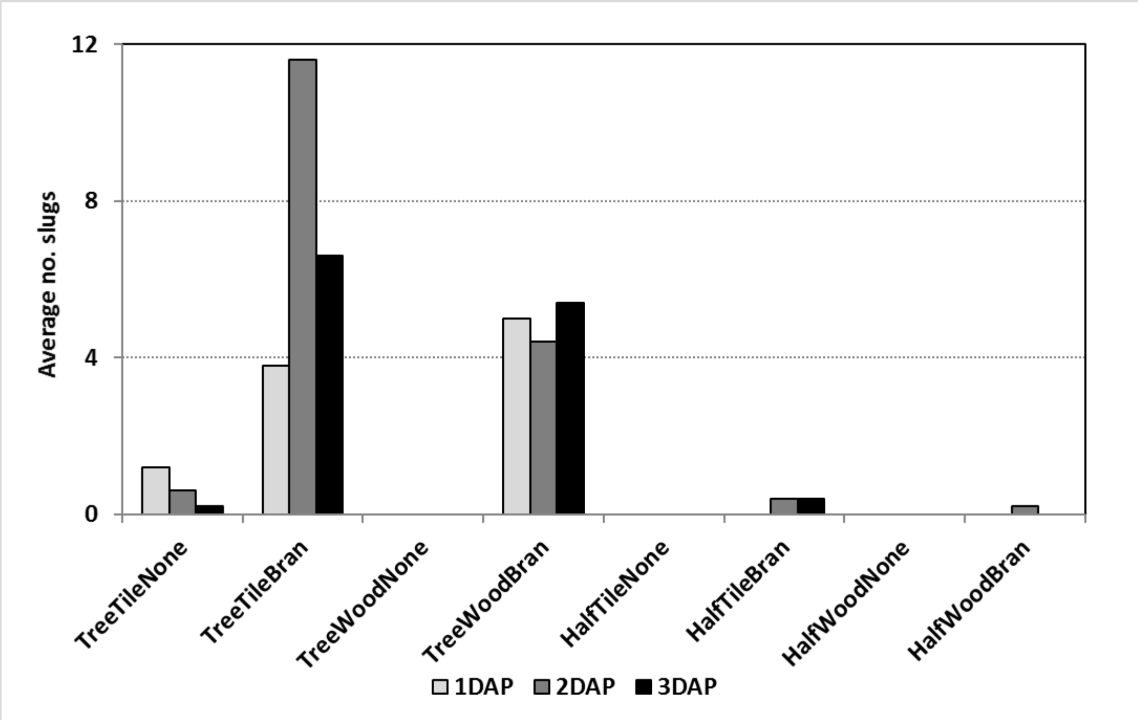


Fig. 2.3.11. The average number of slugs under ceramic tiles and wooden sheets with and without flaky bran under each and adjacent to a hazelnut tree or half way between trees across a bare interrow at 1, 2 and 3 days after placement (DAP).

Table.2.3.2 The average number of slugs under tiles or wooden sheets with and without flaky bran attractant and placed adjacent to a hazelnut tree or halfway across a bare interrow at one, two and three days after placement. Means followed by the same letter were not significantly different at P<0.1; SE is standard error

Treatment	*No. slugs /	SE	Significance
Interrow, Wood	0	0	c
Interrow, Wood,	0	0	c
Interrow, Tile,	0	0	c
Interrow, Tile, +Bran	1	1	bc
Tree, Wood	0	0	c
Tree, Wood, +Bran	24	3	a
Tree, Tile	3	1	b
Tree, Tile, +Bran	37	11	a

* Average based on all dates after set-up, but 2 replications were excluded due to low slug numbers.

The number of slaters under tiles where a range of attractants were compared to determine whether their monitoring efficiency could be improved is given in Fig. 2.3.12 and analysis of the data in Table 2.3 3. The data indicate that cracked wheat was not a suitable attractant for improving the efficiency of tiles in monitoring for slaters. Placing either dog pellets or flaky bran alone or in combination increased the number of slaters recorded. It was observed that dog pellets were also attractive to ants which could interfere with the monitoring of slaters. As was the case described for slugs, flaky bran was chosen as the more acceptable attractant to enhance the use of tiles as a monitoring tool for slaters.

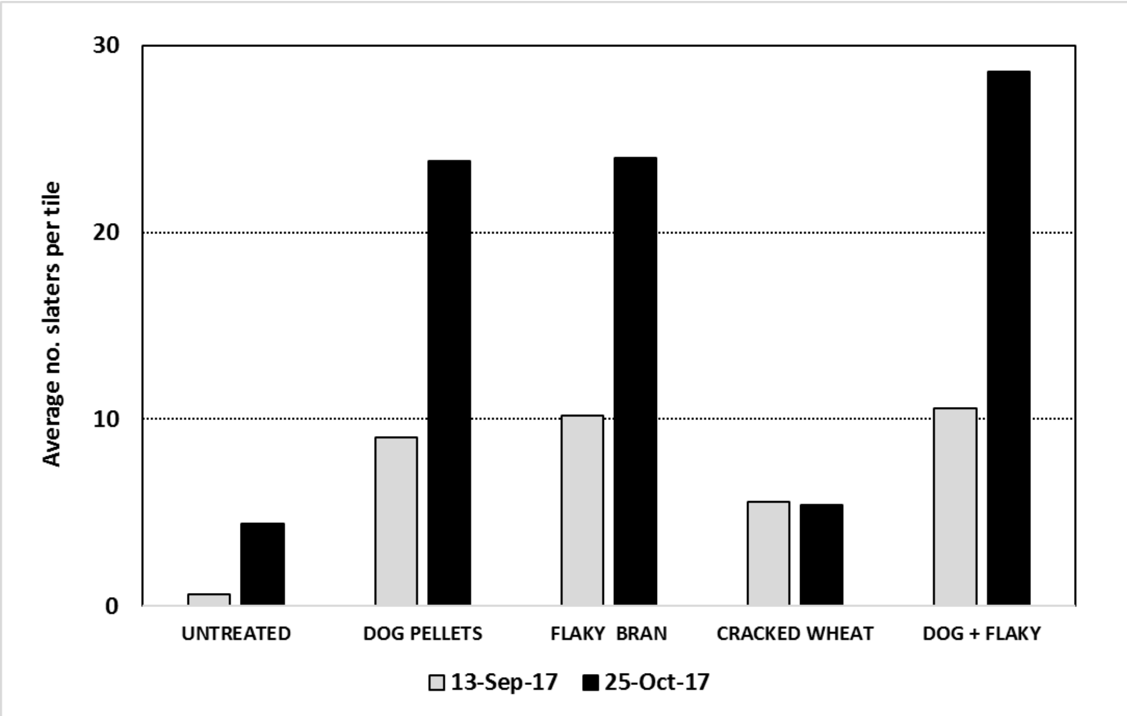


Fig. 2.3.12. The number of slaters under ceramic tiles where different attractants had been placed in a truffle orchard on two occasions.

Table 2.3.3 Statistical analysis of the average number of slaters under tiles with different attractants placed under the tiles and placed adjacent to a truffle host tree at two days after placement on two occasions in 2017. Means followed by the same letter were not significantly different at P<0.1; SE is standard error

Treatment	*Test 1	SE	Significance	*Test 2	SE	Significance
Untreated	1	0	a	4	2	a
Dog pellets	9	4	ab	24	7	b
Flaky bran	10	4	b	24	11	ab
Dog pellets & flaky	11	4	b	29	15	ab
Cracked wheat	6	2	b	5	2	a

*Test 1 on 13 September 2017, Test 2 on 25 October 2017.

Australian truffle beetle

Being an obligate truffle feeder, Australian truffle beetle (ATB) would be expected to have well developed olfactory senses. Dry pitfall traps with truffle pieces added attracted 8-50% more ATB than dry pitfall traps without truffles (Fig. 2.3.13). However, wet pitfall traps using a liquid attractant were on average many times more likely to attract ATB than dry pitfall traps without an attractant (Fig. 2.3.14).

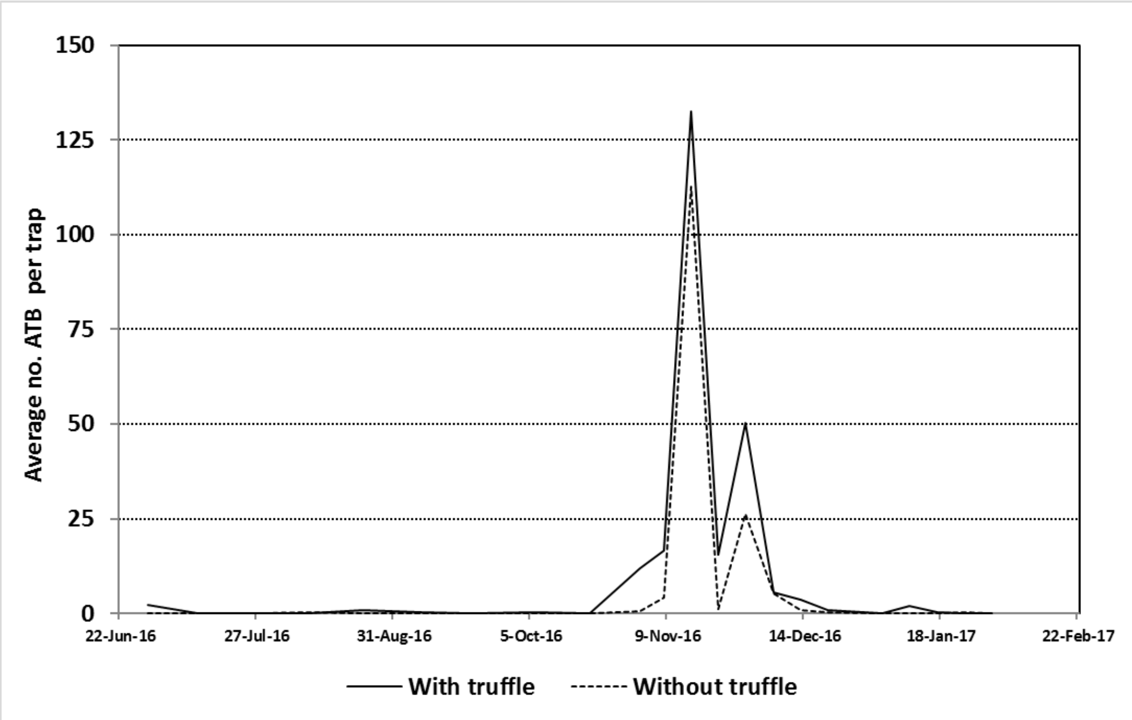


Fig. 2.3.13. The average number of Australian truffle beetles in pitfall traps where pieces of mature truffle were added or not.

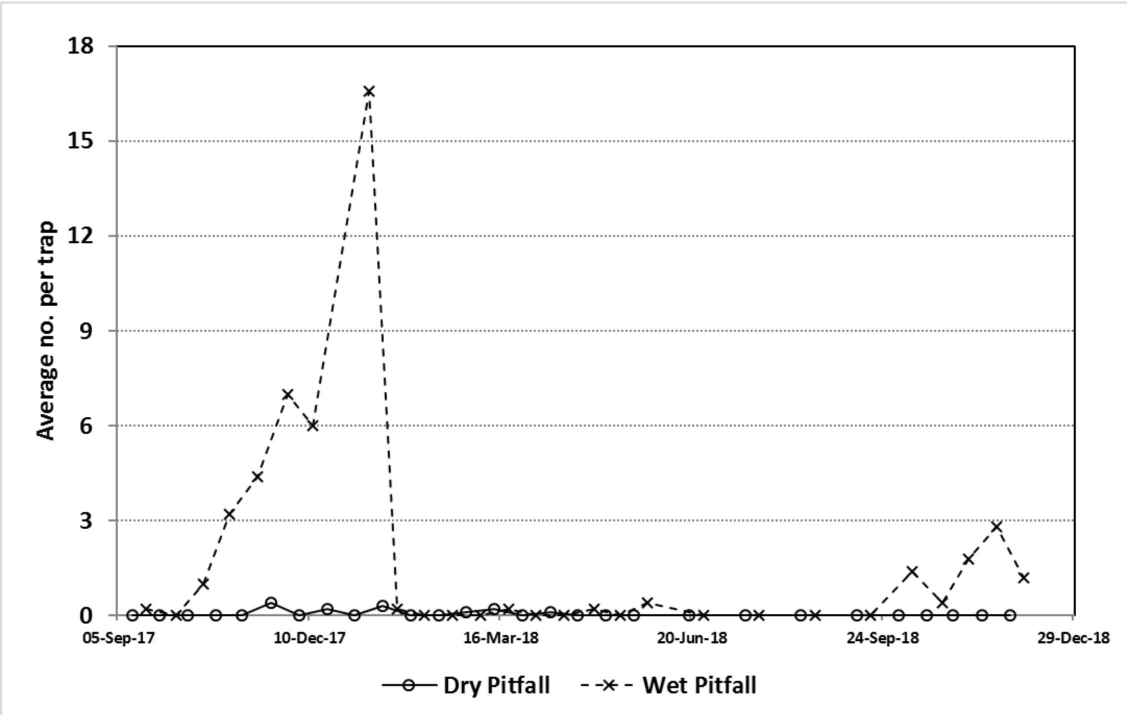


Fig. 2.3.14. The average number of Australian truffle beetles in dry pitfall traps and pitfall traps baited with a liquid attractant (Wet pitfall).

