Final Report

Managing Biting Fly in Vegetable Crop Residues

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Western Australian Agriculture Authority (WAAA)

Project Number: VG12022

VG12022

This project has been funded by Horticulture Innovation Australia Limited using funds from the Australian Government and the following organisations:

APC Vegetable Producers Committee
City of Wanneroo
Gingin Shire
City of Swan
Town of Kwinana
Shire of Harvey
Shire of Capel
Shire of Chittering
Shire of Dandaragan
West Aust Vegetable Growers Assoc Inc T/As Vegetables WA

ISBN 0 7341 3611 0

Published and distributed by:
Horticulture Innovation Australia Limited
Level 8, 1 Chifley Square
Sydney NSW 2000
Tel: (02) 8295 2300
Fax: (02) 8295 2399

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Summary

Stablefly is a serious economic and social pest worldwide, affecting livestock productivity and human lifestyle. This fly is a major pest along the Swan Coastal Plain in Western Australia developing largely from vegetable crop residues left after harvest. This area produces most of WA's vegetables, of which >50% are exported. Both male and female flies regularly blood feed and as few as 20 stablefly/animal cause distress and measurable losses. Typically one to several hundred stablefly develop/1 m^2 of crop residues left to rot untreated (1-3 million flies/ha).

Removing crop residues at harvest would prevent stablefly development, but this is problematic and costly, and would deplete soil organic matter. Hence this project examined techniques to accelerate the breakdown of crop residues left *in situ* after harvest to minimise stablefly breeding. Physical, cultural, chemical and biological options were tested in 13 field trials and other methodologies. These demonstrated that high speed mulching residues and turning overhead irrigation off after harvest reduced stablefly by up to 85%. Stablefly numbers were also reduced by adding to the residues (in decreasing order of effectiveness) (i) calcium cyanamide (CaCN) fertiliser @ 0.5t/ha), (ii) single species of entomopathogenic fungi, (iii) lime or lime sand (2.5t/ha), or (iv) organic matter bio-accelerants. A predatory beetle of stablefly eggs was found in celery residues and shows promise for biological control. The following recommendations were generated for industry and growers, which were incorporated into Vegetables WA's (VWA) 'Good Practice Guide' and reflected in the *Stable Fly Management Plan* (2013) within the *Biosecurity and Agricultural Management (BAM) Act* regulations to minimise stableflies:

- Shorten the harvest period as much as possible
- Turn off all watering when harvesting is complete
- High speed mulch crop residues within 3d of harvest
- Apply either an insecticide, CaCN, fungal agents or lime to mulched residues
- Leave undisturbed for 1 week before incorporating into soil.

Growers indicated that mulching residues reduced further soil tillage, soil wind erosion and the need for a cereal cover crop. Adoption of new BMP's to mitigate stablefly improved productivity (less pesticides, soil tillage and disease) for ≈60% of growers adopting them. Project findings were extended via:

(i) 25 WA Grower Magazine articles to >1,000 vegetable growers
(ii) 12 Brochures for horticultural and livestock producers
(iii) Grower field days (3) with their knowledge and input pivotal to workable solutions
(iv) 36 Presentations to Shires, Industry & Community Forums and the Regulatory Reference Group
(v) 48 page Training Manual provided to 25 Shire Rangers, EHO's and Compliance Officers given regulatory powers under the BAM Act.

Stablefly-affected Shires are more confident in allowing more irrigated horticulture into their Shires, with planning approval conditional on adopting the BMP's relating to stablefly development.

Significant improvements were made to a Walk Through Trap for removing stablefly from livestock. Low side lighting (to target stablefly on animal's legs) and rubber strips in the trap help remove stablefly from animals entering. The use of mass trapping of stablefly using sticky whiteboards and permanent cloth targets (treated with insecticide) can ameliorate peak numbers of stablefly.

A stablefly colony has been established for use in the development of a genetically modified strain of stablefly as part of HIA funded HG13035, which is a long term potential solution (5-10yrs) to stablefly control being adopted by industry.

A recent presentation on this project at the Livestock Insect Workers Conference (Boston, US) generated international interest as the situation in WA parallels Costa Rica, Brazil and parts of Europe. Pineapple residues (Costa Rica) and sugar cane residues (Brazil) are creating huge stablefly problems. These countries are very interested in the next stage, where burial of stablefly larval substrates (eg crop residues) presents the most economic and sustainable management option.

Keywords

Stomoxys calcitrans; stablefly; vegetables; crop residues; bio-accelerant; decomposition; post-harvest; mulching; entomopathogenic fungi.

Introduction

The Stable or Biting Fly (*Stomoxys calcitrans*) is a serious economic and social pest worldwide, reducing the productivity of livestock and disrupting human recreation (Taylor et al. 2012). Most significantly this fly is a major pest in agricultural areas along the Swan Coastal Plain in Western Australia (WA). This blood feeding fly (Figure 1) disrupts livestock productivity and rural communities across many peri-urban shires where the fly develops predominantly from vegetable crop residues left after harvest.


Figure 1. An adult stable fly with prominent, biting mouthpart (L). Female flies nearly triple their bodyweight after completing a blood meal from animals and/or humans (R).

Stablefly were first recorded in Australia in 1881, but not in WA until 1912. This fly has become a serious pest of cattle and horses due to the movement of horticulture into livestock-based regions around Perth (Figure 1). This has produced a situation that favours stablefly development throughout most of the year on our sandy soils. Stablefly is a major pest of livestock in agricultural areas covering the important Swan Coastal Plain whose coarse sandy soils produce >50% of the state's vegetables and > 50% of horticultural exports. Stablefly are capable of developing in rotting organic matter, ageing animal manures and any combination of organic matter and animal manure with an active bacterial community. Vegetative sources of stablefly development include rotting organic material (King and Lenert 1936, Simmons and Dove 1942, Meyer and Petersen 1983, Boire et al. 1988), sugarcane residues (Cancado et al. 2013), hay stacks, hay bales and silage (Bishopp 1913, Williams et al. 1980, Hall et al. 1982, Talley et al. 2009), lawn clippings (Ware 1966, Berkebile et al. 1994), peanut vine litter, freshwater bay grasses and weeds (Simmons and Dove 1941a, b) and residues left after pineapple harvesting.

Stablefly readily develop in the vegetable crop residues left after harvest (leaves, stalks, and reject fruit) (Figure 2) that rot on the hot sandy soils typical of the Swan Coastal Plain. Being of tropical origin, the stablefly larvae are able to tolerate the high temperatures (often up to 55°C) on these sands with regular overhead reticulation. These residues are the most significant source of stablefly in shires along the affected portions of the Swan Coastal Plain (Cook et al. 2011). This is despite the removal of poultry litter for use as a fertiliser in irrigated horticulture, which was the other major source of stablefly development identified along the Swan Coastal Plain by Cook *et al.* (1999).

Figure 2. Eggs of stablefly on rotting carrot tops (L) and stablefly larvae in soil (R).

Stablefly adults developing from crop residues disperse from several km's up to 30km to acquire blood to develop their eggs. The mixed agricultural ecosystem typical of the Swan Coastal Plain allows this fly to readily move from the source of development in crop residues to livestock nearby. Both male and female stablefly require blood 2-3 times/day from livestock (principally cattle and horses), which is very distressing to the animals (Figure 3). Stablefly invokes defensive behaviours in animals such as foot stomping, head tossing, muscle twitching and tail switching (Dougherty et al. 1993a, b; Mullens et al. 2006), which reduces both grazing time and time spent bedded down (Vitela et al. 2006). Cattle typically bunch together when attacked by stablefly in an effort to avoid their painful bites (Wieman et al. 1992). This can lead to heat stress, increased risk of injury (Campbell et al. 1993) and reduced feeding (Catangui et al. 1995, Mullens et al. 2006). This fly continues to impact on livestock and rural communities across many Shires on the Swan Coastal Plain with quite distinct, localised hot spots where the fly maintains its attack on livestock and humans.

Figure 3. Vegetable crop residues left after harvest (L) are a major source of stablefly development, which impacts on livestock such as horses (R), which owners cover in blankets and face veils to protect them from the flies.

Subsequent to this project starting, the stablefly was made a Declared Pest under the Biosecurity and Agricultural Management Act 2007 (BAM Act) in 12 Shires surrounding Perth in 2013. This was in response to the continued impact of stablefly on livestock and rural residents in many shires surrounding Perth, WA. The Act requires vegetable growers to follow recommended practices outlined in the Stable Fly Management Plan, which is part of this legislation. Growers who don't adhere to the practices (developed in consultation with the vegetable industry) may be penalised under the Act. This punitive approach however is the least desirable means of resolving this issue, hence the need to develop approaches to reducing stablefly below levels that affect neighbouring communities and livestock industries.

As few as 20 stablefly/animal on cattle and horses cause measurable reductions in weight gain and cause the animal's great distress (Figure 4). As a consequence, many livestock industries have either shut down or relocated to non-affected areas. Anywhere from 100 to 1,200 stablefly/m^2 can develop from vegetable crop residues left to rot after harvest without any intervention (1.5 – 10 million flies/ha). Even with Best Management Practice (BMP) it is difficult to reduce these numbers below 100/m^2, which is unacceptable given the extent of commercial vegetable production. Where irrigated horticulture is close to livestock, this fly readily migrates from the source of breeding in the crop residue to the animal host. Stablefly also bite humans and infestations have had a major, negative impact on lifestyle in rural and rural residential communities.

Figure 4. Cattle react to stablefly biting by throwing up sand to remove the flies (L) and stablefly blood feeding on a horse leg (R) .

The Swan Coastal Plain produces most of the State's vegetables, of which more than 50% are exported. If stablefly breeding continues at current or increased levels Local Government Authorities (LGAs) may limit future horticultural expansion in affected areas by restricting location, types of activities or months of production. With limited suitable land and water available to the horticultural industry it is important that the potential of further restrictions due to the perceived risk of stablefly breeding to new or existing operation must be mitigated.

The most productive approach to stablefly control is through larval sanitation, i.e., the removal of sources for larval development such as rotting crop residues (Hogsette *et al.* 1987). However removing all vegetable crop residues at the point of harvest is highly problematic and costly and would deplete soils of organic matter recycling. This project will focus on dealing with vegetable crop residues left *in situ* after harvest to reduce stable fly development such that it does not negatively impact on surrounding livestock industries and rural residential communities. The organic matter content of the vegetable matter needs to be returned to the soil for long term sustainability as the sandy soils along the Swan Coastal Plain where vegetable production occurs are typically low in organic matter content.

Use of Bio-accelerants against Stablefly

One of the keys to stablefly management in vegetable crop residues is getting the organic matter broken down as quickly as possible to both a) reduce the potential for stableflies to develop in the material, and b) decrease the time to next planting, which increases productivity. Enhanced biodegradation of vegetable crop residue after harvest was tested using bio-accelerants applied to vegetable residues (leaves, stalks, roots and heads) after harvest. This was compared with current best management practices where unharvested residues are high speed mulched, sprayed with insecticide and then incorporated into the soil.

The focus of this project is to minimize stablefly breeding in rotting vegetable crop residues through both the acceleration and modification of organic matter decomposition to encourage the proliferation of entomopathogenic fungi that are lethal to insects. The two commercial bio-accelerator products A) Digester^{TM} produced by BioStart Pty Ltd NZ and B) Bioprime^{TM} produced by Bioscience Pty Ltd WA, shows promise in terms of being able to accelerate organic matter breakdown as well as altering the microbial populations in the rotting crop residues in such a way that they may have a deleterious effect on stablefly development. See Appendix 1 for detailed information on both Digester^{TM} and Bioprime^{TM} (provided by their manufacturers).

The reach of this project extended primarily to local commercial vegetable growers (8 Shires surrounding Perth) as well as vegetable growers along the Swan Coastal Plain surrounding Perth (120km north and south of Perth). There are national implications with this project's focus on the best management practices for handling post-harvest crop residues to maximise productivity, whilst minimising the social impact on neighbouring enterprises and communities with respect to nuisance fly development, in particular stableflies.

The target audience were commercial vegetable producers, horticulturalists (eg turf farmers, strawberry growers, olive processors), vegetablesWA, rural residential communities, industry groups, livestock producers and owners (cattle, horse, goat, pig, sheep), field extension officers (industry), Local Government (Environmental Health Officers, Rangers and other staff) and the wider community.

Use of Entomopathogenic Fungi against Stablefly

Fungi as a group occupy every niche that arthropods and insects occupy. As part of the decomposer community they inhabit soils in particular, where insects spend most of their life cycle as larvae (mobile feeding phase) and pupae (dormant phase where the adult fly develops). Many fungi have developed the ability to invade one or more of all of the life history stages of insects (egg, larvae, pupae and adult) and in doing so, use the insect host to complete their life cycle. In a 2007 review of the literature, there were over 700 recognised species of fungi that are lethal to insects (i.e., entomopathogenic). Most entomopathogenic fungi produce asexual non-motile spores or conidia, which are capable of surviving for long periods (1-5 yrs) during unfavourable environmental conditions (typically drought). When conditions are favourable, the conidia become infective units, which contact, germinate and pentrate the insect cuticle where they invade the hosts body to later release spores when the insect's body dies. The use of entomopathogenic fungi against stableflies represents an opportunity to deal with a serious pest of livestock and humans by biological means as compared with chemical intervention, which costs the producer, the consumer and the environment.

The literature has examples of specific entomopathogenic fungi impacting on stableflies including *Metarhizium anisopliae*, *Lecanicillium lecanii* (formerly *Verticillium lecanii*) and *Beauvaria bassiana.* Specifically, Moreas *et al.* (2008) showed that *Metarhizium anisopliae* used against stableflies in laboratory bioassays at a concentration of 1×10^8 conidia (spores) per mL caused 100% mortality in stablefly eggs; this strain of fungus however did not have a significant impact on either stablefly larvae or pupae. *M.anisopliae* can significantly impact on adult stableflies when applied to lodging pens for dairy production (López-Sánchez *et al.* (2012)) and directly onto dairy cattle (Cruz-Vazquez et al. (2015).

B. bassiana has been used in several studies against housefly adults and larvae as well as stablefly adults. Housefly adults were more susceptible to *B. bassiana* than stablefly adults at the same conidial concentration (Watson *et al*. 1995). López-Sánchez *et al.* (2012) showed up to 90% mortality in adult stableflies when exposed to the *B.bassiana* fungi. Moreas *et al*. (2010) showed pathogenicity of *B.*

bassiana to immature stages of stableflies. Watson *et al.* (1995) showed that *B. bassiana* helped in the control of housefly and stablefly in sawdust bedding in calf hutches.

Verticillium lecanii together with *V. fusisporum* have been found to be pathogenic against stableflies where the fungus was found in adult populations in Denmark (Steenberg *et al.* 2001). There are some species of entomopathogenic fungi (EF) that clearly have species specific effects on different nuisance flies. For example, 100% of houseflies (*Musca domestica*) and only 2% of stableflies were susceptible to infection by the entomopathogenic fungus *Entomophthora muscae* (Steinkraus & Kramer 1987).

A product from Nutri-Tech Solutions (Yandeena, QLD) called Mycoforce® was sourced as it contained each of the 3 aforementioned entomopathogenic fungi, viz, *M.anisopliae*, *L.lecanii* and *B.bassiana*. This product was made purely for plant health and agronomic benefits and makes no claim whatsoever for controlling biting or stable flies, or any other insect pest affecting plants. In fact, the product label specifically states that Mycoforce® is "A bio-balancing fungal product to assist in the recovery of previously affected insect damaged plants". As this product contained all 3 fungal species (each at 1.5% volume/volume of the product) that have reported a deleterious impact on stableflies (see previously), it was decided to test Mycoforce® on vegetable crop residues to see if it could reduce the development of stableflies. The product was tested as is available commercially (all 3 fungal species mixed together) as well as the manufacturers provided us with each of the 3 single species of fungi (again at 1.5% volume/volume of the product) to test individually against stableflies in crop residues.

Methodology

Field Testing of Bio-accelerants and Entomopathogenic Fungi against Stablefly:

A) Replicate Plot Field Trials:

The trials involved testing over a large area of commercial vegetable production immediately post-harvest. The crop residues were broken down using a high speed mulcher and then either left untreated or treated with a bio-accelerant. Each of the bio-accelerants was applied to vegetable crop residues immediately after harvest according to the product label instructions or immediately after an initial mechanical breakdown of the crop residues using a high speed mulcher and/or rotary hoe. The bio-accelerants were applied by making up a solution of each with a recommended rate by the manufacturers (eg. 4-8L of Digester™ per ha and 50-100L of Bioprime™ per ha). Three target crops were used to assess the bio-accelerants, i.e., broccoli, celery and lettuce. Replicate plots of 100-120m^2 were used in a randomised block design of 4-6 treatments, and a minimum of 4 replicate plots per trial. Field trials were run between November and May (when stablefly numbers are at their highest) over 3 years. Table 1 below summarises the field trials completed as part of this project.

Table 1. Summary of field replicate plot trials completed to assess various treatments on vegetable crop residues against stablefly development.

Time of Year	*Crop*	*Location*	*Treatments*
November 2012	Celery	Property 1, Gingin	BP
February 2013	Lettuce	Property 3, Gingin	BP, DG, Lime, SU
March 2013	Cabbage	Property 4, Gingin	DG, SU
May 2013	Broccoli	Property 5, Gingin	BP, DG
December 2013	Cauliflower	Property 5, Gingin	BP, DG, LS, PER
February 2014	Cabbage	Property 6, Gingin	BP, DG
March 2014	Celery	Property 6, Gingin	BP, DG
April 2014	Broccoli	Property 1, Gingin	MYCO
November 2014	Broccoli	Property 1, Gingin	BP, DG, ±Water
January 2015	Celery	Property 1, Gingin	EF (3)
March 2015	Broccolini	Property 7, Capel	EF (3), CON
April 2015	Broccoli	Property 2, Gingin	EF (2)
May 2015	Broccoli	Property 2, Gingin	EF (2), VEN, CON

BP=Bioprime™; DG=Digester™; LS=Lime Sand; PER=Perlka® (CaCN), MYCO = Mycoforce® (contains 3 entomopathogenic fungal species); SU=Success® (120g/L spinosad); EF=Entomopathogenic Fungi; CON=Confidor® 200 SC (200g/kg imidacloprid); VEN=Venom 100 EC (100g/kg bifenthrin).

Purpose-built fly traps were placed over each treatment area (5-7 traps/plot) to capture adult flies as they emerge from the soil over the following 2-4 weeks post-treatment (Figure 5). The mean ± se number of stablefly will be compared across treatments using standard statistical analyses.

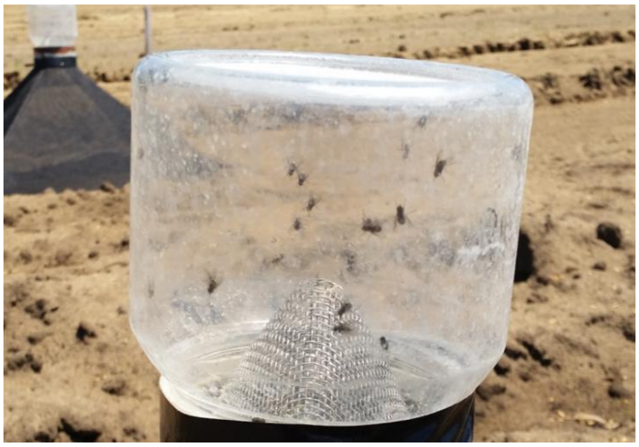

Figure 5. Adult fly emergence cages placed over replicate plots (L) and a close-up of a trap canister where adult stable flies have been caught after emergence from the soil (R).