The number of ATB recorded from pitfall traps and pipe traps where the liquid beetle attractant was used indicate that either trap type was equally efficient in capturing ATB (Fig. 2.4.15). In terms of servicing the traps, the pipe traps were considered to be more easily accessed and maintained than the pitfall traps which required he addition of a lattice wall and corflute lid.

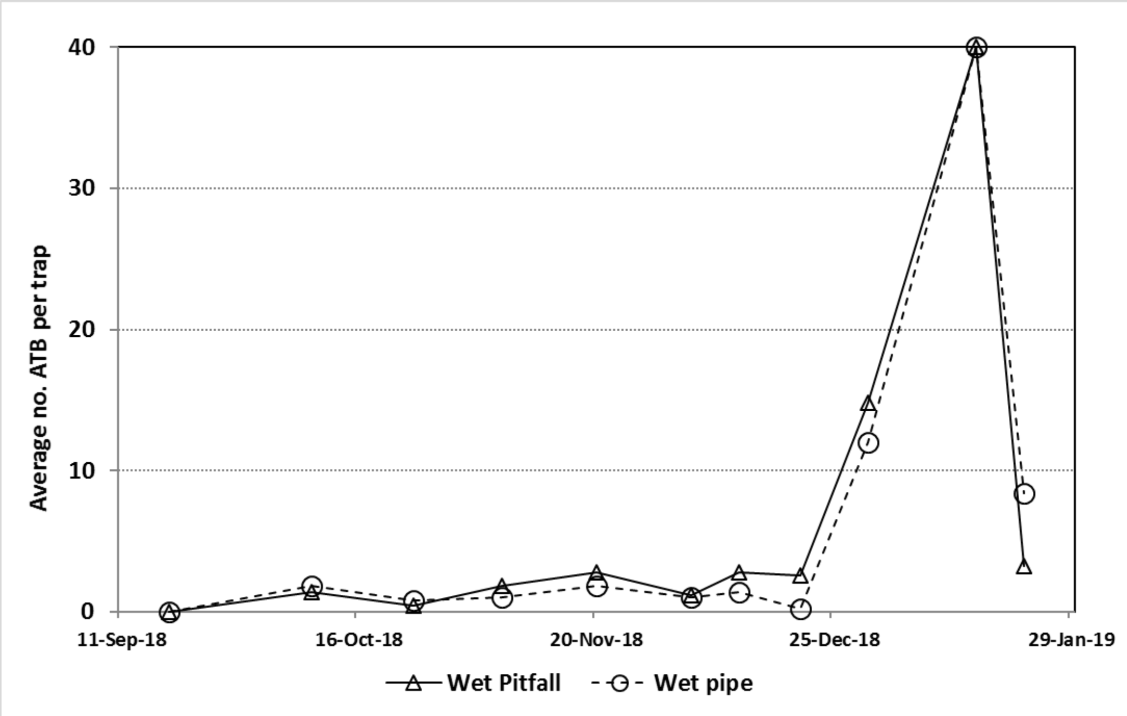


Fig. 2.3.15. The average number of Australian truffle beetles in pitfall traps (Wet pitfall) and pipe traps (Wet pipe) baited with a liquid attractant.

Use of attractants to monitor for ATB is more efficient than using flight intercept traps. If ATB were found in dry pitfall traps with truffles they were also captured in flight intercept traps (Fig. 2.3.16). However, the number of beetles recorded from the flight intercept traps was around half that for the truffle baited pitfall traps (Fig. 2.3.16). The time required to service the flight intercept traps on a regular basis was much greater than that required in using the pitfall traps and because their efficiency in monitoring ATB was on most occasions inferior to that of pitfall traps, their use was discontinued.

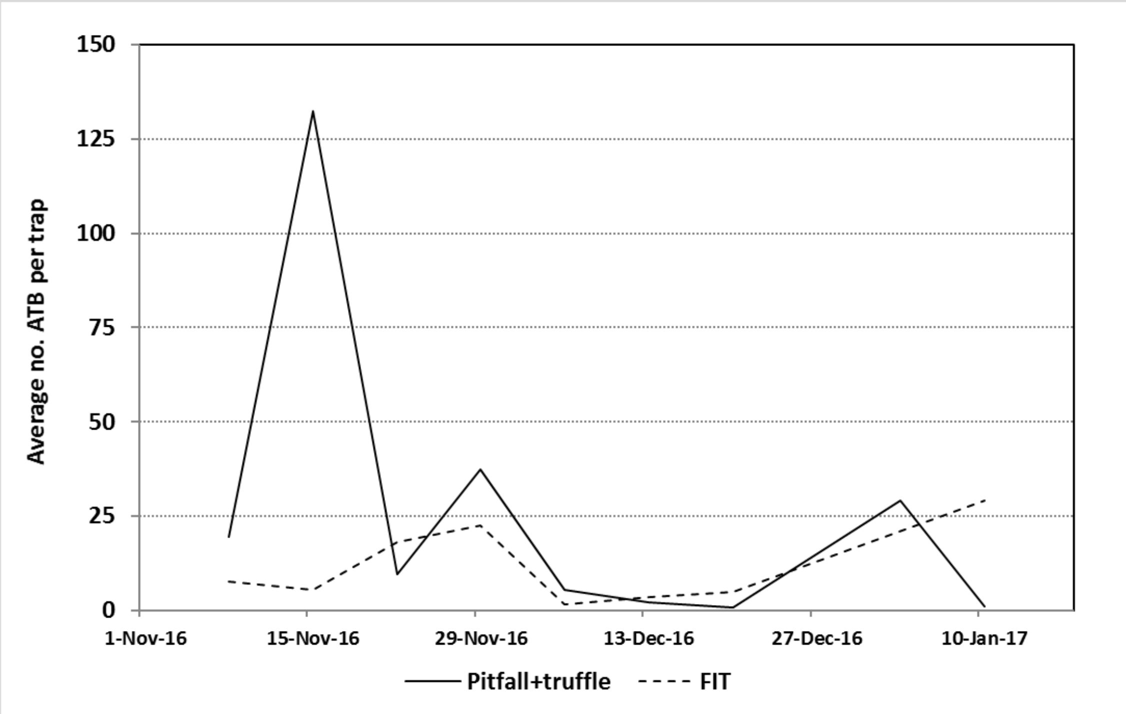


Fig. 2.3.16. The average number of Australian truffle beetles in pitfall traps baited with pieces of mature truffle and the number recorded in flight intercept traps (FIT).

Management

Effect of orchard management practices on ground dwelling invertebrates

Monitoring of slugs in an orchard commenced after the desuckering and leaf litter was removed in one section and remained in another section. Initially the abundance of slugs in plots in which hazelnut trees were desuckered and leaf litter in the tree line was removed increased (Fig. 2.4.17). The somewhat surprising result of recording a higher number of slugs under tiles where suckers and leaf litter were removed, was interpreted as the tiles being a refuge in an area where there was no alternative. In the areas where suckers and leaf litter were not removed, this provided an alternative shelter for the slugs so numbers were lower in the adjacent tiles.

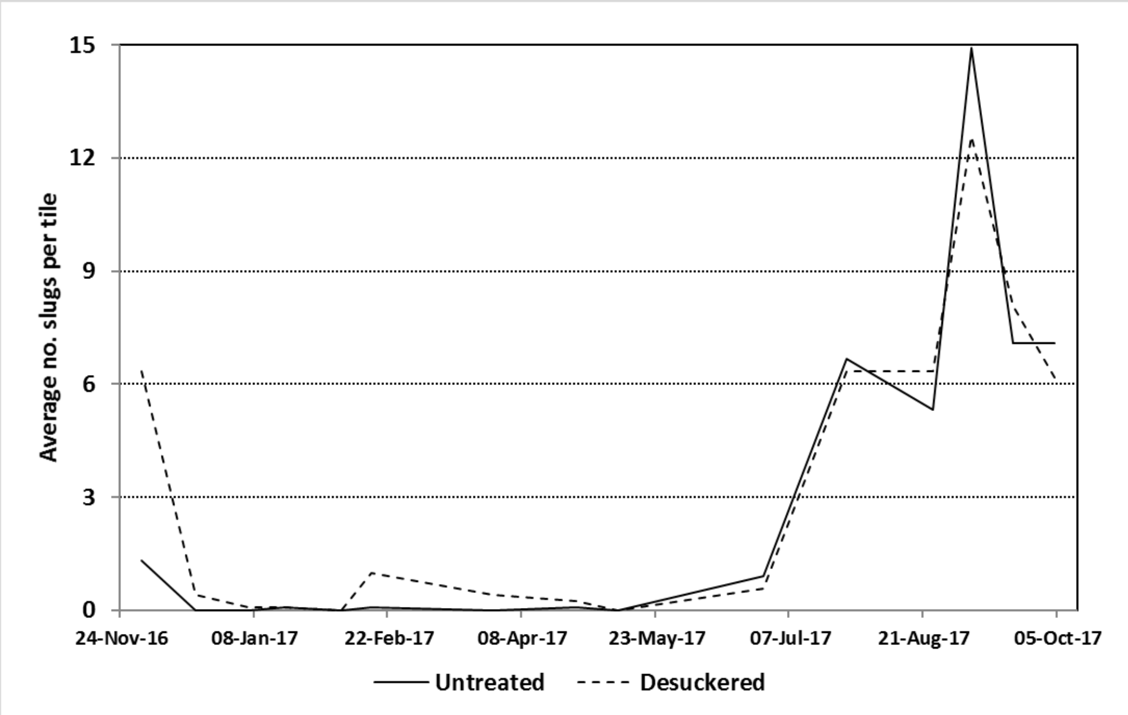


Fig. 2.3.17. The average number of slugs under tiles in areas of a truffle orchard where hazelnut trees were either desuckered with leaf litter between trees removed or untreated.

After nearly half a year, slug abundance in both areas was equivalent. The fact that the plots where suckers and leaf litter was removed were not maintained in this relatively clean state after the initial removal, may have contributed to this unexpected non-result. Further investigation on the role of desuckering hazelnut trees is warranted and in any case this practice is desirable from the point of view of aiding truffle hunting.

Truffle orchardists often remove all but a few suckers from hazelnut trees (Fig. 2.4.18) to reduce the possibility of losing the whole tree should the main trunk become infected with disease At the same time this pruning to a minimal number of suckers reduces the potential for the trees to act as a leaf trap which may otherwise enhance invertebrate presence.

Fig. 2.3.18. All but a few major secondary trunks or suckers are removed from hazelnut trees so that leaf litter does not accumulate and it also aids tree survival should disease affect the main trunk.

Another management practice of orchardists is the use a range of blowers and pick-up machines to remove leaf litter in order to help make the orchard floor less suitable for pest invertebrates including slugs, slaters, millipedes and springtails. See Figs. 2.4.19 and 20 for examples of machines used.

Fig. 2.3.19. A high volume air blower to remove leaf litter from the floor of a truffle orchard.

Fig. 2.3.20**.** Pick-up machine for removing leaf litter from the floor of a truffle orchard.

The effect of cultivation on inducing truffles to form deeper in the soil and thereby being less susceptible to attack by ground-dwelling invertebrates was examined in a preliminary way only. Fewer truffles required covering in those interrows that had been cultivated (Fig. 2.3.21). It is expected that more of the truffles that formed in the uncultivated area and breaching the soil surface would have sustained more invertebrate feeding damage than those that formed deeper in the cultivated area.

Fig. 2.3.21. The effect of cultivating the interrow of a truffle orchard on the left hand side of the tree row where almost no covering of exposed truffles was required compared to the many truffles covered with soil on the uncultivated interrow on the right.

Truffle growers are employing practices for pest control that have not been tested to determine the exact impact they have on pest populations. Some truffle growers in eastern Australia advised they use low temperature ground cover burning in spring to reduce the abundance of ground-dwelling invertebrates. They also apply rice hulls on the orchard floor to interfere with the mobility of invertebrates. Neither of these practises with the objective of reducing damage to truffles by ground-dwelling invertebrates was assessed.

Effect of seasonal conditions on ground dwelling invertebrates

While the objective of using a video camera/weather station in the field was unsuccessful in collecting data that would clarify the effect of seasonal conditions on the activity of ground dwelling invertebrates, it demonstrated graphically the nocturnal behaviour of slaters and to some extent millipedes (Mathews, 2018). These invertebrates are attracted to light and this attribute could be studied in terms of managing their abundance. Solar powered lights placed over pitfall traps if spaced appropriately across a truffle orchard, may help reduce their numbers.

Effect of predators on slugs and ground dwelling invertebrates

Predatory beetles have been reported to attack slugs (Anon. 2006, Horne, 2007). Predatory beetles belonging to the families Carabidae and Staphylinidae were recorded from all truffle orchards during the project (Table 1.2.2.). Carabid beetles were sometimes very abundant in orchards (Fig. 2.3.22) and their ability to prey on slugs was observed in the laboratory during the project (Fig. 2.3.23).

***Fig. 2.3.22*.** The catch of carabid beetles in a pitfall trap over a two week monitoring period in a truffle orchard.

***Fig. 2.3.23*.** A carabid beetle collected from a truffle orchard and the remains of a slug it had been feeding on in the laboratory.

It is possible that high carabid beetle numbers may suppress slug populations. Monitoring of slugs and carabid beetles in NSW orchards and in Western Australia suggests that if high slug catches occur in one month, the following month higher numbers of carabid beetles are captured (Figs. 2.3.24 and 25). However, pitfall traps are not an effective means to monitor for slugs so further work is required to determine how much of an impact carabid beetles have on slug populations.

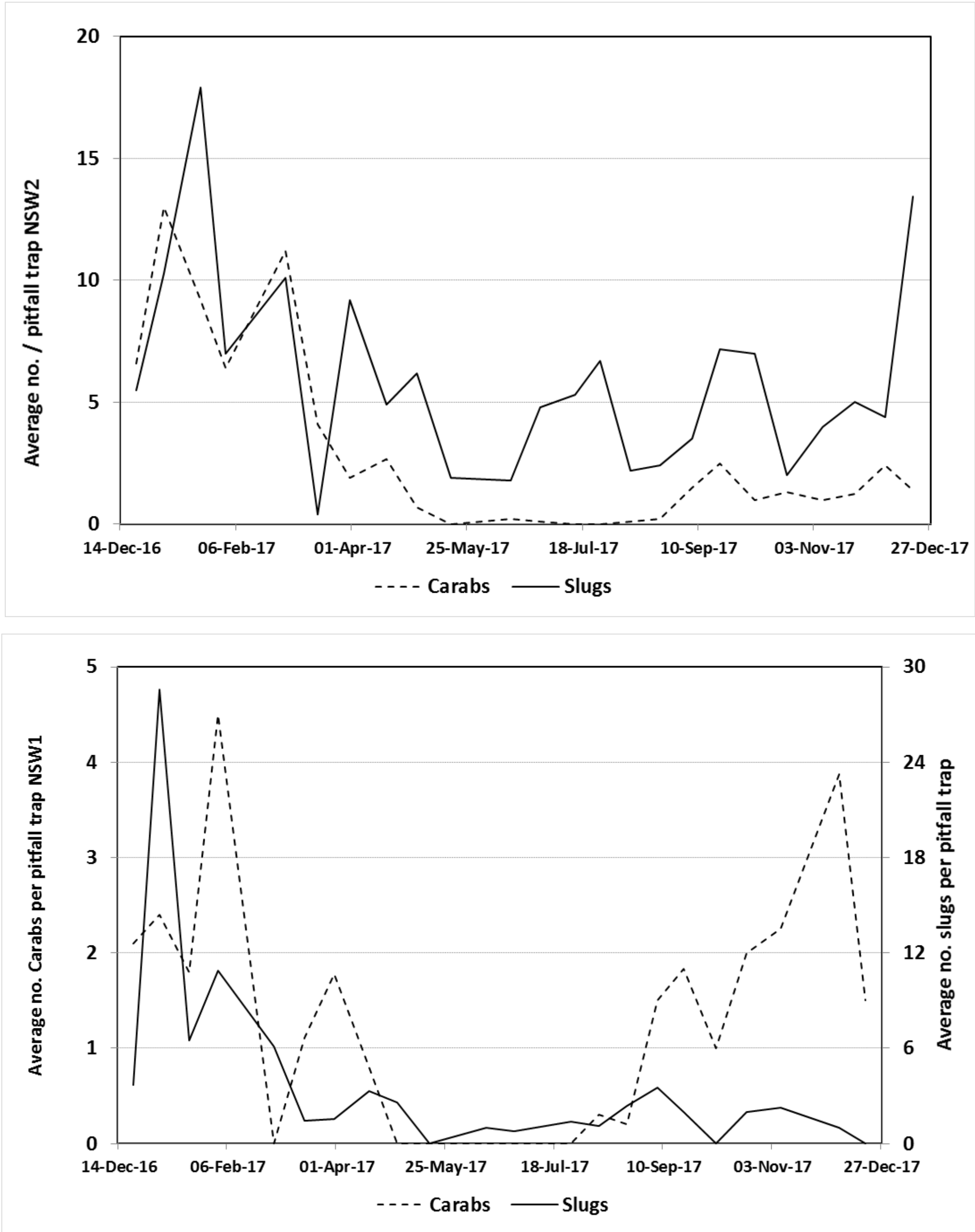
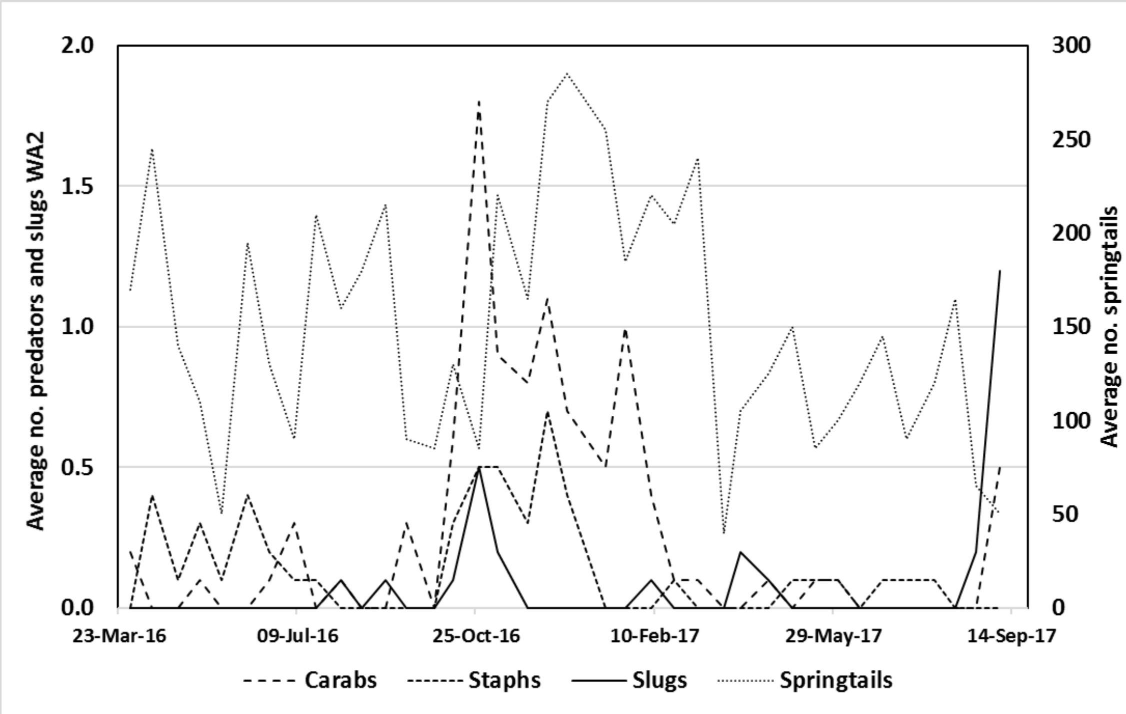


Fig.2.3.24. The abundance of predatory beetles from family Carabidae and slugs recorded in pitfall traps in two truffle orchards in NSW.



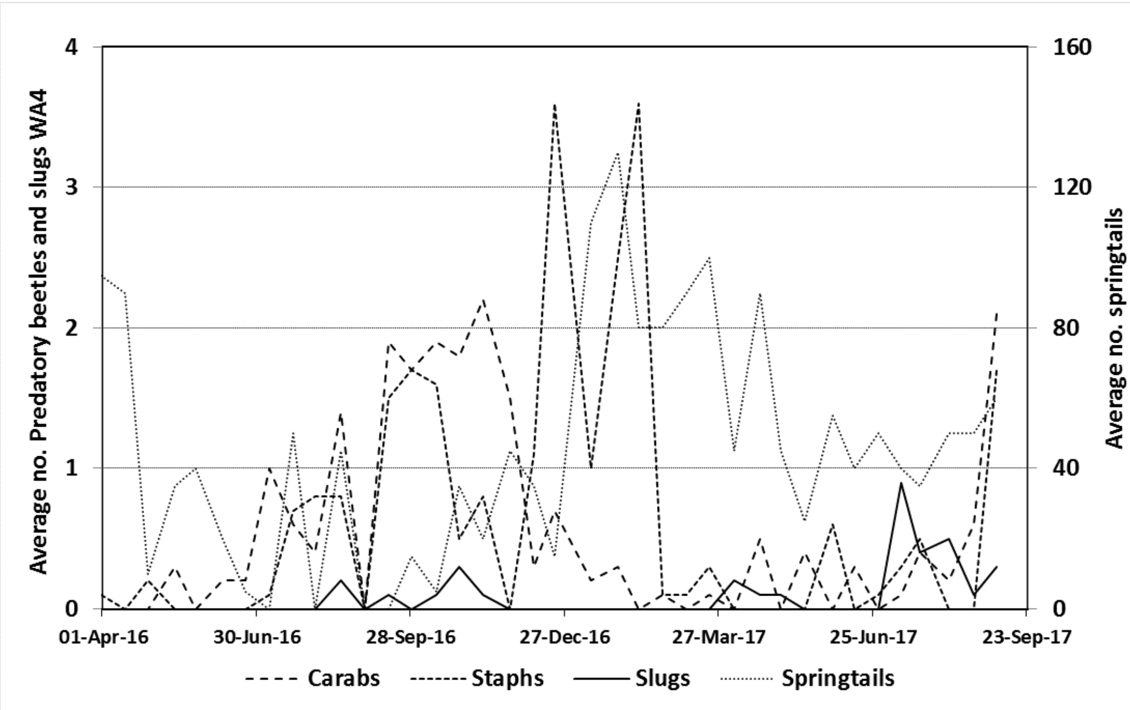


Fig.2.3.25. The abundance of predatory beetles from families Carabidae and Staphylinidae and slugs and springtails recorded in pitfall traps in two truffle orchards in WA.

No information on carabid beetles feeding on springtails was found. However, there also appears to be a relationship between the abundance of these predatory beetles and the changes in the abundance of springtails. If higher numbers of springtails are captured in one month, higher numbers of carabid beetles were also captured (Fig. 2.3.26). Further work is required to determine whether this is due to carabid beetle feeding or on environmental conditions. Some species of smaller staphylinid beetles, particularly stenines and pselaphines, are reported to be predatory on springtails (Seago, pers. comm.).

While the extent of the survey to determine whether parasitic nematodes were present in slugs in WA was limited in time and space, no nematodes were found. Further surveys could be undertaken to confirm this finding.

The use of poultry in truffle orchards could be considered where heavy populations of invertebrates are encountered. To overcome concerns of poultry causing damage to the developing truffles though disturbance to soil and the root systems of the host trees, poultry use could be restricted to short intervals. This could start immediately after harvest for around a fortnight before poultry are withdrawn, either to other parts of the orchard or removed completely. Such short intervals of poultry access could complement other sustainable management methods and at a time when some slug species are breeding – see below for comments on this timing.

Understanding the lifecycle of slugs

The heavier a slug is, the older it is and the more likely it is that the slug is laying eggs. Based on this interpretation of a combination of slug average weight and the number of slugs present, the time of year when each of the five species identified is breeding, can be estimated (Fig. 2.3.26):

- For black keeled slugs and reticulated slugs those collected in January to February had the lowest weight, whereas the highest number were found from July to August
- Brown and hedgehog slugs had a low average weight in June but at the same time slug numbers had increased from January suggesting that in June immature slugs were present
- The average weight of striped slugs increased from January to June, then declined indicating breeding was occurring in July to September.

In general, for all species slug abundance was lowest during summer and early autumn. Numbers and the average weight of slugs increased in autumn, presumably as breeding and growth occurred. As further breeding occurred resulting in an increase in number, the average weight of slugs declined then increased again towards the end of spring and early summer as slugs developed.