B) Box Trials: A simple method of exposing vegetable crop residues was employed using large, black tote boxes (60L) (Figure 6). Freshly harvested vegetable crop residues are placed onto a 15cm bed of sand in the self-draining tote boxes. After application of various treatments (eg Bioprime^{TM}, Digester^{TM}, lime, lime sand) the boxes are then placed in a long line under overhead irrigation and left exposed to stableflies for 2 weeks to enable the flies to lay eggs and larval development to commence. Ten replicate boxes will be set up for each treatment and the treatments randomised along the line of boxes. After 2 weeks, the remaining crop residues (Figure 6 R) and soil to a depth of 5cm below the residues is removed and placed into smaller plastic boxes (25 x 25 x 10cm deep) and covered with fine mesh lids to allow any adult stablefly eggs/larvae (and any other fly species) to develop and emerge over the next 4 weeks. The adult flies that emerge in the smaller boxes simply die from desiccation and are easily removed, identified and counted. A summary of the box trials completed assessing various treatments on vegetable crop residues against stablefly development is provided in Appendix 2.

Figure 6. Vegetable crop residues in black tote boxes (L) & after 2 weeks exposure (R).

Soil Biodiversity: Soil biodiversity (as a result of the application of organic matter bio-accelerants) was assessed using ARISA testing (Automated Ribosomal Intergenic Spacer Analysis). This test was done on soil samples taken before and after the addition of the two organic matter bio-accelerants. Looking at soil microbiology using DNA shows it is very complex. There are tens of thousands of different species in each gram of soil, which can change with each testing point, time of the year and sampling place. So rather than looking at single species of micro-organisms, the ARISA test provides an assay of soil microbial diversity and looks at the major groups of soil microbes and the diversity within each group. The ARISA testing procedure identifies 5 major functional groups of soil micro-organisms (see Appendix 3 for full details)

Organic Matter Fractions: Measurements were taken of the organic matter fractions in soil before and after application of the organic matter bio-accelerants Digester™ or Bioprime™. Each plot had 15 sub-samples taken of the soil with an augur (5cm in diameter and to a depth of 10cm). The 15 sub-samples of soil were pooled and homogenised before 4 replicate sub-samples were sent for analysis by CSBP Ltd Laboratories (Bibra Lake, Perth WA) for the organic matter content (dry matter content) of the soil. The total organic matter content of the soil was measured and divided into 2-5mm fractions and >5mm fractions as these would best indicate the amount of vegetable matter on the soil surface and down to 15cm deep.

Subsequent Crop Yield: Marketable head yields (kg/head) of crops that organic matter bio-accelerants were applied to once (at early growth) or twice in split applications (early and mid-growth) were assessed for both cabbage and celery.

Reducing the Impact of Stableflies on Livestock

The main focus of VG12022 has been on reducing the development of stablefly from commercial vegetable production. But there is also a need to look at reducing the impact of stablefly on animals that supply the blood for their egg development and subsequent breeding.

Cattle Walk through Trap: Research into reducing the impact of a similar blood–feeding fly, the buffalo fly (*Haematobia exigua*), led to the development of a walk through trap. Affected cattle were encouraged to walk through a darkened tunnel. This helped to remove these voracious blood feeders off cattle without the need for chemicals. Based on the simple principle of adult flies being photopositive (i.e. they always move towards light), the buffalo flies moved off the cattle in the darkened trap tunnel and went off into narrow ports that opened into larger areas of light. Once in this area, most flies stayed as they tried in vain to escape through the transparent windows, where they ultimately died from heat and desiccation. Use of these traps resulted in 70-85% reduction in the numbers of buffalo flies on the cattle (Hall & Doisy 1989; Tozer & Sutherst 1996). The work on buffalo fly was adapted to help reduce the effect of stablefly on livestock. With support from vegetablesWA, this project worked on a non-chemical means of reducing the stress on animals by modifying the existing buffalo fly walk through trap in order to remove and catch stableflies from cattle outside of Perth, WA.

Stable Fly Population Monitoring: Linked in with reducing the impact of stableflies on livestock involved setting up a trapping grid of stablefly monitoring traps. 25 stablefly monitoring traps were set up within a 2.5km^2 Case Study area along Caraban Road and within Woodridge Estate in the Shire of Gingin. Each of the 25 monitoring traps was serviced weekly to record the local stablefly populations. Trapping and monitoring was done over a period of 34 weeks, with the first trapping week of data on the 8th October, 2014 and the final week of data collected on the 27th May, 2015.

Stable Fly Mass Trapping: A series of stablefly capturing systems were employed during the summer of 2015 to reduce the impact of this fly on cattle in properties close-by to commercial vegetable production operations in the Shire of Gingin. These included cloth-treated target traps (impregnated with insecticide) and sticky white boards with non-drying glue. Cloth-treated target traps are an effective means of presenting a semi-permanent stable fly killing trap in the field (Foil & Younger 2006; Hogsette et al. 2008).

Outputs

1. A total of 13 Field Replicate Plot Trials and 14 Box Trials were conducted over the duration of this project in evaluating various options for the treatment of vegetable crop residues to minimise stable fly breeding in the field. The full details of all Field Trial results and Box Trial results are given in Appendices 4 and 5 respectively. A summary report on these options forms the major basis of the recommendations put forward to industry from this research. These options included removal of overhead irrigation after harvest was complete; high speed mulching of vegetable crop residues and addition of organic matter bio-accelerants, lime, lime sand, calcium cyanamide or entomopathogenic fungi (single species) to freshly mulched residues. Entomopathogenic fungi did not have a significant impact on active stable fly larvae (see Appendix 6), but had a much greater impact on stableflies when applied to residues before stableflies even laid eggs on the residues as they aged. Organic matter bio-accelerants were also applied twice during the growth cycle of the crop; once at the mid-point and again on the post-harvest residues. The impact of organic matter bio-accelerants on both soil organic matter fractions and marketable yield was assessed during the field trials (for full details see Appendix 7).

2. Best Management Practices for the handling of vegetable crop residues after harvest have been significantly updated both in DAFWA publications, Webpages and Extension material as well as in the Vegetables WA's (VWA) 'Good Practice Guide' (2008). Furthermore, these practices have been reflected in the Stable Fly Management Plan that sits alongside the BAM Act Legislation for Stable Fly (as a Declared Pest under this Act). This has been done through a Regulatory Reference Group of relevant stakeholders, including representatives of the vegetable industry.

3. A total of 25 articles were published in the WA Grower Magazine (circulated to 1400 vegetable growers in WA) as well as 6 articles in local newspapers. In addition, some 12 Extension brochures and pamphlets were produced for vegetable growers, strawberry growers, olive producers, livestock producers (cattle, horse, pig) and local community on methods for reducing stable fly development across a range of industries and breeding substrates. A complete list of all Extension material generated from this project is given at the end of the Outputs section.

4. Field days for vegetable growers were co-ordinated and advertised through vegetablesWA and run in both November 2013 (at Bogdanich Farms, Cowalla Road, Gingin) and November 2014 (TC Do & Sons, Caraban Road, Gingin). In addition, a Vegetable Growers Discussion night was held at Bogdanich Farms in the initial phase of this project commencing (May 2012) for their input, knowledge and how workable solutions would be to them on farm.

5. Presentations (n=36) were made by Dr David Cook to stakeholders in this project and other interested parties. This included regular Council meetings and Industry Forums to most of the Shires that contributed funding towards this project, including the Shire of Gingin (4 presentations), Shire of Dandaragan (3), Shire of Capel (1), City of Swan (1), Shire of Chittering (1), as well as the Shire of Victoria Plains, who have not contributed to this project but wanted to be kept informed on what was going on with respect to stable fly in neighbouring Shires. In addition, the Stable Fly Action Group was kept regularly informed of research progress and all issues relating to this pest fly at both their Annual General Meetings (Oct 2012- Oct 2014 inclusive), and most monthly meetings (6 presentations). Dr Cook has also presented on this project's research and findings at meetings of the Regulatory Reference Group on Stablefly at DAFWA (2 presentations), DAFWA Meetings (3 presentations), Stablefly Compliance Officers Training Workshops (3 presentations and workshops), the Chittering Landcare Expo, the WA Horse Industry Council and 3 Public Meetings held in Capel from Feb to April 2015.

6. A Stablefly Training Manual was produced by Dr David Cook as a source of information for any BAM Compliance officers, Shire Rangers and Local Government Environmental Health Officers that have powers related to the implementation of the '*Biosecurity and Agriculture Management Act 2007*'and the associated '*Biosecurity and Agriculture Management (Stable Fly) Management Plan 2013'*. This 48 page

Training Manual was given to over 25 individuals that underwent Training over a series of 3 Workshops and Training Sessions.

7. Significant improvements have been made to the Cattle Walk through Fly Trap for removal of stablefly from affected livestock. Lowered side lighting (to better target stablefly on animals legs and underbelly) and rubber strips in the middle of the trap have greatly improved removal of flies from animals walking through the trap. Improvements still need to be made to the capture of stablefly in the side windows where the flies are encouraged to move towards as part of their photopositive response. Full details of the modifications to the trap are given in Appendix 8.

8. A trapping grid of 25 stable fly monitoring traps were set up within a 2.5km^2 Case Study area along Caraban Road and within the western half of the Woodridge Estate in the Shire of Gingin. Each trap was serviced weekly to record the local stablefly population and monitoring was done over a period of 34 weeks (8th October, 2014 to 27th May, 2015). The traps gave an invaluable measure of the localised population of stablefly in area surrounding a large commercial vegetable growing operation and demonstrated a typical pattern of a series of peaks in stable fly numbers. Full details of the trap locations and trapping results are given in Appendix 9.

9. Mass trapping methods were used to help reduce stableflies within the Case Study area monitoring stablefly numbers). with sticky whiteboards and permanent cloth-treated target traps (impregnated with insecticide and effective at killing stable flies for up to 3 months) A series of 10 white boards (with non-drying glue) were put out along Caraban Road in December 2014 followed by cloth treated target traps (impregnated with insecticide) (n=20) exposed during late January to early February 2015 (weeks 16-18 of trapping period). Full details of the mass trapping systems used are given in Appendix 10.

10. A stablefly colony has been established at DAFWA with a steady adult population of around 200 flies being maintained. Daily blood meals are provided with citrated pigs blood (non-coagulating) presented at 40°C on a circular sponge. This has proved extremely effective and quite simple to employ with feeding the flies. Other details relating to the colony logistics (e.g. egg laying medium, larval rearing medium, pupal extraction and adult fly emergence) are detailed in Appendix 11.

11. Dr David Cook presented at the 59th Livestock Insect Workers Conference in Boston, Massachusetts (US) (11th -14th July, 2015). His talk entitled "Managing stable fly development from vegetable crop residues in Western Australia" was very well received and generated a lot of interest from conference delegates. Most notable was the fact that the stablefly situation reported in Costa Rica by Dr David Taylor (USDA-ARS, Lincoln, Nebraska) and Jose Arroyo (Ministerio de Agricultura y Ganaderia Costa Rica, San Jose, Costa Rica) had uncanny parallels with the stable fly situation in Western Australia. In Costa Rica, pineapple residues left after harvest are creating huge stable fly outbreaks, whereas in Western Australia, vegetable crop residues are producing stable fly outbreaks. Both researchers and agencies from Costa Rica and Brazil (where stablefly are developing from sugarcane residues as a by-product of ethanol production plants) expressed great interest in our proposed research in the next stage, where they agreed that burial of stablefly larval development substrates (such as crop residues) presents the most economic and sustainable management option. A more detailed report on the Conference is given in Appendix 12.

Extension Material generated from this Project

1) **WA Grower Magazine**

Table 2. Extension articles in the WA Grower Magazine

Issue	**Article**
Winter 2012 Vol 47 (2)	Growers chew over stable fly pp 52 (in Vietnamese pp 110)
Spring 2012 Vol 47 (3)	Biting back against Stable Fly pp 42 (in Vietnamese pp 92)
Summer 2012 Vol 47 (4)	New stable fly regulatory reference group pp 44 Minister declares fly pest pp 45 A new approach to stable fly control pp 46
Autumn 2013 Vol 48 (1)	vegetablesWA Presidents Report stable fly bad pp 11. Stable fly regulations moving forward pp 48 (in Vietnamese pp 92) Stable fly project takes to the field pp 53
Winter 2013 Vol 48 (2)	Essential for vegetable growers to reduce Stable fly breeding pp 42 Stable fly regulations update pp 43 (in Vietnamese pp 87)
Spring 2013 Vol 48 (3)	Lowering stable fly numbers pp 66-67 (in Vietnamese pp 99) New Stable fly regulations now in force (in Vietnamese pp 98)
Summer 2013 Vol 48 (4)	Stable fly pp 43
Autumn 2014 Vol 49 (1)	Modified practices effective in controlling stable fly pp 20 (in Vietnamese pp 92) More insecticide options for stable fly control pp 54 (in Vietnamese pp 88) Research shows promising stable fly controls pp 32 (in Vietnamese pp 89)
Winter 2014 Vol 49 (2)	Biological options for stable fly appear promising pp 14-15
Spring 2014 Vol 49 (3)	First year of new stable fly regulations pp 18-19 Using fungi against stable flies pp 52-53
Summer 2014 Vol 49 (4)	Stable fly management plan pp 48 Hay under stable fly group focus pp 49
Autumn 2015 Vol 50 (1)	vegetablesWA Presidents Report pp 5 Attacking stable fly on all fronts pp 16-17 *Spalangia*: a new tool in the fight to control stable fly pp 18 (Bugs for Bugs) Predatory Beetle offers promise against stable fly pp 19

2) **Local Newspaper Articles**

The Advocate, October 31st, 2012

"State acts on stable fly: pest control essential"

The Advocate, November 13th, 2014.

"Invasion starts to bite – stable flies out in force", and
"Fight against flight of flies", and
"Removing residues the key"

Northern Valley News, November 2012

"The Stable Fly Action Group (SFAG)"

3) Brochures and Pamphlets

Cook, DF (2012). Biting Fly Traps. 4 page colour brochure.

Cook, DF (2012). Biting Flies & Cattle. 4 page colour brochure.

Cook, DF (2012). Biting Flies & Horses. 4 page colour brochure.

Cook, DF (2012). Biting Flies from Rotting Crop Residues. 4 page colour brochure

Cook, DF (2013). Managing Stable Flies in Olive Pressing Residue. 2 pp colour pamphlet (A4)

Cook, DF & McPharlin, IR (2013). Managing stable lies in reject produce. 2 pp colour pamphlet (A4)

Cook, DF & McPharlin, IR (2014). Managing stable flies in reject strawberries. 2 pp colour pamphlet (A4)

Cook, DF & McPharlin, IR (2014). Managing stable flies in cattle feedlots. 2 page colour pamphlet (A4)

Cook, DF & McPharlin, IR (2014). Feeding out reject vegetables to livestock. 2 pp colour pamphlet (A4)

Cook, DF & McPharlin, IR (2015). Stop the Stable Fly Brochure. 6 panel DL fold-out (A5)

Outcomes

The major outcome of this project has been the delivery of a range of management tools to vegetable growers for reducing the development of stableflies from their post-harvest crop residues. Over 3 years of field trials, it was demonstrated that high speed mulching of vegetable crop residues and turning overhead irrigation off immediately after harvest could reduce stablefly development by as much as 85%. The development of adult stablefly was also ameliorated (% reduction in parentheses) by adding various treatments to the residues including (i) calcium cyanamide (high value N-fertiliser) (85%), (ii) single species of entomopathogenic fungi (70%), (iii) lime and lime sand (50%), and (iv) organic matter bio-accelerants (40-80%). These options represent a more sustainable approach to stablefly control than conventional pesticides. A predatory beetle (staphylinid of the genus *Aleochara*) feeding on stablefly eggs was found in rotting celery crop residues near Lancelin (120km north of Perth). This beetle offers promise as a biological control agent with development of mass rearing protocols and use in inundative releases.

Several growers commented that high speed mulching of crop residues reduced the need for further tillage of the soil. In addition, the layer of residues left on the soil after mulching reduced soil wind erosion and often obviated the need for a cereal cover crop to be planted. In several instances, where growers have followed the new BMP's for mitigating stablefly (including turning off overhead reticulation after harvest), growers have not had to apply a pesticide as the combination of mulching and turning off water significantly reduced stablefly development. Adoption of the new BMP's to mitigate stablefly has delivered improved economic outcomes for up to 60% of growers adopting them (improved productivity, less use of pesticides, less tillage of soil, less incidence of disease, no need for a cereal cover crop). The application of organic matter bio-accelerants did not realise any measurable yield improvements after use on a single crop. Measurements taken of the organic matter fractions in the top 15cm of soil before and after application of organic matter bio-accelerants did not show any significant difference up to 4 weeks post-treatment. Similarly, there was no measurable difference in in marketable head yields (kg/head) of crops that organic matter bio-accelerants were applied to once (at early growth) or twice in split applications (early and mid-growth) of both cabbages and celery.

All the intended outcomes of the project were achieved with additional agronomic benefits to growers from adopting the BMP options. The impacts of grower adoption of the BMP's for stablefly has seen the number of complaints decrease by around 50% in areas typically badly affected by stablefly. This is based on the number of stablefly complaints received by Local Government Shires and the DAFWA Pest and Disease Information Service). The costs of monitoring and enforcing stablefly compliance in some shires are a significant financial burden. However, the increased awareness, education and adoption of BMP's against stablefly have seen complaints decline in several areas around Perth. This has reduced regulation and enforcement costs by between 30-50% in some Shires. Regular engagement and presentations to Shires where stablefly is a persistent problem has given them more confidence in allowing more irrigated horticulture into their Shires, with planning approval being conditional on adopting BMP's relating to stablefly development from their operations.

Significant reductions in stablefly development by adoption of the new BPM's have reduced the risk to vegetable growers from prosecution under the new Biosecurity and Agricultural Management Act (Stable Fly) 2013. This is reflected by the fact that no vegetable grower to date has been prosecuted under the BAM Act for stablefly breeding. There has been a measurable decline in the numbers of stablefly complaints from areas in Shires badly affected by this fly (for example, the Shire of Gingin's registry of complaints relating to stablefly has declined from a high of 1,650 in 2011/2012 (prior to the project commencing) down to 515 by the second year of the project (2013/2014). Despite some success in reduced levels of complaints, there are some areas where stablefly continues to significantly affect livestock and residents. The numbers of stablefly developing from vegetable residues is still unacceptable and above both economic and social thresholds. Greater grower adoption of BMP's relating to stablefly control will further reduce stablefly in the longer term as compliance and enforcement increases its coverage along the Swan Coastal Plain, but this will be a slow process and those being affected by stablefly want a much quicker resolution of the problem.

The work from VG12022 needs to be backed up with further methodologies to ensure that stablefly development is consistently below 10 stablefly/m^2 of vegetable crop production to reduce their numbers below economic and social thresholds. Greater grower adoption of BMP's relating to stablefly reduction will only succeed with management options that present a real and significant productivity benefit to growers. Hence, the next stage of research proposes to assess new machinery that is capable of burying crop residues to a depth of up to 30cm. It is assumed that stablefly are unable to either (i) penetrate soil to lay eggs on rotting residues or (ii) lay eggs in soil above residues, where the larvae that hatch can burrow down to the residues. Proving that stablefly cannot access deeply buried residues will promote the uptake of this new machinery, which has clear agronomic and productivity benefits (eg organic matter retention from residues, less tillage of soils and reduced pesticide use). The research from VG12022 and that proposed in the next stage is world-first for stablefly and has global implications for its management in countries where this fly is a major economic and social pest (eg pineapple residues in Costa Rica, sugar cane residues in Brazil).

A laboratory colony of stablefly has been established at DAFWA (South Perth) to supply Oxitec in the United Kingdom with stablefly for developing a genetically modified strain of stablefly as part of HIA funded HG13035 (*Novel genetic control of the biting fly, Stomoxys calcitrans*) with DAFWA support. This colony is an integral component of HG13035, which is a long term potential solution (5-10yrs) to stablefly control for use by industry and government agencies alike.