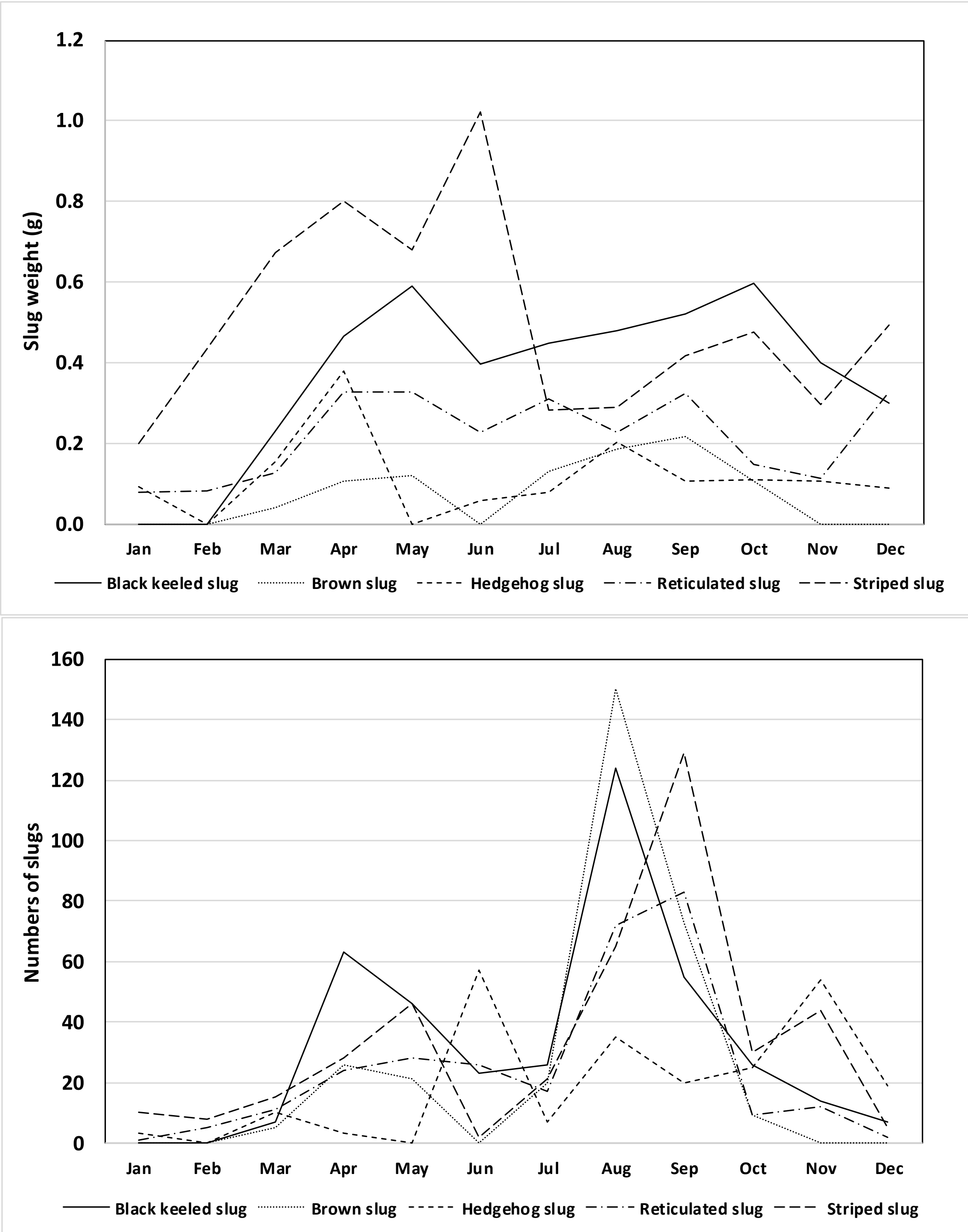


Fig. 2.3.26. The top graph is the average weight of each of the five species of slugs collected each month and the lower graph is the number of slugs weighed. Slugs were collected from all field activities in Western Australia during the project from 2016 to 2018.

Australian truffle beetle

The damage caused to truffles in Europe by the European truffle beetle has not been reduced significantly to date by any method communicated either in the literature (Pérez-Andueza et al, 2013; Morcillo et al, 2015) or by discussing the situation locally when visiting the region (Mitchell, pers. comm.). However, research is underway in Spain to clarify the role of attractant traps to reduce the abundance of truffle beetle (María Martin Santafé, pers. comm.).

This current project has shown that pipe traps and the use of liquid beetle attractant does capture ATB, hence there is an opportunity to increase the density of these traps to attract and kill ATB. Further work is required on this approach.

Where the level of truffle production is low in some regions in Spain, orchardists have sought to redress this by replacing cylinders of orchard soil with truffle fungus spore/peat mixture. Reports are that this procedure results in an increase in truffle production and coincidentally little damage by the truffle beetle. The explanation given for this apparent success is that the adult beetle finds it difficult to excavate the peat mixture compared with the soil it replaced.

European truffle beetles are suspected of not being strong fliers. This is not quite true for ATB where the beetle has been observed to fly readily in the laboratory. The distance between the most heavily infested truffle orchard and the nearest area of native forest from which the beetle would have originated is about 150m. This is thought to be well with the flight capability of ATB. What is not known is how high the beetle will fly. It may be possible to install barriers around the edge of a truffle orchard to impede the dispersal of beetles.

Many aspects of the biology of ATB remain a mystery. More detailed knowledge on its mating habits may enable management options that disrupt the lifecycle of this pest. This beetle has been observed in copulation in the laboratory so it is assumed that mating is necessary for reproduction. It is not known whether mating would take place in the soil or if beetles are required to come to the surface. If the latter is the case, it may be that some sort of aggregation or sex pheromone exists which would be used as an attract-and-kill management method. Also, placing material on the orchard floor such as weed mat or shadecloth may provide a physical impediment to beetles finding a mate.

Conclusions

This project has clarified monitoring methods for the major invertebrate pests of Australian truffles – tiles with flaky bran attractant for ground dwelling invertebrates and a liquid lure in ‘pipe traps’ for Australian truffle beetle. These will assist in deciding whether a potential pest situation occurs in a truffle orchard and can be used for further studies such as more detailed understanding of their biology. The trap for the truffle beetle offers a means of management also. ATB has been tentatively identified as *Thalycrodes* sp. nr. *australe* (Seago, pers. comm.). This genus of beetles has been described for Australia by Kirejtshuk & Lawrence (1992), but more investigation is required to confirm the identity of ATB due to its potential significance to the Australian truffle industry.

The effect of cultural and biological controls and utilising insect behaviour to manage invertebrate pests of truffles were not studied in detail but suggestions here indicate which aspects are worthy of further investigation. Studies such as those included in broadacre cropping areas in WA (Smith, 2019) provide background information and experiences of farmers that would assist in reviewing the commercial implementation and assessment of management options.

One of the major outputs of this project is the publication of an IPDM Manual for truffles and their host trees. This includes information on suggested monitoring and management methods for invertebrate pests of truffles. From activities reported in this chapter, a summary of the findings and recommendations for further studies are given in Table 2.3.4.

Table 2.3.4 The invertebrates and their development stage(s) implicated in damage to truffles and a summary of the recommended monitoring methods and potential management practices

Common name	Monitoring	Potential management practices
'Australian truffle beetle', adult, larva	'Pipe trap' baited with liquid beetle attractant (see text for details).	To be assessed - barriers to prevent entry. Intense deployment of 'wet pipe traps'. Artificial orchard floor covers to inhibit movement a research project. Attract and kill with aggregation/sex pheromone.
Slugs, slaters, millipedes, earwigs	Flaky bran under ceramic tile.	Cultural – prune the canopy to open up for light, desucker hazelnuts, litter removal, cultivation for deep truffle formation, cover exposed truffles, irrigate only as required. Biological - preserve insect predators, strategic use of poultry. To be assessed - light attract and kill for slaters, millipedes and possibly earwigs.
Springtails	Pitfall trap or equivalent	Cultural – as for slugs. Biological - preserve insect predators. The role of interrow ground covers should be assessed.
Fungus gnat, larva	Level of infestation/damage in truffles when grading	Cultural – as for slugs. Review control methods in mushroom cultivation.
African black beetle, adult, Apple weevil, larva Fuller's rose weevil, larva garden weevil, larva whitefringed weevil, larva	Opportunistic feeding on truffles	-
Click beetle/true wireworm, larva	Opportunistic feeding on truffles	-
Flies, not otherwise classified, larva	Most common in rotten truffles	Cultural – as for slugs.
Pot worm	Only present in rotten truffles	-
Garden centipede	Not considered a primary pest	-

References

Anon. 2006. Slug predator identification. In ‘Arable Extra, March 2006’. *Foundation for Arable Research: Canterbury, New Zealand*. 2pp.

Fortea, V.P., Santafé, M.M. 2018. La Sanidadad en Truficultura – Guía de identificación de agentes nocivos en truficultura (Heathy truffles – an identification guide for harmful agents in truffle farming). Truffle Association of Teruel, Spain. Ed Government of Aragon. ISBN: 978-84-8380-365-3. 156pp.

Georgis, R., Koppenhöfer, A.M., Lacey L.A., Bélair, G., Duncan, L.W., Grewal, P.S., Samish, M., Tan, L., Torr, P., van Tol, R.W.H.M. 2006. Successes and failures in the use of parasitic nematodes for pest control. *Biological Control* **38**: 103–123.

Glen, D., Bamber, G. Batchelor, C., Bohan, D., Fisher, J., Foster, V., Godfrey, M., Green, D., Gussin, E., Meredith, R., Oakley, J., Port, G., Wiltshire, C. 2006. Integrated slug control in arable crops: Risk assessment, trapping, agronomy and chemical control. Home-Grown Cereals Authority. Project Report No. 393. 263pp.

Hall, I., Brown, G., Zambonelli, A. 2007. Taming the truffle: the history, lore, and science of the ultimate mushroom. Timber Press, Portland. 304pp.

Horne, P.A. 2007. Carabids as potential indicators of sustainable farming systems Australian Journal of Experimental Agriculture. 47(4) 455-459.

Kirejtshuk, A.G. & Lawrence, J.F. 1992. Review of the *Thalycrodes* complex of genera (Coleoptera: Nitidulidae) endemic to the Australian Region. *Journal of the Australian Entomological Society.* **31**: 119–142.

Mathews, A. 2018. Truffle Pest and Disease Project Newsletter #7 on DPIRD web site. https://www.agric.wa.gov.au/newsletters/trufflepestsdiseases/australian-truffle-pest-disease-newsletter-issue-7-december-2018?page=0%2C7; refer page 8.

Morcillo, M., Sanchez, M., Vilanova, X. 2015. Truffle Farming Today, a Comprehensive World Guide. Publisher: Micologia Forestal & Aplicada. ISBN 978-84-617-1307-3. 351pp.

Pérez-Andueza, G., Ruíz, J.I., Ortiz, A., Saucedo, C. and Herrero, F. 2013. The truffle beetle Leiodes cinnamomeus (Coleoptera, Leiodidae), key pest of black truffle (Tuber melanosporum) in Central Spain: bioecological contributions and mass trapping control. Paper presented at the 1st International Congress of Trufficulture, Tereul, 5-8 March 2013.

Smith, A. 2019. Mitigating Snails, Slugs and Slaters in Southern Western Australia - Grower Case Studies. Grains Research and Development Corporation. ISBN: 978-1-921779-83-1. 80pp.

Tulli, M.C., Carmona, D.M., López, A.N., Manetti, P.L., Vincini, A.M., Cendoya, G. 2009. Predation on the slug Deroceras reticulatum (Pulmonata: Stylommatophora) by Scarites anthracinus (Coleoptera: Carabidae). *Ecología Austral (Asociación Argentina de Ecología). 19:55-61.*

2.4 Managing diseases of truffles

Introduction

The following information on factors that affect truffle rot and how some of them may be managed to reduce the level of truffle rot is based on research and experience in a truffle orchard in WA.

Reducing truffle rot

This note is based on the work of Harry Eslick (2012, 2013) and other reference material as noted.

Introduction

Truffle rot (Fig. 2.4.1) is a disease syndrome resulting in the spoilage and loss of truffles before harvest. Truffle rot has been observed across Australia, resulting in losses of greater than 50% of marketable truffle yield in some orchards.

Truffle rot typically presents as discolouration, softness and wetness of the truffle tissues and a foul smell, though sometimes may begin with a dry crumbly texture. A wide range of symptoms are observed which indicate that multiple primary or secondary disease causing agents are involved.

Truffle rot was the second most reported cause of damage and loss of value in the survey conducted as part of this project**.**

Fig. 2.4.1. One of the many symptoms of a rotten truffle.

Truffle rot is also observed in Europe, where truffles are found naturally. Observations in Spain show that rot is more likely to occur when autumn is warm. High levels of rot have recently been reported in truffle plantations in the Pacific North-West region of the USA, where the truffle industry is relatively new. The true levels of truffle rot experienced in Europe is difficult to ascertain. Little research on truffle rot has been conducted outside Australia, though it is thought that warm autumn and winter soil temperatures leads to increased oxygen demand by the truffles making them more susceptible to hypoxia.