Significant improvements were made to a Cattle Walk through Fly Trap for removal of stablefly from affected livestock. Lower side lighting (to better target the flies on the animals) and rubber strips in the middle of the trap have greatly improved removal of stablefly from animals walking through the trap. Improvements still need to be made in capturing the stablefly in the side windows where the flies are encouraged to move towards.

Use of mass trapping with sticky whiteboards and permanent cloth target traps (impregnated with insecticide) ameliorated the peak numbers of stablefly through the summer months (Jan-Feb) as evidenced by stablefly trapping data following their implementation. The peaks in numbers of stablefly were ameliorated after exposure of 20 cloth-treated target traps, which remain active in killing any stable flies that land on them for up to 3 months under field conditions. In particular, the huge summer peak in stablefly around Christmas/New Year was significantly reduced in the following 3 months, with 3 lesser peaks in stablefly numbers.

There was a lot of international interest generated by Dr David Cook's presentation at the 59th Livestock Insect Workers Conference in Boston, Massachusetts (US) (July 2015) on "Managing stable fly development from vegetable crop residues in Western Australia". Most notably was the fact that the stablefly problems reported in Costa Rica (pineapple residues) and Brazil (sugarcane residues) have uncanny parallels with the situation in WA. A significant outcome of presenting at this conference was exposure of this project's research and representation at the conference both funded by HIA. Conference delegates working in the area of stablefly were particularly interested in our proposed next stage of research into determining the depth at which residues need to be buried to prevent stablefly access as well as the timing of the burial after harvest before stablefly lay eggs onto the residues. They saw the simplicity and practicality of such an option and would be very keen to implement this management option in Costa Rica and Brazil and all wanted to be kept up to date on the findings.

Evaluation and Discussion

The project activities undertaken in this project have been totally effective in delivering all the outputs that were promised as a result of this project, viz:

1. An evaluation of various options for treating of vegetable crop residues to minimise stablefly breeding in the field.
2. Best Management Practices for the handling of residues updated in the Vegetables WA's 'Good Practice Guide'.
3. Training manual on Stable Fly prepared for Local and State Government Inspectors.
4. Report on the effect of mass trapping and cattle walk through traps on the stable fly impacts on cattle properties adjacent to vegetable enterprises.
5. Report on the establishment and maintenance of the stable fly colony for the RIDL work.

Grower field days and one to one contact with growers gave the best feedback on the activities and project outputs. These talks took into account the crops grown be each grower, their typical management of post-harvest crop residues, available machinery, scale of production and scheduling of events (harvesting, pesticide applications, machinery usage). A grower discussion night prior to the project starting (April 2012), sought their feedback on how best to manage crop residues (see Appendix 13). Their feedback was incorporated into the methodologies tested during this project.

Increased vegetable production combined with their movement into traditional livestock areas around Perth have created the stablefly problem, rather than as a direct result of poor industry practice. Evoking change to traditional harvesting methods has not been easy, but acceptance of the need to change is evidenced by the project variations, which reflect an attitudinal change by the vegetable industry in acknowledging their role in creating the stablefly problem. The fact that vegetablesWA put up money from their own levies towards minimising the impact of stableflies on livestock near commercial vegetable growing operations is testimony to this attitudinal change.

Growers were not convinced for a long time that crop residues were responsible for the stablefly problem with many feeling that they were being targeted unfairly over the stablefly issue. Stableflies typically emerge before dawn and disperse away from vegetable farms to locate livestock, so not many stableflies are present on their farms. Hence the perception is that the stablefly problem is being exacerbated. This attitude has changed quite considerably over the course of the project.

Several informal grower meetings on-farm determined how practicable and economic some of the post-harvest machinery options were in dealing with crop residues. Feedback from these meetings were included in the current project direction and will be proposed in the next stage of research (focused on using new machinery to completely bury crop residues after harvest). Adoption of the new BMP's to mitigate stablefly development has delivered improved economic outcomes for up to 60% of growers adopting them (improved productivity through less use of pesticides, less tillage of soil (multiple passes with rotary hoes), less disease carryover into the following crop, no need for a cereal cover crop).

The ultimate benchmark of this project's success has been the number of stablefly complaints from affected livestock owners and landowners. Complaint data collected from Gingin (the Shire most severely affected by stablefly) showed that 1,650 complaints were recorded in 2011/2012 (prior to the project commencing) and declined to 515 by the final year of the project (70% reduction) as a result of improved management practices in combination with compliance inspections by BAM Officers and Shire Environmental Health Officers. Similarly, in the Shire of Capel, the first public meeting of affected livestock owners and Shire residents (19th February 2015) was attended by over 70 people, with subsequent meeting attendances (26th March 2015 and 30th April 2015) declining as did the number of stablefly complaints to the Shire. This was primarily due to grower engagement and improved crop residue management from vegetable producers in the Shire where stablefly numbers were reduced to 8 stablefly/m^2 (from emergence trap data).

Total removal of residues at the point of harvest would be an ideal solution to preventing stableflies, but this is both high problematic and costly and would deplete the sandy soils of organic matter. That is why this project focused on managing crop residues left *in situ* after harvest. This project delivered a range of management tools to vegetable growers for reducing stablefly from post-harvest crop residues, viz, high speed mulching of residues and turning overhead irrigation off after harvest. In situations where water cannot be turned off, stablefly development can be ameliorated by adding either calcium cyanamide (N-fertiliser), single species of entomopathogenic fungi, or lime amendments to residues.

This project has significant relevance to industry as the vegetable industry is perceived negatively with regards to the stablefly issue. This project gives vegetable growers some flexibility in dealing with crop residues and there are some agronomic benefits to be realised from adopting the improved BMP's as a result of this research. For example, high speed mulching residues often reduced the need for further tillage of the soil. Furthermore, the layer of residues left on the soil after mulching reduced soil wind erosion and often obviated the need for a cereal cover crop to be planted. Turning off overhead irrigation after harvest is not always possible as reticulation systems varied across vegetable producers and often did not allow for precise control of watering. Adoption of the new BMP's to mitigate stablefly has delivered improved economic outcomes for up to 60% of growers adopting them.

There were several delivery mechanisms for this projects findings. At least one article was produced in every issue of the WA Grower magazine (circulated to >1,000 vegetable growers in WA) during the project. Grower field days and discussion nights were invaluable to the project to draw on their knowledge and discuss how workable solutions would be in commercial operations. Regular one to one contact with growers was the most effective method for extension with assistance from vegetablesWA Field Extension officers playing a pivotal role.

The methodology applied to this project was highly specific and appropriate. For example, the use of adult fly emergence cages to determine stable fly emergence from the soil is a novel and accurate method previously used by Cook et al. (2011). The methodology also fitted in with normal grower practices and timeframes so that it was practical to employ.

Recommendations

Treatment of Vegetable Crop Residues left after Harvest

- Shorten the harvest period as much as possible from individual bays to prevent old crop residues and reject produce being available to stableflies
- Turn all overhead reticulation off as soon as possible after harvest is complete
- High speed mulch the remaining crop residues as soon as possible after harvest (Figure 6)
- Apply an insecticide to the mulched residues (rotate between the 4 different chemical classes with minor use permits for use against stableflies) to minimise resistance developing
- Leave the residues undisturbed for at least 1 week (this will minimise soil wind erosion) before deep incorporation into the soil (Figure 7).

Figure 6. Two different high speed mulchers used by vegetable growers (Top L & R) and the mulched residue left behind (Bottom L (broccoli) and R (cauliflower)).

Figure 7. Mulched residue dried out after 1 week with overhead irrigation turned off.

Non-Insecticidal Treatment of Crop Residues

Instead of the need to apply an insecticide to post-harvest residues for stablefly control, growers are encouraged to apply either of the following biological and cultural treatment options:

- Calcium cyanamide (500kg/ha)
- Entomopathogenic fungi (single species) (1kg/ha)
- Lime or lime sand (2.5t/ha)
- Organic matter bio-accelerants (apply twice during the crop cycle: mid-point and post-harvest)

Treatment of Reject Vegetable Produce

With accumulations of reject produce (particularly with crops that are sequentially harvested over several weeks to months), the reject produce must be:

- Collected at least weekly
- Placed into a deep pit
- Sprayed with an approved insecticide
- Covered with a minimum of 30cm of soil (to prevent fly access)

If reject vegetable produce is fed out to livestock, it must be:

- fed out in long, thin lines to maximise consumption by livestock
- not fed out in the same area of a paddock/pen/yard

These two strategies will prevent the accumulation of residues that when mixed with animal manure and urine can decompose over time and provide an ideal environment for stablefly development.

Trapping to Reduce Stablefly Populations

Exposure of multiple traps (10-15/ha) specific to stableflies can reduce their numbers in localised areas. Place the following traps near animal yard/paddocks where animals can't contact: Glossy or bright white materials/surfaces are the best for attracting stableflies after they have blood-fed including:

- White boards (corflute, melamine, polycarbonate) coated with non-drying glue (Stik-em®)
- Cloth target traps ($1m^2$) soaked in 0.1% solution of lambda-cyhalothrin or other permethrin-based insecticides

Scientific Refereed Publications

None to report

Intellectual Property/Commercialisation

‘No commercial IP generated’

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Acknowledgements

We would like to thank all the Local Government Shires and their staff including CEO's Presidents, Councilors, Environmental Health Officers, Rangers, Office Administration and others for their support of this project. This support has included both financial and human resources to help deal with complaints from residents and ratepayers, livestock owners and producers affected by stablefly. Thanks to all the input from industry representatives on the Regulatory Reference Group established to develop the Stable Fly Management Plan (that sits in the BAM Act Stable Fly) for use by BAM Compliance Officers in reducing stablefly across various horticultural and livestock industries that contribute to stablefly development. Thanks to vegetablesWA (in particular John Shannon (CEO), Jim Turley, Rebecca Blackman, Maureen Dobra) and the Vegetable committee of the APC in support of this research both in terms of financial contributions and the great support from vegetablesWA field extension officers, namely Sarah Houston, Dominic Jenkin and Truyen Vo for his great help with the Vietnamese grower community and removal of bogged vehicles on more than one occasion.