Research performed in an orchard in Western Australia has shown that truffles which breach the soil surface are at much higher risk of developing rot (Eslick, 2012, 2013). Surface exposure increases the susceptibility to a wide range of disease causing agents, including insect pests, microbial pathogens, as well as weather-related factors such as drying and sunburn. Many of these factors may cause rot alone, or the combinations of stressors interact to reduce the truffles' natural defences to a point at which disease symptoms develop. Another factor observed in Europe is the occurrence of frost and freezing of the upper layers of soil, which when deep enough, freezes the truffle. This presents initially as patchy, pale discolouration of the truffle gleba and ultimately rotting. Given the shallow nature of the truffles in question, frosts, even mild ones, may increase the level of rot in an orchard with a significant proportion of shallow or surface truffles.

Fig. 2.4.2. Immature truffle affected by frost.

Formation of shallow/exposed truffles

Truffles are naturally produced below the surface of the soil, with signs sometimes of the developing truffle visible from the surface in the form of cracking and mounding of the overlying soil. Australian orchards experience a higher number of shallow forming truffles (Fig. 2.4.3) compared to those in Europe. Australian truffle productions systems have developed in relative isolation to the European industry resulting in significant differences in the methods of truffle cultivation. It is possible that the rapid and almost 100% adoption of irrigation explains the observations of many shallow truffles in Australia. Many other differences also exist between the environment and production systems of European and Australian. Australian plantations are established on different soils types, which are naturally acidic but amended with large quantities of calcium carbonate (lime) prior to establishment. The presence of lime only in the upper soil profile may be another contributing factor. It is not yet known whether this or other differences contribute in unison or alone.

Fig. 2.4.3. Developing truffle breaching the soil surface.

Research supported by AgriFutures, the Truffle and Wine Co. in Manjimup WA and ATGA demonstrated that the incidence of surface truffle formation at the Truffle and Wine Co.'s property could be reduced through soil tillage, as well as reducing the rate and/or frequency of irrigation. Soil tillage in particular was shown to be highly effective in reducing surface truffle formation and truffle rot (Eslick, 2012, 2013).

Interestingly both reduced irrigation frequency and cultivation treatments also resulted in a reduction of the incidence of truffle rot observed in deep truffles found below the soil surface. Cultivation has been used in Europe to disrupt pests such as slugs (P. Sourzat pers. comm.), among other reasons.

It is well known in other horticultural crops such as viticulture that the frequency of irrigation influences the depth of feeder roots. Too frequent irrigation results in very shallow feeder roots, which render the plant more susceptible to water stress. It is possible that since the truffle is dependent on the feeder root supplying nutrients from initiation to maturity, frequent irrigation may be playing a role in enhancing the development of truffle rot.

Hypoxia and Truffle Rot

The results from soil cultivation and irrigation trials suggest a relationship between those factors and soil oxygen and gas exchange. Soil hypoxia (lack of oxygen) at depth may be driving truffle formation towards the shallower soil layers, where oxygen is more readily available. Furthermore, lack of oxygen at depth may be directly responsible for rot development in truffles which do manage to develop below the soil surface.

Managing truffle rot

Site selection

Soil type, aspect and climate are all likely to have a significant impact on the risk of rot development. Previous European work has demonstrated the importance of soil texture, soil structure and aeration to truffle production. Truffles require a free draining soil with a well-developed soil structure, without excess clay content e.g. <30% (Bonet et al, 2009) to support truffle production. These factors are likely to be even more important in Western Australia where increased winter temperatures may increase the truffle respiration rate resulting in higher oxygen demand compared with truffles in the natural cooler environment of Europe. Managing existing orchards in Australia will involve techniques to improve drainage and aeration.

Irrigation

High volumes and frequent irrigations are associated with shallow truffles and a greater incidence of rot at all depths. High soil water content may lead to truffle rot through displacement of soil air spaces leading to hypoxia. Less frequent irrigation also results in drier soil in the top of the profile, resulting in less feeder roots surviving long enough to nurture a developing truffle. While soil type does influence soil moisture retention, irrigation will influence soil oxygen and feeder root resilience.

Truffles themselves are surprisingly tolerant of drought conditions with various studies showing truffle production is unaffected by soil moisture potentials as low as -300 kPa (Le Tacon et al, 1983) and -1500kPa (Oliach et al, 2016). Irrigation rates however must also meet the needs of the host tree, which will vary depending on the species of host used. Supporting the survival of the host tree is important as it in turn supports the truffle throughout its development and maturity. Anecdotal evidence that lack of water in a dry summer results in a dramatic reduction in the number of truffles reaching maturity in a producing orchard supports this hypothesis.

The impact of irrigation on truffle rot as well as truffle productivity and host vigour will vary depending on site conditions. For example, sites with deep soils and a high soil water holding capacity can sustain longer periods between irrigation events, which may reduce truffle rot, before adverse stress of the host tree is induced, compared with soils with a low water holding capacity. Altering plantation spacing, leaf area reduction, or choice of host species can also reduce tree water use, reducing the required irrigation frequency.

Currently there is a huge variation in the frequency of irrigation in Australian truffle orchards. Fig.2.4.4 shows the results of the recent national survey where most growers are irrigating between daily and weekly.

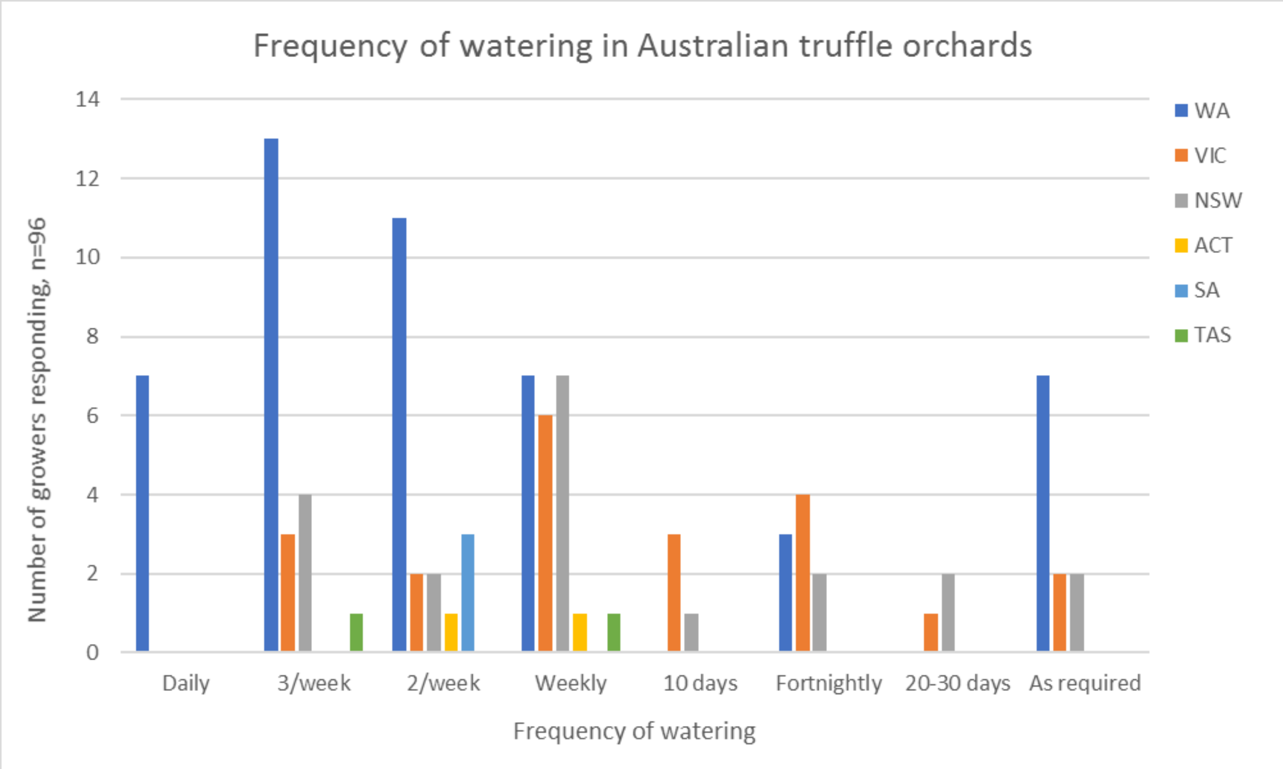


Fig. 2.4.4. Frequency of watering in Australian truffle orchards; collation from national grower survey.

Soil cultivation

Experimental results show remarkable reduction in truffle rot following soil cultivation. However caution is advised regarding cultivation as a management practice. Once host trees are established, the presence of large lateral roots will be established in the surface soil layers. Soil cultivation will generally cause severe damage to these lateral roots which may compromise tree vigour and have long term impacts on truffle productivity, or even result in tree death. Furthermore, many of the soils used in Australia for truffle production are prone to compaction when repeatedly cultivated because the soil structure is poor. These soils are often softer directly after cultivation, however, in the long term they generally become more compacted than they were prior to cultivation. Furthermore organic material that is critical for binding the soil particles together is rapidly lost as CO_2 following soil cultivation resulting in a permanent decline in soil quality, which will likely be counter-productive over the long term.

Truffle covering

Truffles which become exposed at the soil surface can be effectively protected by covering truffles with a layer of soil. Experiments have demonstrated that covering truffles had a significant effect on the percentage of truffles with rot at harvest. The timing of covering is also critically important. Truffles should be covered as soon as possible after becoming exposed to reduce the time exposed to harsh surface conditions. The type of soil used to cover truffles is important, however little work has been done to compare soil types. Ideally field soil should be used. Soils with high clay contents can hold moisture and prevent oxygen reaching the truffles which may promote truffle rot, whereas sandy soils can hold too little water resulting in desiccation of the developing truffle.

More work required

- The study of truffle rot was conducted in a single orchard – this work needs to be replicated in different conditions
- Key factors to study – from above points:
    - Effect of the range of soil types in which truffles are cultivated
    - Effect of different climates – sub tropical to temperate
    - The effect of different material used for covering truffles.
- Management techniques for improving soil structure in the truffle plantation, grass interrows, litter, organic amendments - do these cause rot?
- Are there other pathogenic fungi that can damage and cause rot in truffles?
- How does host tree and plantation design influence irrigation requirements?
- Does hypoxia cause truffle rot?

References

Bonet, J.A., Oliach, D., Fischer, C., Olivera, A., Martinez de Aragon, J., Colinas, C. 2009. Cultivation methods of the black truffle, the most profitable Mediterranean non-wood forest product; a state of the art review. EFI Proceedings no. 57: 57-71.

Eslick, H. 2012. Identifying the cause of rot in black truffles and management control options. RIRDC publication no. 12/005.

Eslick, H. 2013. Identification and management of the agent causing rot in black truffles – Part 2. RIRDC publication no. 13/111.

Le Tacon, F., Delma,s J., Gleyze R Bouchard, D. 1983. Effect of soil water regime and fertilization on fructification of the black truffle of Perigord (*Tuber melanosporum* Vitt.) in south east of France. *Acta Oecol, Oecol. Appl.* **3** (4): 291-306.

Oliach, D., Fischer, C.R., Colinas, C. 2016. Soil water potential relation to truffle productivity. In: Abstract book of ‘The eighth international workshop on edible mycorrhizal mushrooms’ (IWEMM8). Oral presentation. 10-17 October 2016, Cahors, France.

Chapter 3: Technology transfer

Extension of information to growers and other truffle industry stakeholders was a key part of the project. The main extension channels used were:

- DPIRD website, with links provided to ATGA and TPWA to extend information to their readership
- Australian Truffle Pest and Disease Project Newsletter
- Articles in other DPIRD publications and external media
- Oral presentations, including annual ATGA conferences and TPWA field days, Manjimup WA Truffle Kerfuffle grower forums and various field trips, meetings and workshops
- MyPestGuide Reporter App and Direct Enquiries.

Details on what was included in each of these is outlined below.

DPIRD website

Over the course of the project, four webpages were published on the following topics:

- Pests and diseases of truffles and their host trees
- Truffle orchard on-farm hygiene
- Pest monitoring in truffle orchards
- Assessing invertebrate damage to truffles at harvest and grading.

Analytics show high numbers of page views particularly for the 'Pests and diseases of truffles and their host tree' page which received over 6000 unique page views between 30 October 2015 and 29 January 2019 (Table 3.1). The average time spent on each page suggests that those viewing them where reading the pages and not simply landing on the pages and moving straight on.

Table 3.1 Webpage analytics for four truffle pest and disease topics produced as part of this project and located on the DPIRD website

Article Title	**Page views**		**Unique Page views**		**Avg. time on page**
	Total	**Avg. per**	**Total**	**Avg. per**	
Pests and diseases of	7185	184	6092	156	3:08
On-farm hygiene	644	17	568	15	2:43
Pest monitoring	232	15	128	9	2:48
Assessing damage at	149	19	110	14	2:16

The number of views per day of the 'Pests and diseases of truffles and their host trees' remained consistent over the life of the project showing there was regular engagement with the extension resources being produced (Fig. 3.1).