There are many growers who kindly allowed us to use their properties to carry out the many field trials relating to this project and for their honest and robust feedback. In particular thanks to Andrew and Michael Bogdanich and Ralph Trimboli (Bogdanich Farms), Tran Do, Linda Do and Ali Al-Boraich (TC Do & Sons), Mick & Luciano Monte and Frank (on-site manager, Gingin) and David Blakers (Capel Farms, Capel).

Thanks for the valued input from many vegetable producers over the stablefly issue and the management of crop residues including: Tony Colotti (Brookrise Farms), Kevin Dobra (Sativa Gardens), Loose Leaf Lettuce Company (Maureen Dobra), Glenn Dobra (Asher Road, Gingin), Binh (Chitna Road, Gingin), Giang (Asher Rd Bullsbrook and Bookine Place, Gingin), Francis Tedesco (Sun City Farms, Gingin), Luca Biocich (West Hill Farms, Lancelin), Tan (Jambanis Rd, Wanneroo), Tran Nam (Duffy Rd, Gingin, Dang Thanh Quang (Old West Rd, Bullsbrook), Arthur Trandos and Vulcan De Villiers (Goodwell Farms, Gingin), Tran Hua (Kirby Rd, Bullsbrook), Manlee (Cowalla Rd, Gingin), Tan (Asher Road, Bullsbrook), Tony Cosentino (Cowalla Rd, Gingin), Scarfo brothers (Acre Fresh, Gingin), Odeum Melons (Chitna Rd, Gingin), Roy Humfrey (Quinn Rd, Gingin), Bao (Asher Rd, Bullsbrook), Charles Hildebrand (Farmland Greens, Bullsbrook), Richard Lovegrove (Lovegrove Turf Farms) and West Coast Turf (Chitna Rd and Brand Hwy, Gingin), Thanks also to the Stable Fly Action Group for their valuable input into community concerns and complaints relating to stablefly and representation with relevant government agencies, Ministers and Ministerial advisers.

Appendices

Appendix 1. Information on the two commercial organic matter bio-accelerants Digester™ and Bioprime™ tested over 3 years in field trials in minimizing stablefly.

The following information on Digester™ was supplied by Biostart Pty Ltd NZ.

Digester™: Digester™ is based on molasses fermentation that accelerates organic matter breakdown by "activating the primary decomposers of OM". Digester™ is powered by signal molecule technology and contains a combination of enzymes, signal molecules, bacteriocins (proteinaceous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s)) and secondary metabolites from the fermentation of beneficial soil bacteria including *Pseudomonas putida*. These organic compounds activate the soil microbes responsible for decomposition. By speeding up the natural decomposition process, Digester™ improves nutrient recycling and soil structure and reduces the opportunity for disease over wintering. It also improves soil structure by maximizing aeration through activation of the microbes responsible for repairing soil structure and busting open compacted soil and improving drainage. Digester™ also suppresses disease as it contains defence proteins called bacteriocins; these antimicrobial compounds attack and clean out pathogens such as rhizoctonia and pythium as well as simply removing the substrates that pathogens would traditionally over winter on.

The following information on Bioprime™ was supplied by Bioscience start Pty Ltd.

Bioprime™: Bioprime™ is a soil conditioner made in Perth, WA, which allows growers to change the nature of soil biology. It is a way to weed out the bad bugs and give the good bugs a boost. The outcome is better soil health, better plant production and better root disease management. Bioprime™ was originally developed as a way to control root disease in vegetable production, particularly pythium and sclerotinia. The last decade has seen many advances in understanding what microbes are in soil, and what they are doing. This has come about due to the so-called DNA revolution. Bioprime™ is made by fermentation of molasses using baker's yeast; the amount of oxygen in the fermentation determines how much alcohol, acid or CO_2 is made from the sugar in molasses. Bioprime™ is made with the amount of air controlled to make a maximum amount of both alcohols and acids. These then condense to form esters. Bioprime™ is full of esters. Also, with Bioprime™ we want lots of yeast, so nutrients are added to make the yeast grow quickly. At the end of the process we have a lot of biomass, because just like in Vegemite, yeast biomass contains a lot of protein and vitamins. The fermentation process doesn't stop completely, but slows down as it becomes pickled in its own acids. We then arrest the process by bursting the yeast cells, making a stable, long-life product. When Bioprime™ is added to soil, the Actinobacteria and Dikarya get a boost because they get a dose of vitamins. The proteobacteria get suppressed, firstly because the way they communicate with each other, a process called quorum sensing, is interrupted. Secondly, they can't grow as easily on the complex esters, but higher bacteria and fungi can. When we look at the way group diversity changes, we see short term and longer term impacts. In the short term, anaerobic bacteria are quickly suppressed. Then we see Dikarya and Actinobacteria start to build up numbers. The best time to use Bioprime™ is when you sow your crop as during this time, the presence of proteobacteria can be harmful, as they can feed on the exudates produced by a germinating seed and make a local anaerobic zone in wet soil, slowing down emergence. On the other hand, you want Dikarya active, for within this group are the mycorrhyzal fungi which colonize roots, protecting them and helping nutrient uptake. There is plenty of competition in soil, so the quicker they can colonise the root, the more likely they will persist. There are a number of important pathogenic organisms which lurk in soil, and they often go unnoticed until stress hit the plant. An important control against pathogens is the Actinobacteria which feed on the pathogens and antagonise simple bacteria which mineralise nitrogen. Application to vegetable crop residues will require 50% more Bioprime™ than at the time of sowing a crop as there are more background microbes living off the organic matter in the residues.

Appendix 2. Summary of Box Trials assessing various treatments on vegetable crop residues against stablefly development.

Table 3. Summary of the box trials completed assessing various treatments applied to vegetable crop residues and exposed to stableflies during VG12022.

Trial	Crop	Application	Treatments & Rates of Application
#1	Broccoli	Sprayed on surface	Bioprime and Digester
#2	Celery	Sprayed & Mixed	Bioprime and Digester
#3	Celery	Mixed & Incorporated	Bioprime and Digester
#4	Celery	Sprayed & Mixed	Bioprime, Digester and Lime
#5	Silverbeet	Sprayed & Mixed	Bioprime, Digester and Lime
#6	Broccoli	Sprayed on surface	Pre-compost products
#7	Broccoli	Sprayed on surface	Bioprime, Digester, Lime and DE
#8	Celery	Sprayed on surface	Lime Sand, Lime, CaCl, $MgSO_4$ and DE
#9	Cauliflower	Broadcast + water	CaCN (250-1000kg/ha)
#10	Cauliflower	Broadcast - water	CaCN (250-000kg/ha)
#11	Celery	Sprayed on surface	Mycoforce (0.5-8kg/ha)
#12	Celery	Sprayed on surface	Mycoforce (0.5-8kg/ha)
#13	Celery	Sprayed on surface	Mycoforce®, Bioprime Digester, CaCN
#14	Celery	Sprayed on surface	Mycoforce®, Bioprime, Digester, CaCN

Appendix 3. ARISA Testing Methodology to Measure Soil Biodiversity

The ARISA Testing methodology to measure soil biodiversity identifies 5 major functional groups of soil micro-organisms.

- Proteobacteria: These are simple, fast growing bacteria (formerly called gram negatives), can dominate anaerobic (no oxygen) environments, live off simple organic matter and are not particularly good for soil health and plant growth.
- Fermicutes: More complex, also fast growing (formerly called gram positives), some are important pathogens, can be anaerobic or aerobic, also are not particularly good for soil health and plant growth.
- Archea: These are tough and ancient life forms, slow growing, but live in extreme environments (hot, salty, acidic etc). Their importance in soil was only realised with DNA methods. They build up to significant numbers in cultivated soil.
- Actinobacteria: These are quite complex bacteria which are involved in soil carbon dynamics. They are slow growing but feisty, as they are the chemical warfare specialists in soil. (Most of the antibiotics and drugs we use come from this group). When there are plenty present, root pathogens are suppressed.
- Fungi: These are more complex than bacteria, and fall into two groups, the lower fungi (slimes and moulds) and the higher fungi or Dikarya of which mushrooms and the mycorrhiza are common examples.

ARISA Testing Methodology

The ARISA testing methodology consisted of the following: 200 g of soil (removed as a subsample of a minimum of 20 sampling points across each replicated plot (100m^2) taken from the top 100 mm is to be placed into a refrigerator and delivered for analysis within 24hrs. The DNA is extracted from the soil and measured to determine how many types of microbes are present in significant numbers in each of the 5 major groups. From this we can derive a biodiversity index. The ARISA assay has PCR primers made which are specific for (i.e. they only bind to) Group conserved DNA sequence on each side of the Intergenic spacer of ribosomal DNA. We use 6 individual PCR's (2 for proteobacteria), then analyse PCR products using an ABI 3730 capillary electrophoresis system and Genemapper software. We only consider peaks with height greater than 100 rfu's and size between 100 and 1200 base pairs. After normalising each PCR result, we derive a biodiversity index based on total peaks from 6 PCR's, corrected against peaks with greater than 20% of the total integrated fluorescence score (dominance index). The Health Index is determined from the ratio of Dikarya and Actinobacteria peaks to combined Proteobacteria peaks. The more diverse a soil's microbial population, the healthier the soil is. All the five major groups should be present in soil, but because the Actinobacteria and Fungi are beneficial to plant growth, high diversity in these groups is particularly important.

Appendix 4. A summary of all the Large scale Field Trails (with replicate plots) carried out over 3 years on vegetable crop residues to minimize stablefly development.

FIELD TRIAL RESULTS (2012/2013)

Field Trial #1 – Property 1, Gingin, Nov 2013. Celery crop residues.