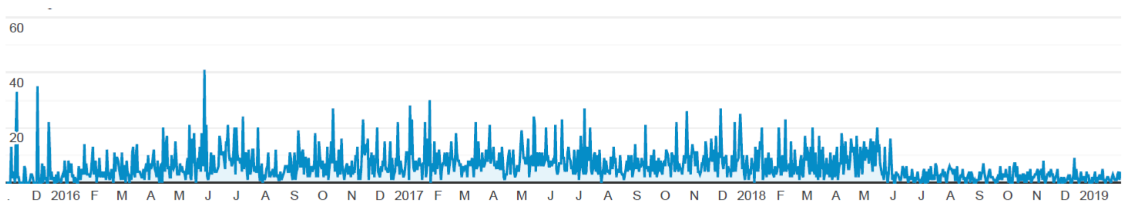


Fig. 3.1. Daily hits to the 'Pests and diseases of truffles and their host trees' bulletin on the DPIRD web site.

The information in these pages has been combined into the Australian Truffle Orchard IPDM Manual and Australian Truffle Orchard Pest Identification Guide. These documents will be made available on the DPIRD website and the above pages will be removed.

Australian Truffle Pest and Disease Project Newsletter

The newsletter was used to provide growers with the latest information on project research and extension activities, inform them of resources available, provide information on specific pests and diseases and explain IPM techniques that could be used in orchards.

Seven project newsletters were produced from December 2015 to December 2018. They were sent out as an email that re-directed people to the DPIRD website where the newsletter was hosted and archived.

The first newsletter was sent to the email addresses of growers and other stakeholders known to the project team as well as to the Secretaries of ATGA and TPWA. People were able to self-subscribe to the newsletters and that along with an expanding list of known growers lead to the number of direct recipients more than doubling over the life of the project (Table 3.2).

Table 3.2 The number of recipients on the email list for each Australia truffle pest and disease project newsletter issue

Newsletter issue - date	No. of recipients
1 – Dec 2015	85
2 – Sep 2016	na
3 – Jan 2017	na
4 – Aug 2017	183
5 – Dec 2017	182
6 – Jul 2018	185
7 – Dec 2018	183

Tables 3.3 to 3.9 below list the article headlines in each newsletter. These tables also contain the number of page views, unique page views and average time spent on each page for all of the articles. Note that due to the set-up of the newsletter webpages not all page views can be attributed to specific articles so the page views for all individual articles does not always add up to the total page views.

As was the case for the Pest and Disease bulletin, the average time spent on each page suggests that for the majority of articles, those viewing them where reading the articles and not simply landing on the pages and moving straight on. For each newsletter edition, the first article always received more page views. This was anticipated and the article order was managed to keep the most pertinent information for growers in the leading articles.

The number of page views did decline towards the end of the project. The reason for this is unknown. However, there were 385 unique views of articles for the final issue, meaning on average everyone on the mail list read at least two articles from this newsletter.

Table 3.3 The articles and readership details for Australian Truffle Pest and Disease Project Newsletter Issue 1 – December 2015

Article Title	Page views	Unique page views	Avg. time on page
Total	1074	880	
Welcome to the project	472	380	1:50
Pest and disease field guide and enquiry line	234	189	1:29
National grower survey	124	105	1:04
ATGA conference and AGM – Pest and disease project presentation and field activity	113	94	1:05
Basics of on-farm hygiene	108	94	1:03

Table 3.4 The articles and readership details for Australian Truffle Pest and Disease Project Newsletter Issue 2 – September 2016

Article Title	Page views	Unique page views	Avg. time on page
Total	1384	1094	
Introduction – Breaking news	512	405	2:06
MyPestGuide Reporter - Truffle Survey	180	151	0:51
National Grower Survey	115	90	0:34
Mapping Australian Truffle Orchards	129	96	0:35
Field Activities – Tree diseases - Fauna in truffle orchards and truffle pests	151	116	2:06
Biosecurity	100	82	0:49
2016 ATGA Conference and AGM, Manjimup WA 9 – 11 September	95	79	1:04

Due to the format of newsletter Issue 3, analytics could not be done on individual pages.

Table 3.5 The articles and readership details for Australian Truffle Pest and Disease Project Newsletter Issue 3 – January 2017

Article Title	Page views	Unique page views	Avg. time on page
Total	300	196	2:37

Articles from issue 3 – January 2017

- Identifying and cataloguing pests and diseases of truffles and their host trees
- National pest and disease survey - top pest species across the country
- Mapping & monitoring sites of Australian truffle orchards
- Field activities.

Table 3.6 The articles and readership details for Australian Truffle Pest and Disease Project Newsletter Issue 4 – August 2017

Article Title	Page views	Unique page views	Avg. time on page
Total	935	773	1:42
Pest monitoring continues - what are we finding in orchards?	265	196	1:43
More primary pests of truffles confirmed	122	107	1:36
What's been damaging my truffle – Our assessments so far this season	124	106	1:51
Truffle beetle update	133	117	1:06
Out and about with the project team - Extension activities in 2017	121	107	2:26
Project team and support	98	80	1:03

Table 3.7 The articles and readership details for Australian Truffle Pest and Disease Project Newsletter Issue 5 – December 2017

Article Title	Page views	Unique page views	Avg. time on page
Total	614	441	
DIY orchard pest monitoring	184	121	1:30
Truffle damage assessments in Western Australia - 2017 harvest	104	71	1:37
Orchard monitoring in the east	68	51	1:14
Slug and slater field trials	63	51	1:19
Using wet traps to attract truffle beetle	62	47	1:28
Out and about with the project team - Spring extension activities	85	61	3:17

Table 3.8 The articles and readership details for Australian Truffle Pest and Disease Project Newsletter Issue 6 – August 2018

Article Title	Page views	Unique page views	Avg. time of page
Total	518	381	
How costly is invertebrate damage to truffles?	173	121	1:54
Assessing invertebrate damage to truffles at harvest and grading is a guide to prevention	88	64	1:15
A new foliar disease of *Quercus robur* (European or English oak) in Western Australia	91	73	1:05
French truffle growers visit Manjimup in June	96	68	2:14
Extension Activities – Autumn and Winter 2018	67	52	1:49

Table 3.9 The articles and readership details for Australian Truffle Pest and Disease Project Newsletter Issue 7 – December 2018

Article Title	Page views	Unique page views	Avg. time of page
Total	455	385	1:47
The truffle pest and disease project winds up	102	72	2:49
2018 Harvest damage assessment	63	54	1:54
Fungi important in disease on hazelnut trees	46	41	1:48
Integrated Pest and Disease Management	30	27	1:09
Disease or drought?	30	28	0:45
Springtails in Truffle orchards	36	35	1:31
Spring beetles in truffle orchards	30	27	0:46
What happens in the orchard when you're not there?	46	42	2:10
Recent observations from WA disease survey	29	24	0:55
Eastern states' road trip July 2018	41	33	1:52

Articles in other DPIRD publications and external media

To broaden the audience reached by the project the following articles were produced for other newsletters and publications:

- 2015, DAFWA (now DPIRD) AgMemo South West Agricultural Region, April - New project to protect bourgeoning truffle industry
- 2015, RIRDC (now AgriFutures Australia) Rural Diversity, issue 18 – New biosecurity project to protect flourishing truffle industry
- 2015, WA government media statement, June - Research to protect valuable truffle industry
- 2015, Manjimup-Bridgetown Times newspaper article, June – Boost for truffle growers
- 2017, DPIRD AgMemo - National truffle pest and disease project activities to August 2017
- 2017, The Truffle and Wine Co, Truffle Business, edition 03 June, TWC newsletter article - Update on the RIRDC project
- 2018, DPIRD AgMemo, August - Clarity on pests and diseases to yield quality truffles.

Oral presentations and workshops

Two grower workshops and 30 presentations were given by project team members from 2015-2019. A very broad geographical spread of growers were able to attend presentations as they were conducted in every truffle growing state (except Qld and SA due to them being smaller sectors of the industry) over the life of the project. The ATGA national conference and Truffle Kerfuffle, held in Manjimup WA, provided excellent forums for presentation of technical information to truffle growers.

ATGA national conference presentations and workshops

- 2015 – Queanbeyan, ACT, 13-15 November
    - Truffle pests and diseases and the WA mapping project - S. Learmonth.
- 2016 – Manjimup, WA
    - Pests and diseases of truffles and their host trees, grower workshop – H. Collie, A. Davey, P. Dal Santo, S. Learmonth, C. Linde, A. Mitchell and A. Seago
    - Pests and diseases of truffles and their host trees in Australia, one year on - S. Learmonth and A. Mitchell
    - Pests and diseases of truffles, biosecurity and other tools - A. Davey
    - Diseases and disorders of truffle fruiting bodies - H. Eslick
    - Tree diseases in truffle production - C. Linde
    - Truffle orchard irrigation - R. Prince.
- 2017 – Ballarat, Victoria, 14 October
    - Pest and diseases workshop and research communication - A. Davey, P. Dal Santo, A. Mathews, A. Mitchell,
    - Pest and diseases part 1 - A. Davey
    - Pest and diseases part 2 - A. Mathews
    - Truffle grower survey - A. Mitchell.
- 2018 – Medlow Bath, NSW, 15 September
    - [#] Truffle tree diseases - C. Linde
    - [#] Biosecurity Threats to the Truffle Industry – C. Linde
    - [#] Pests of truffles - A. Mathews
    - [#] Pests and diseases of truffles and their host trees - A. Mitchell
    - [#] *Xylella fastidiosa* and its diseases – A. Davey (ATGA sponsored talk).

Eastern state road trips

- 2017 – 2-14 July: Sutton and Bathurst, NSW; Woori Yallock, VIC; Deloraine TAS.
    - P&D Project ES Road Show Intro & Tree Pests July 2017
    - P&D Project ES Road Show Tree Diseases & Truffle Rots July 2017
    - P&D Project ES Road Show Australian Truffle Beetles July2017
    - P&D Project ES Road Show Invertebrate Pests other than Truffle Beetles July 2017.
- 2018 – 2-15 July: Sutton and Bathurst, NSW; Ballarat, VIC; Deloraine TAS.
    - Pests and diseases in Australian truffle orchards – S. Learmonth.

Truffle Kerfuffle growers' forum

- 2017 – Manjimup, 25 June
    - Integrated pest management in truffle orchards - S. Learmonth
    - Truffle tree hosts diseases, including notes on exotic diseases - C. Linde.
- 2018 – Manjimup, 25 June
    - Pests and diseases in Australian truffle orchards - S. Learmonth
    - Tree health and truffle production - C. Linde
    - [#] Pests of truffles - A. Mathews.

Truffle Producers of Western Australia (TPWA) field days

- 2017 TPWA AGM October - Pests and diseases of truffles and their host trees in Australia, project update - A. Mathews (repeat of presentation at the ATGA conference)
- # 2018 TPWA field day April - Truffle pest and disease project update - A. Mathews
- 2018 TPWA AGM October - Pests of truffles - A. Mitchell and A. Mathews (repeat of presentation at the ATGA conference).

Other presentations

- 2017 WA Slug seminar February - Slugs in truffle orchards - M. Nash and S. Learmonth
- 2017 WA Seminar Truffle P&D Project Australian Truffle Beetles 23June2017 – S. Learmonth
- #2018 Gordon Victoria Grading workshop - Pests and diseases in Australian truffle orchards - A. Mitchell and S. Learmonth.

MyPestGuide Reporter App. and Direct Enquiries

The MyPestGuide Reporter App. is produced and managed by DPIRD. It is a free app that allows any member of the public to submit pictures and descriptions on unknown pests for identification. The availability of this App. was widely promoted to the truffle industry through the project newsletter and presentations. However, there was minimal uptake by industry in the use of the MyPestGuide Reporter App. for getting unknown pests identified (Table 3.10). Over the life of the project 29/05/2015 to 10/01/2019, thirty-nine reports were received via MyPestGuide Reporter, thirty from Western Australia and nine from NSW/ACT.

Table 3.10 The number of truffle related enquiries received through the MyPestGuide Reporter App. from 2015-2018

Year	No. of enquiries
2015	1
2016	15
2017	16
2018	7

The reason for the low uptake of the MyPestGuide Reporter App. by truffle growers is unknown. Several explanations are that the project team also encouraged direct correspondence with growers and would regularly receive emails with photos of pests for identification, bypassing the need for the App. Also, the pests found to be the most abundant and widespread were ones that are easily identified, namely slugs and slaters.

Chapter 4: IPDM Manual and Identification Guide

The two major extension outputs from this project are the:

- Australian Truffle Orchard IPDM Manual
- Australian Truffle Orchard Pest Identification Guide.

These documents bring together information already published on DPIRD truffle webpages as outlined in Chapter 3 as well key outcomes from to the project collated into formats that are practical and applicable for truffle producers.

Australian Truffle Orchard IPDM Manual

The Manual is designed to be a comprehensive reference for integrated pest and disease management for Australian truffle orchardists. It will be available in a digital and print friendly format on the DPIRD website. A digital format has been decided upon as it will be widely accessible and more easily updated.

The Manual explains integrated pest and disease management, and describes the steps involved in using IPDM and how it can be applied in truffle orchards.

The first step in an IPDM program is to understand the orchard environment and what agents are present. Hence the core of the Manual is a section on the pests and diseases that have been identified in truffle orchards over the course of this project. Information is provided on each agent including physical description, lifecycle, damage, and monitoring and management techniques. Following this is information on exotic pests and diseases of truffles and their host trees.

Prevention and monitoring are the next steps in an IPDM program and a section on each will be included in the Manual. The prevention section will look at establishing and maintaining a healthy orchard as well as the importance of on-farm biosecurity and how to implement it. The monitoring section describes why monitoring in the orchard and at harvest and grading is so important. Instructions are given on how to conduct in-orchard pest monitoring with techniques devised and honed in this project for the most common and important pests of truffle orchards; as well as how to conduct truffle damage assessments at harvest and grading. Monitoring scoresheet templates are included.

Australian Truffle Orchard Pest Identification Guide

The Guide is produced as a ‘ute guide’ style publication. Like the Manual, the format is digital and print friendly and available on the DPIRD website. A single run of hard copies will be printed and made available to truffle orchardists, researchers and personnel who provide services to truffle growers. A combination of digital and hard copy format have been chosen as a digital format is widely accessible and more easily updated, yet having a small, portable document that can be taken into the field is very useful when wanting to identify agents at the time they are observed in the orchard.

The information in the Guide is designed to be more concise than the Manual, focussing on descriptions and photographs. It is intended that this document will be used by truffle producers to identify the invertebrates and diseases in their orchard and then determine if they are a pest, beneficial or benign.

Exotic pests and diseases are included. Monitoring of orchards has been promoted during the project and is further encouraged in the Manual and Guide. It is important to include descriptions and photographs of exotic pests and diseases so that if an incursion into a truffle orchard does occur it is more likely to be noted, reported and acted upon to improve the chance of eradication.

The ‘Manual’ will be available on the DPIRD web site and revised periodically.

The ‘Guide’ will be printed in hard copy by DPIRD for distribution to relevant parties. Updates will be reprinted as required.

Chapter 5: Biosecurity/Export/PHA Biosecurity Manual

Introduction

A review of the biosecurity threats of exotic pests and diseases that would affect the well-being of the Australian truffle industry has been undertaken by Plant Health Australia (PHA). This review has been compiled into the document *Biosecurity Plan for the Truffle Industry*. A copy of latest version of the plan, 1.0, 2016, is available by contacting PHA by phone 02 6215 7700 or email admin@phau.com.au. The Australian truffle industry is a signatory to the PHA Emergency Plant Pest Response Deed which allows the industry to establish a mechanism to facilitate the making of rapid responses to, and the control and eradication of, emergency plant pests.

The Australian truffle industry is free of some notable pests and diseases which are indicated in the biosecurity plan. Detail on the more important agents is provided in the form of fact sheets available on the PHA website (http://www.planthealthaustralia.com.au/industries/truffles/).

As well as considering and minimising the risks from incursions of such exotic agents, the Australian industry, primarily through the export of truffles, should consider threats posed to receiving countries of pests and diseases that occur in Australia but not overseas. As a result of the current project, clarification of some relevant agents has been made.

This section considers some of the main diseases and pests exotic to Australia. Following this, the relevance of the risk to exports of Australian truffles by endemic agents that growers and exporters should be aware of is discussed.

Exotic Diseases

The three main exotic diseases that are a threat to truffle host trees in Australia as listed by PHA and included in table 5.1. For each of these, information on identification of symptoms is provided as well as notes on their importance.

Table. 5.1 Plant Health Australia Truffle Biosecurity Plan - risk rating for the three most threatening exotic diseases of truffle host trees

Disease	Host Tree	Affected plant part	Entry potential	Establishment potential	Spread potential	Economic impact	Overall risk
Sudden Oak Death	Oak and Hazel	Stems, branches, leaves	HIGH	HIGH	HIGH	HIGH-EXTREME	HIGH-EXTREME
Filbert Blight	Hazel	Branches and stems	MEDIUM	MEDIUM	HIGH	EXTREME	HIGH
Xylella (Pierce's)	Oak and Hazel	Whole plant	MEDIUM	MEDIUM	HIGH	HIGH	MEDIUM

Sudden Oak Death, *Phytophthora ramorum*

Phytophthora ramorum, (Anon., 2019b) as the name of the disease implies, causes a sudden death in oak trees native to North America. It is a newly emerged pathogen belonging to the Stramenopila (Oomycetes). Symptoms include a stem or trunk canker with red sap oozing from the bark surface, leaf blight and twig dieback. Infection typically result in blackened shoots and dark blotching of leaves. Infection results in host death in the most susceptible tree species, whereas in less susceptible species foliar infection leads to premature leaf death and leaf drop (Rizzo et al., 2002). On *Q. ilex* infected young leaves are water-soaked, dull grey in appearance, and petioles are blackened. Lesions start at leaf margins, tips, or petioles, often progressing into the midrib veins. Initial infections also occur on shoots and extend into the petioles. If shoots are infected, they are blackened at first, but later in the season clusters of dry, dead leaves and twigs characterize branch tips. Infected mature leaves bore dry, reddish-brown, restricted lesions (Denman et al., 2005).

Host range: Phytophthora ramorum is known to infect over 130 plant species representing approximately 40 genera. Common ornamental host species include azalea (*Rhododendron* spp.), *Camellia* spp., common beech (*Fagus sylvatica*), elder (*Sambucus nigra*), fir (*Abies* spp.), horse chestnut *(Aesculus hippocastanum*), larch (*Larix* spp.), *Magnolia* spp., maple (*Acer* spp.), oak (*Quercus* spp.), *Pittosporum undulatum* and *Viburnum* spp. Common commercial host species include avocado (*Persea americana*), blueberry (*Vaccinium* spp.), chestnut (*Castanea sativa*), hazelnut (*Corylus* spp.) and macadamia (*Macadamia integrifolia*) (Grünwald et al., 2008). While most foliar hosts are not killed by the disease, they do play a key role in the spread of *P. ramorum*, acting as source of fungal spores. Many native plants in Australia, especially Proteaceae, are very susceptible to *P. ramorum*.

Distribution: Phytophthora ramorum is found throughout North America and Europe.

Transmission: Unlike most other phytopthoras, *P. ramorum* is airborne and can be disseminated by wind and rain splash. Long distance dispersal of the pathogen can occur in rivers and streams and human activities such as the movement of infected plant material, e.g. infected nursery material. Animals (e.g. deer, squirrels, birds, snails) might also act as vectors.

Management: Once *P. ramorum* infects trees, there is no known way to cure them. Phosphonate applications will suppress disease progression in early infections, but will not cure the tree. Therefore, most of the management practices are directed at preventing the spread of the disease to new plants or areas and protecting susceptible trees before they are infected.

Eastern filbert blight, *Anisogramma anomala*

Eastern filbert blight (Anon., 2019a) causes potentially fatal cankers on the trunk and branches on cultivated hazel, *Corylus avellana,* as well as on wild hazel (*Corylus* spp.) (Figs. 5.1 and 5.2). In the 1960s, the disease spread on infected plant material to Oregon, where it then threatened US hazelnut production in the Willamette Valley. In the US, entire hazelnut orchards have been lost to the disease.

Once cankers form, they can expand at a rate of 30cm/year. This causes canopy and yield loss and can cause death of mature trees in 5-15 years with younger trees being killed within 4-7 years. As the canopy dies back, new shoots and suckers may emerge from the tree base and these in turn become infected and die. Infected trees may not show symptoms for up to two years.

Host range: Coryllus spp.

Distribution: Eastern filbert blight is caused by a fungus known as *Anisogramma anomala*, native to Eastern North America and currently only present in North America.

Transmission: New infections are usually as a result of infected nursery stock. The fungus cannot spread over large distances as cool wet weather and rain splash is needed for dispersal and infection.

Fig. 5.1. Eastern filbert blight dieback in hazelnut trees (Photo by CropLife Foundation).

Fig. 5.2. Eastern filbert blight fruiting bodies on an infected hazelnut branch (Photo by Jay W. Pscheidt, Oregon State Univ., Pacific Northwest Pest Management Handbooks).

Management: Breeding for resistance led to the selection of resistant cultivars in the US, but these are unavailable at present in Australia. In Oregon, scouting for cankers, therapeutic pruning, and copious fungicide applications are reported to be necessary (but costly measures) to continue hazelnut production in the presence of the disease.

Xylella fastidiosa

Symptoms vary depending on the host plant species and its degree of susceptibility, but include marginal leaf scorch, wilting of foliage and withering of branches. Severe infections in some of the most damaging combinations of host plant and *Xylella* sub-species can result in dieback, stunting and eventual death, e.g. with olive trees or grape vines, on which it is known as Pierce's disease, (Fig. 5.3). *Xylella fastidiosa* is considered by biosecurity specialists to be potentially the most significant threat ever faced to plant-based industries and national flora. Even eucalypts and Acacias native to Australia, are susceptible to *Xylella* ((EFSA), 2018). Australia has at least 350 native, commercial and ornamental plant species that are susceptible, potentially providing multiple hosts for transmission and evolution.

Fig. 5.3. *Xylella fastidiosa* infection in an olive orchard in Italy (Photo by USDA-ARS).

Host range: Xylella fastidiosa is a bacterium that can cause significant dieback and death in more than a 100 woody plant species, representing more than 50 plant families. Many strains of the pathogen exists. Hosts include almonds, blueberries, cherries, citrus, macadamias, olives, grapes, walnuts etc. Symptoms range from leaf scorch (browning) to dieback and death. Not much is known about the susceptibility of truffle host trees, although a recent report suggest *Quercus ilex* is susceptible to a strain of *X. fastidiosa*. (Jeger et al., 2016), as well as *Quercus robur* and *Q. suber* ((EFSA), 2018).

Distribution: The disease is widely distributed in North and South America, but was also introduced to Taiwan (pre 2013), Italy (2013), Iran (2014), France (2015) and Spain (2016). It is under eradication in some of the European countries.

Xylella fastidiosa affects its host plants by invading their water-conducting systems, moving both upstream and downstream. In so doing, it restricts or blocks the movement of water and nutrients through the plant, with serious consequences, including death, for some host plants.

Transmission: The disease is transmitted by anthropogenic movement of plant material and insect vectors such as sharpshooters, spittlebugs, leafhoppers and aphids which feed on plants' xylem fluid. There are several species of insects in Australia which could vector (spread) *Xylella fastidiosa*. Although such insects usually only fly short distances of up to 100 metres, they can be carried much longer distances by the wind.

Long-distance spread can occur by the movement of infected plants for planting. These plants can act as a source of the bacteria for the feeding insects, which can then transmit it to other hosts. There can also be some transfer of the bacterium between neighbouring plants via root grafts.

Management: Disease management employs the same general set of tools as for other vector-borne plant pathogens. Because there are currently no truly *Xylella*-resistant varietals available, management relies on a combination of efforts to control vectors and reduce pathogen supply. Vector control can take the form of biological control, chemical control, and other methods of reducing vector abundance or activity. Reducing pathogen supply can include removing infected material and reservoir hosts in adjacent habitats.

Exotic Insects

There are two insects that cause high levels of damage to truffles in Europe but which do not occur in Australia. These are European truffle beetle, *Leiodes cinnamomea*, and truffle fly, *Suillia* spp.

European truffle beetle, *Leiodes cinnamomeus*

This beetle causes similar damage to truffles in Europe as Australian truffle beetle (ATB), potentially rendering individual truffles a complete loss. ATB has been recorded as an important pest in one Australian orchard only to date. Unlike ATB, taxonomically the European truffle beetle (ETB) belongs to a different family of beetles, Leiodidae. The Australian beetle belongs to the family Nitidulidae. The main distinguishing characters between the two beetles are that adult ATB has club like antennae and many rows of spines along its back, whereas ETB has neither of these features (Fig. 5.4). Detail on the life history and habits of ETB can be found in the books by Morcillo et al (2015) and Fortea and Santafé (2018).

Fig. 5.4. European truffle beetle (ETB) (left side) and Australian truffle (ATB) beetle showingthe differences with ATB having clubbed antennae (indicated by arrow) and rows of short spines on its back. (Photo of ETB coutesy Wikipedia).

Host range

Both larvae and adults of European truffle beetle are obligate truffle feeders and is an important pest of black truffle (*T. melanosporum)*, the main culinary truffle cultivated in Australia. It is likely that any native truffles that occur in the forests of Europe, and possibly Australia, would be a suitable host for the beetle.

Distribution

As the common name implies, these beetles are native to Europe, the home of black truffle and other native European truffles that occur in native forests and some of which are commercially farmed in truffle orchards.

Dispersal

Like ATB, ETB is capable of flight but this is only relevant for short dispersal flights so adults can locate truffle near to where beetles emerge in their native habitat. Because ETB is an obligate truffle feeder, it is most likely to be moved over long distances as adults or larvae in infested truffle. Depending on the longevity of adults or larvae, they may be able to survive for reasonable periods of time in imported truffles.