Treatments: Bioprime^{TM} @ 50L/ha, 100L/ha or 2000L/ha applied in a total water volume of 1500L/ha

Table 4. Total numbers of stablefly (SF) developing from celery crop residues treated with Bioprime^{TM} at either 50L, 100L or 200L/ha (Field Trial #1).

Treatment	Total Number of SF	% SF reduction
Control	374 (n=30)	
Bioprime 50L/ha	548 (n=30)	Nil
Bioprime 100L/ha	531 (n=30)	Nil
Bioprime 200L/ha	465 (n=30)	Nil

n=number of emergence traps

Field Trial #2 – Property 3, Gingin, Feb 2013. Lettuce (iceberg) crop residues.

Treatments: Bioprime @ 50L and 100L/ha, Digester @ 4L and 8L/ha, Lime (2.5t/ha) and Success® (120g/L spinosad) @ 400mL/ha.

Table 5. Total numbers of stablefly (SF) developing from lettuce crop residues treated with either Bioprime^{TM}, Digester^{TM}, Lime, or the insecticide Success® (Field Trial #2)

Treatment	Total Number of SF	% SF reduction
Control	75 (n=15)	
Bioprime 50L/ha	104 (n=15)	Nil
Bioprime 100Lha	72 (n=15)	4.0
Digester4L/ha	89 (n=15)	Nil
Digester 8L/ha	115 (n=15)	Nil
Lime (2.5t/ha)	36 (n=15)	52.0
Success®	52 (n=15)	30.6

n=number of emergence traps

Field Trial #3 – Property 4, Gingin, March 2013. Cabbage (Savoy) crop residues

Treatments: Digester^{TM} @ 4L and 8L/ha, Bioprime @ 50L and 100L/ha, Success® (spinosad) @ 400mL/ha.

Table 6. Total numbers of stablefly (SF) developing from cabbage crop residues treated with either Bioprime^{TM} (50L and 100L/ha), Digester^{TM} (4L and 8L/ha) or Success® (spinosad)(Field Trial #3).

Treatment	Total Number of SF*	% SF reduction
Control	632 (n=20 traps) 31.6	
Success®	762 (n=18 traps) 42.3	Nil
Digester 4L/ha	499 (n=20 traps) 25.0	20.9
Digester 8L/ha	694 (n=20 traps) 34.7	Nil
Bioprime 50L/ha	762 (n=18 traps) 42.3	Nil
Bioprime 100L/ha	807 (n=21 traps) 38.4	Nil

n=number of emergence traps; *adult stablefly emergence was incomplete.

In this trial, the growers did not high speed mulch the cabbage crop residues into the soil after harvest was complete and left the overhead irrigation on. There was a substantial number of reject cabbages left behind and the material was simply rotary hoed into the soil. At the time of collecting the adult fly emergence traps, there was evidence of stablefly larvae still in the soil in rotting cabbages, so adult stablefly emergence was incomplete and underestimated.

Field Trial #4 – Property 5, Gingin, May 2013. Broccoli post-harvest crop residues.

Treatments: Bioprime^{TM} @ 50, 100 & 150L/ha; Digester^{TM} @ 4L, 8L & 12L/ha

Table 7. Total numbers of stablefly (SF) developing from broccoli crop residues treated with either Bioprime^{TM} or Digester^{TM} (Field Trial #4).

Treatment	Total Number of SF	% SF reduction
Control	28 (n=16) 1.8	
Digester 4L/ha	22 (n=18) 1.2	33.3
Digester 8L/ha	16 (n=17) 0.9	50.0
Digester 12L/ha	26 (n=17) 1.5	16.7
Bioprime 50L/ha	32 (n=18) 1.8	Nil
Bioprime 100L/ha	23 (n=16)1.4	22.3
Bioprime 150L/ha	25 (n=17) 1.5	16.7

n=number of emergence traps

FIELD TRIALS (2013/2014)

Field Trial #5 – Property 5, Gingin, December 2013. Cauliflower crop residues.

Treatments: Bioprime^{TM} (150L/ha) Digester^{TM} (12L/ha), Bioprime + Digester (BP + DG) (at rates previously), Lime Sand (2t/ha), CaCN (Perlka®) (500kg/ha). Overhead irrigation turned off immediately post-harvest.

Table 8. Total numbers of stablefly (SF) developing from cauliflower crop residues treated with either Bioprime^{TM}, Digester^{TM}, Bioprime^{TM} + Digester^{TM}, Lime Sand (5t/ha) or CaCN (Perlka®) (Field Trial #5).

Treatment	Total Number of SF	% SF reduction
Control	328 (n=19)	
Bioprime	199 (n=20)	42.4
Digester	170 (n=19)	48.2
Bioprime + Digester	210 (n=19)	36.0
Lime Sand	170 (n=20)	50.8
CaCN (Perlka®)	49 (n=19)	85.1

n=number of emergence traps

Field Trial #6 – Property 4, Gingin, February 2014. Cabbage crop residues.

Treatments: Bioprime^{TM}, Digester^{TM} or Bioprime + digester (each applied either once (post-harvest) or twice (half way point of crop and post-harvest).

Table 9. Total numbers of stablefly (SF) developing from cauliflower crop residues treated with either Bioprime^{TM}, Digester^{TM} or both products either once (post harvest) or twice (half way to harvest and post-harvest) (Field Trial #6).

Treatment	Total Number of SF	% SF reduction
Control	248 (n=20)	
Bioprime	114 (n=19)	51.6
Bioprime 2X	131 (n=20)	47.2
Digester	51 (n=20)	79.5
Digester 2X	82 (n=20)	66.9
Bioprime + Digester 2X	80 (n=19)	67.8

Field Trial #7 – Property 6, Gingin, March 2014. Celery crop residues (centre-pivot irrigated)

Treatments: Bioprime^{TM}, Digester^{TM} or Bioprime + digester (each treatment applied either once (post-harvest) or twice (half way point of crop and post-harvest).

Table 10. Total numbers of stablefly (SF) developing from cauliflower crop residues treated with either Bioprime^{TM}, Digester^{TM} or both products either once (post harvest) or twice (2X) (half way to harvest and post-harvest) (Field Trial #7).

Treatment	Total Number of SF	% SF reduction
Control	893 (n=20)	
Bioprime	784 (n=20)	12.2
Bioprime 2X	664 (n=19)	21.7
Digester	1057 (n=19)	Nil
Digester 2X	484 (n=18)	39.8
Bioprime + Digester 2X	769 (n=20)	13.9

n=number of emergence traps

Field Trial #8 – Property 1, Gingin, April 2014. Broccoli crop residues.

Treatments: Mycoforce® (contains 3 different entomopathogenic fungi) @ 1kg/ha (MYCO 1), 2kg/ha (MYCO 2) and 4kg/ha (MYCO 4).

Table 11 . Total numbers of stableflies (SF) developing from broccoli crop residues treated with Mycoforce® at either 1kg, 2kg or 4kg/ha (Field Trial #8).

Treatment	Total Number of SF	% SF reduction
Control	1,809 (n=28 traps)	
Mycoforce (1kg/ha)	1,418 (n=28 traps)	21.7
Mycoforce (2kg/ha)	1,376 (n=28 traps)	24.0
Mycoforce (4kg/ha)	1,180 (n=27 traps)	32.4

n=number of emergence traps

FIELD TRIALS (2014/2015)

Field Trial #9 – Property 1, Gingin, November 2014. Broccoli crop residues (var. Brumby)
Treatments: Bioprime^{TM}, Digester^{TM} plus or minus watering post-harvest. Both bioaccelerants were applied to the soil either half-way through the growing crop cycle (at 6 weeks in early November 2014) and to the freshly mulched crop residues immediately after harvest (mid December 2014).

Table 12. Total numbers of stablefly (SF) developing from broccoli crop residues treated with either Bioprime^{TM} or Digester^{TM} with watering turned on or off immediately after harvest was complete (Field Trial #9).

Treatment	+ watering	- watering	% SF reduction
Control	273 (n=20)	46 (n=18)	83.2
BioprimeTM	381 (n=20)	113 (n=18)	70.3
DigesterTM	695 (n=20)	58 (n=19)	91.7
Total	1349	217	84.0

n=number of emergence traps

Field Trial #10 – T&C Do & Son, Caraban Rd, Gingin, January 2015. Celery crop residues. Watered for 48hr post harvest.

Treatments: *Lecanicillium lecanii, Metarhizium anisopliae, Beauvaria bassiana*

Table 13. Total numbers of stablefly (SF) and their respective headwidths (size in mm) developing from celery crop residues treated with either *L. lecanii*, *M.anisopliae* or *B. bassiana* (Field Trial #10).

Treatment	Total Number of SF	Mean headwidth	% SF reduction
Control	14,550 (n=29)	1.92	
L.lecanii	10,806 (n=30)	1.88	25.7
M.anisopliae	12,546 (n=30)	1.81	13.8
B.bassiana	11,208 (n=29)	1.81	23.0

n=number of emergence traps

Field Trial #11 – Capel Farms, Capel, March 2015: Broccolini crop residues.

Treatments: *Lecanicillium lecanii, Metarhizium anisopliae, Beauvaria bassiana*

Table 14. Total numbers of stablefly (SF) developing from broccolini crop residues treated with either *L. lecanii*, *M.anisopliae* or *B. bassiana* or Confidor® (imidacloprid) (Field Trial #11).

Treatment	Total Number of SF	% SF reduction
Control	138 (n=25)	
L. lecanii	144 (n=25)	Nil
M. anisopliae	168 (n=25)	Nil
B. bassiana	167 (n=24)	Nil
Imidacloprid	72 (n=20)	35.1

n=number of emergence traps

Field Trial #12 – TC Do & Son, Croot Place, Gingin, April 2015. Broccoli crop residues.

Treatments: *Lecanicillium lecanii* (1kg & 4kg/ha): *Metarhizium anisopliae* (1kg & 4kg/ha)

Table 15. Total numbers of stablefly (SF) developing from broccoli crop residues treated with either *L. lecanii* (1kg/ha or 4kg/ha) or *M.anisopliae* (1kg/ha or 4kg/ha) (Field Trial #12).