Management

Research is underway in Europe to determine methods that will reduce the damage the beetles cause. Insecticides are not used to control the insect in Europe, with the main focus currently on whether trapping adults with attractants in specially designed traps will be effective in reducing populations (M. Martín Santafé, pers. comm.).

Truffle fly, *Suillia* spp.

These truffle fly species belong to the fly family Heleomyzidae. There are many species of flies in this genus in Europe. Six have been associated with damage to black truffle in Spain (Fortea and Santafé, 2018) (Fig. 5.5). No obligate truffle feeding flies were identified in Australia during this project, but species of flies in this family do occur in Australia. All flies observed on truffles during this project were regarded as opportunistic species that were either generalist fungus feeding flies such as sciarids or flies that feed on decaying organic matter (Table 1.2.9).

Fig. 5.5. Adult truffle flies are about 10mm long; their larvae are obligate truffle feeding insects and are an important pest of truffles in Europe (Photos courtesy Wikipedia and Flickr).

Host range

Apart from truffles, the host range of this fly is not known. The flies are well adapted to locating truffles and this ability has been used by people to harvest truffles. The flies use the scent of ripe truffles to indicate where they should lay eggs. Larvae dig after hatching to feed on truffles.

Distribution

Truffle fly occurs in Europe; none of the species that damage truffles has been recorded in Australia.

Dispersal

Descriptions of the flight of flies as jumping rather than flying indicate that they can disperse by flight but not over long distances. If these insects are truly obligate truffle feeders, the most likely entry to Australia is via the larval stage in infested truffle. Depending on the longevity of larvae, they may be able to survive for reasonable periods of time in imported truffles.

Management

An attractant and sticky paper and are available for attracting and killing truffles flies in Europe (Fortea, V.P. & Santafé, 2018), but their effectiveness is not known.

Endemic agents and export truffles

As far as known, all Australian truffles are made ready for sale after being washed and brushed to remove soil, then air dried before being graded. The intensive process of grading involves individual examination of truffles including the removal of portions of any truffle with gouges by invertebrates or rot. Any holes in truffles are examined carefully to ensure no live agents are present within truffles. Such an infestation may result in the flesh of truffles containing soil particles, as has been observed in truffles infested with Australian truffle beetle (ATB).

The larger invertebrates such as slugs, slaters, millipedes and earwigs are easily removed, usually during harvest in the truffle orchard, but the smaller and internal feeding invertebrates pose a risk of not being detected. This risk is overcome in the grading room by careful examination of any truffle with holes caused by the invertebrates such as Australian truffle beetle. Cracks in truffles may occur during truffle formation due to environmental stresses such as variable temperature or soil moisture changes. Truffles infected with rot or that have cracks are predisposed to being infested with springtails, fly larvae and potworms. These truffles require special attention during grading to ensure any invertebrates are removed before being sold. For a list of invertebrates that could infest truffles, see Table 1.2.9.

References

Anon. 2019a. Eastern filbert blight. Plant Health Australia Fact Sheet. http://www.planthealthaustralia.com.au/wp-continuedent/uploads/2013/03/Hazelnut-blight-or-Eastern-filbert-blight-FS.pdf

Anon. 2019b. Sudden oak death. Plant Health Australia Fact Sheet. http://www.planthealthaustralia.com.au/wp-continuedent/uploads/2015/07/Sudden-oak-death-FS-Plantation-forestry.pdf

EFSA 2018. Update of the *Xylella* spp. host plant database. *EFSA Journal* **18:** 5408.

Denman, S., Kirk, S.A., Brasier, C.M., Barton V.C., Hughes, K.J.D., Webber, J.F. 2005. *Phytophthora ramorum* on *Quercus ilex* in the United Kingdom. *Plant Disease* **89:** 11, 1241.

Fortea, V.P., Santafé, M.M. 2018. La Sanidadad en Truficultura – Guía de identificación de agentes nocivos en truficultura (Heathy truffles – an identification guide for harmful agents in truffle farming). Truffle Association of Teruel, Spain. Ed Government of Aragon. ISBN: 978-84-8380-365-3. 156pp.

Grünwald, N.S.J., Goss, E.M., Press, C.M., 2008. *Phytophthora ramorum:* a pathogen with a remarkably wide host range causing sudden oak death on oaks and ramorum blight on woody ornamentals. *Molecular Plant Pathology* **9:** 729-40.

Jeger, M., Bragard, C., Caffier, D.*, et al.*, 2016. Susceptibility of *Citrus* spp., *Quercus ilex* and *Vitis* spp. to X*ylella fastidiosa* strain CoDiRO. *EFSA Journal* **14.** 4601. 19pp.

Morcillo, M., Sanchez, M., Vilanova, X. (2015). Truffle Farming Today, a Comprehensive World Guide. Publisher: Micologia Forestal & Aplicada. ISBN 978-84-617-1307-3.

Rizzo, D.M., Garbelotto, M., Davidson, J.M., Slaughter, G.W., Koike, S.T. 2002. *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and L*ithocarpus densiflorus* in California. *Plant Disease* **86:** 205-14.

Results and Discussion

The database of Australian truffle growers was achieved with partial success. Information regarding the truffle industry in Western Australia was very accurate because the distribution of growers is over a much smaller are than for eastern Australia and a high proportion of growers are members of TPWA. The information regarding the truffle industry in eastern Australia was not as accurate or complete due to the wide area over which truffle growers are located – from Queensland through NSW, Victoria and South Australia to Tasmania.

The data generated on the Australian truffle industry from the national survey was comprised of a high percentage of participation by growers. The information on results of the survey collated in this report is therefore considered to be a good reflection of the industry. The full report on the survey is available as a separate document appended with the final report: *The Australian Truffle Industry – a snapshot*.

Through the range of tech transfer modes established to facilitate two-way exchange of information between truffle growers and the research project team, an effective dialogue was established. This was enhanced by conducing the one year in-depth monitoring on nine truffle orchards across Australia as well as less regular field visits to gain insight to pests and diseases of truffle host trees and truffles.

To confirm and supplement information gathered from the national grower survey, the focussed, regular truffle orchard pest monitoring yielded valuable information on the presence, abundance and seasonality of pest of trees and truffles. This information was further enhanced by one-off truffle orchard visits, both as enquiries were received and could be acted on and two study tours by members of the project team through eastern Australia in 2017 and 2018. The information on pests and diseases gather during the project was collated into two separate publications – management and identification. While the original objectives of the project included weeds as a pest agent in truffle orchards, it was quickly realised that to document the range and prevalence of weeds and investigate their management was beyond the scope of resources and expertise of the research team. Therefore, no studies were undertaken with respect to weeds. Despite this, and compared to the effects of other pest and disease agents, weeds seemed of minor importance to growers.

At the outset, the project sought to conduct investigations to manage what were considered to be major pests - slugs, snails and collembolans (springtails). This was followed-up for slugs only. It was apparent during the early stages of the project that snails presented a minor threat to truffle host tress and truffles, especially in comparison to other agents discovered to be very important but were not considered earlier. Likewise, springtails were not regarded as being important but this view changed towards the end of the project and springtails represent one pest group where further investigation is required. New agents identified during this project to have an effect on truffle host trees and truffles include slaters, an obligate truffle feeding beetle, millipedes and a fungal tree disease.

Investigations on the management of key pests and diseases concentrated on developing identification kits and monitoring methods for what are considered to be the key truffle pests slugs, slaters and the obligate truffle-feeding beetle, ‘Australian truffle beetle’. Field studies confirmed the pest status of what are considered to be the major diseases of truffle host trees.

Information on endemic pests and diseases identified in this project can be cross-referenced with overseas information and the Plant Health Australia Biosecurity Manual for the truffle industry.

Results for this project have been collated into two publications for use by Australian truffle growers and others who support them in the field and in preparing truffles or sale. These publications are: Australian Truffle Orchard IPDM Manual and Australian Truffle Orchard Pest Identification Guide.

The main outputs for this project are the two publications mentioned above. These have the objective of giving growers information and recommendations regarding pest management so they have the confidence to implement it to produce quality truffles. Pest management is a dynamic and this was no more apparent that what was learned while this project was being conducted. As growers implement the management plan suggested, they will become more familiar with current pests on their own farms and be more responsive to potential incursions. The sooner new pests are discovered, the more likely they can be contained and hopefully eradicated before they become established and add to the pest burden already present.

Implications

To protect trees and truffles from pests and diseases, Australian truffle growers have the tools to monitor and help manage the important agents that have been identified to occur currently in orchards. By implementing the recommendations for monitoring and management, growers will be better placed to have healthy truffle host trees and to produce quality truffles. The reputation of the Australian truffle supply both at home and in overseas markets will be maintained. By becoming more familiar with pest agents in their orchards, there is a greater chance that growers will be able to detect exotic agents sooner after their arrival and try to eradicate them.

With what is considered to be more assured production of quality truffles, growers, marketers and consumers of truffles will be more secure with their involvement in the industry. Financial reward from all phases of the industry will be maintained – at the production level, from markets both domestic and export as well as culinary events for the general public which has the add-on component for any product for human consumption.

The project sought to provide management guidelines for current pests and diseases of Australian truffle orchards. It is hoped that the communication established between project team members and truffle growers will ensure that growers will access and implement management guidelines, the main outputs from the project. Committees of both major truffle grower organisations ATGA and TPWA have been kept informed of project activities and therefore are well placed to act as conduits for such information for any new or existing growers not aware of the project outputs and outcomes.

For other pest agents, the objective of finding management solutions was commenced but not realised fully in this project. For these agents, more investigations are required. These are highlighted in the Recommendations section of this report.

Investigations carried out in this project have some relevance for other industries where the same pest agents are important. This is particularly the case for broadacre agriculture in Australia where liaison with researchers would benefit both industries; for example, slugs, slaters and to a lesser extent, millipedes are of increasing concern as establishment pests in broadacre agriculture as that sector has moved towards adoption of minimum tillage techniques.

With the clarification of pests and diseases important to the truffle industry, companies supplying inputs for management either as advise or goods, will be better placed to help growers achieve improvements if crop management and crop protection.

While there are some differences in the suite of pest agents that occur in truffle industries in other countries, there will undoubtedly be some relevance for techniques to be tested in Australia. Now that the Australian industry has made some contribution to the better understanding of pest agents and their management, researchers and growers in other countries may have more interest in sharing ideas with their Australian counterparts to improve pest management to the advantage of all.

Recommendations

Australian truffle growers should be made aware of and be encouraged to adopt an integrated pest and disease management strategy as described in the Australian Truffle Orchard IPDM Manual which is complemented with the Australian Truffle Orchard Pest Identification Guide.

Australian truffle growers should be encouraged to maintain liaison with associations they belong to and other reliable sources of information on truffle pests and diseases, in order to keep the channels of communication open. Truffle growers should pro-actively seek out new and updated information on pest and disease agents and management.

Detailed research is required on:

- The biology and management of slugs; including cultural and biological control
- The biology and management of slaters; including cultural and biological control
- The biology and management of springtails; including cultural and biological control
- The taxonomy, biology and management of Australian truffle beetle (ATB); including cultural and biological control as well as research on lifecycle, current pest status in orchards, distribution and risk analysis of further impact on industry.

  The project team suggests that this research on ATB is best undertaken initially through a post graduate study because:

  - ATB is a potentially very significant pest for the Australian truffle industry
  - To date only one orchard is affected
  - ATB and truffles is a most unusual crop/pest association requiring individual and detailed attention.
- The biotic and abiotic causes of truffle rot.

Supplementary investigations to findings in the current project could be conducted on:

- Truffle host tree health and the impact of this on pests, diseases and truffle production
- The management of orchard establishment pests, in particular African black beetle and apple weevil
- The biology and management of millipedes; including cultural and biological control
- Management of dieback
- Clarification of the species of “powdery mildew” found in oaks and management strategies
- Investigate introducing filbert (hazelnut) blight resistant varieties to truffle orchards given the very likely event of this disease being introduced to Australia – this includes identifying suitable, cost effective varieties to import into Australia and any modifications to inoculation required
- Work with nurseries to develop seed selection protocols for the use of disease resistant seed genotypes, for example powdery mildew resistance in *Quercus robur*.

AgriFutures™
Emerging
Industries

Pests and diseases of truffles and
their host trees in Australia

by Stewart Learmonth, Celeste Linde, Anne Mitchell, Alan Davey, Alison Mathews, Ainsley Seago and Helen Collie

September 2019

AgriFutures Australia Publication No. 19-040
AgriFutures Australia Project No. PRJ—009832
ISBN: 978-1-76053-060-0
ISSN 1440-6845

AgriFutures Australia

Building 007
Tooma Way
Charles Sturt University
Locked Bag 588
Wagga Wagga NSW 2650

02 6923 6900
info@agrifutures.com.au

@AgriFuturesAU
agrifutures.com.au