Treatment	Total Number of SF	% SF reduction
Control	486 (n=24)	
L. lecanii (1kg/ha)	232 (n=24)	52.3
L.lecanii (4kg/ha)	393 (n=24)	19.1
M.anisopliae (1kg/ha)	145 (n=24)	70.0
M.anisopliae (4kg/ha)	137 (n=24)	71.9

n=number of emergence traps

Field Trial #13 – TC Do & Son, Croot Place, Gingin, May 2015. Broccoli crop residues

Treatments: Venom® 100EC (100g/L bifenthrin), Confidor® (200g/kg imadacloprid), Venom® + Confidor® and 2 entomopathogenic fungi (supplied by BASF Global).

Table 16. Total numbers of stablefly (SF) developing from broccoli crop residues treated with either bifenthrin, imidacloprid, bifenthrin + imidacloprid, or two entomopathogenic fungi (20 traps in total) (Field Trial #13).

Treatment	Total Number of SF	% SF reduction
Control	12 (n=20)	
Bifenthrin	3 (n=20)	75%
Imidacloprid	139 (n=20)	Nil
Bifenthrin + Imidacloprid	2 (n=20)	83%
EF #480	7 (n=20)	42%
EF #479	10 (n=20)	17%

n=number of emergence traps

The two insecticides Venom® and Confidor® were applied to the broccoli crop 10 days before harvest (to meet the 7d withholding period prior to harvest of broccoli). Imidacloprid was applied as a foliar application to the broccoli crop and the bifenthrin was applied mostly onto the soil with some foliar application (due to the size of the fully grown plants blocking the soil). Bifenthrin remains active in soil for long periods and has the longest residual time in soil of any insecticide currently available in agriculture, so it is anticipated that this insecticide should impact on stable fly larvae that may be present in the soil.

Imidacloprid is a systemic pesticide that is widely used in agriculture and is taken up by the plant on foliar application. It was anticipated that residues of imidacloprid in vegetable matter left after harvest may have an impact on stable fly eggs and/or larvae that develop in the vegetable matter as it rots after harvest. The 2 fungal agents supplied by BASF Global (EF #479 and 480) were applied immediately after the crop residues were high speed mulched and then incorporated by rotary hoe into the soil.

Appendix 5. A summary of all the Box Trials carried out assessing a range of treatments applied to vegetable crop residues on stablefly development over 3 years.

BOX TRIAL #1.

Table 17. Total numbers of stablefly (SF) developing from broccoli crop residues sprayed on the surface with either Bioprime™ or Digester™.

Treatment	Mean Number of SF	% SF reduction
Control	116 ± 22 (n=10)	
Bioprime 25L/ha	86 ± 26 (n=10)	25.9
Bioprime 50L/ha	102 ± 28 (n=10)	12.1
Digester 4L/ha	88 ± 23 (n=10)	24.1
Digester 8L/ha	97 ± 14 (n=10)	16.4

n=number of replicate boxes

BOX TRIAL #2.

Table 18. Total numbers of stablefly (SF) developing from celery crop residues treated on the surface and mixed either Bioprime™ or Digester™ with no further overhead irrigation.

Treatment	Mean Number of SF	% SF reduction
Control	0.2.± 0.1(n=10)	
Bioprime 25L/ha	0.1 ± 0.1 (n=10)	50.0
Bioprime 50L/ha	0.7 ± 0.4 (n=10)	Nil
Digester 4L/ha	0.1 ± 0.1 (n=10)	50.0
Digester 8L/ha	0 (n=10)	100

n=number of replicate boxes

BOX TRIAL #3.

Table 19. Total numbers of stablefly (SF) developing from celery crop residues sprayed, mixed with either Bioprime™ or Digester™ before incorporation into the soil and no further overhead irrigation.

Treatment	Mean Number of SF	% SF reduction
Control	2.4 ± 1.5 (n=10)	
Bioprime 25L/ha	10.4 ± 2.7 (n=10)	Nil
Bioprime 50L/ha	7.3 ± 2.3 (n=10)	Nil
Digester 4L/ha	0.7 ± 0.4 (n=10)	70.1
Digester 8L/ha	0.4 ± 0.4 (n=10)	83.4

n=number of replicate boxes

BOX TRIAL #4.

Table 20. Total numbers of stablefly (SF) developing from celery crop residues sprayed and mixed with either Bioprime™, Digester™ or lime.

Treatment	Total Number of SF	% SF reduction
Control	3.9 ± 1.8 (n=10)	
Bioprime 50L/ha	4.1 ± 2.2 (n=10)	Nil
Digester 8L/ha	0.4 ± 0.3 (n=10)	89.7
Lime 2.5t/ha	2.1 ± 1.7 (n=10)	46.2
Lime 5t/ha	1.4 ± 0.8 (n=10)	64.2

n=number of replicate boxes

BOX TRIAL #5.

Table 21. Total numbers of stablefly (SF) developing from silverbeet crop residues sprayed and mixed with either Bioprime™, Digester™ or lime.

Treatment	Total Number of SF	% SF reduction
Control	78 ± 14 (n=10)	
Bioprime 100L/ha	95 ± 16 (n=10)	Nil
Digester 8L/ha	66 ± 12 (n=10)	15.4
Lime 2.5t/ha	32 ± 8 (n=10)	59.0
Lime 5t/ha	39 ± 10 (n=10)	50.0

n=number of replicate boxes

BOX TRIAL #6.

Table 22. Total numbers of stablefly (SF) developing from broccoli crop residues treated (surface application) with several pre-compost (PC) products.

Treatment	Total Number of SF	% SF reduction
Control	17 ± 4 (n=10)	
PC #1	59 ± 32 (n=10)	Nil
PC #2	24 ± 11 (n=10)	Nil
PC #3	121 ± 50 (n=10)	Nil
PC #4	85 ± 36 (n=10)	Nil

n=number of replicate boxes

BOX TRIAL #7.

Table 23. Total numbers of stablefly (SF) developing from broccoli crop residues sprayed with either Bioprime^{TM}, Digester^{TM}, diatomaceous earth (D Earth) or lime.

Treatment	Total Number of SF	% SF reduction
Control	58 ± 8 (n=10)	
Bioprime 50L/ha	35 ± 5 (n=10)	29.7
Digester 8L/ha	53 ± 8 (n=10)	8.7
D Earth 0.1t/ha	36 ± 9 (n=10)	38.0
Lime 5t/ha	43 ± 9 (n=10)	25.9

n=number of replicate boxes

Table 24. Reduction in fresh weight of broccoli crop residues 2 weeks after exposure after treatment with either Bioprime^{TM}, Digester^{TM}, diatomaceous earth (D Earth) or lime.

Treatment	Reduction in Fresh Wt of Residue
Control	74.9 ± (n=10)
Bioprime 50L/ha	78.5 ± (n=10)
Digester 8L/ha	76.5 ± (n=10)
D Earth 0.1t/ha	73.2 ± (n=10)
Lime 5t/ha	43 ± 9 (n=10)

10 replicate boxes per treatment

BOX TRIAL #8.

Table 25. Total numbers of stablefly (SF) developing from celery crop residues treated with either lime, lime sand, CaCL, $MgSO_4$ Bioprime^{TM} or diatomaceous earth without further overhead irrigation.

Treatment	Total Number of SF	% SF reduction
Control	No Flies Developed	
Lime 2.5t/ha	No Flies Developed	NA
Lime sand 2.5t/ha	No Flies Developed	NA
CaCl 200kg/ha	No Flies Developed	NA
$MgSO_4$ 350kg/ha	No Flies Developed	NA
Bioprime 100L/ha	No Flies Developed	NA
Diatomaceious Earth 0.1t/ha	No Flies Developed	NA

10 replicate boxes per treatment; NA=Not Applicable

BOX TRIAL #9.

Table 26. Total numbers of stablefly (SF) developing from cauliflower crop residues treated (surface broadcast) with several rates of CaCN (Perlka®) and left without further overhead irrigation.

Treatment	Total Number of SF	% SF reduction
Control	1 ± 2 (n=10)	
CaCN 250kg/ha	0.4 ± 1 (n=10)	60.0
CaCN 500kg/ha	0.6 ± 2 (n=10)	40.0
CaCN 750kg/ha	2.7 ± 7 (n=10)	Nil
CaCN 1000kg/ha	3.3 ± 8 (n=10)	Nil

n=number of replicate boxes

BOX TRIAL #10.

Table 27. Total numbers of stablefly (SF) developing from cauliflower crop residues treated (surface broadcast) with several rates of CaCN (Perlka®) and left under overhead irrigation.

Treatment	Total Number of SF	% SF reduction
Control	46 ± 31 (n=10)	
CaCN 250kg/ha	14 ± 11 (n=10)	69.6
CaCN 500kg/ha	6 ± 5 (n=10)	87.0
CaCN 750kg/ha	5 ± 4 (n=10)	89.1
CaCN 1000kg/ha	9 ± 12 (n=10)	80.5

n=number of replicate boxes

BOX TRIAL #11.

Table 28. Total numbers of stablefly (SF) developing from celery crop residues sprayed on the surface with different rates of Mycoforce® and left under overhead irrigation.

Treatment	Total Number of SF	% SF reduction
Control	39 ± 20 (n=10)	
Mycoforce 0.5kg/ha	33 ± 12 (n=10)	15.4
Mycoforce 1kg/ha	46 ± 44 (n=9)*	Nil
Mycoforce 2kg/ha	27 ± 13 (n=9)*	30.8
Mycoforce 4kg/ha	17 ± 16 (n=7)*	56.5
Mycoforce 8kg/ha	25 ± 19 (n=10)	35.9

n=number of replicate boxes; *=box drain holes blocked

BOX TRIAL #12.

Table 29. Total numbers of stablefly (SF) developing from celery crop residues sprayed on the surface with different rates of Mycoforce® and left without any further overhead irrigation.

Treatment	Total Number of SF	% SF reduction
Control	No Flies Developed	
Mycoforce 0.5kg/ha	0.1 ± 0.3 (n=10)	Nil
Mycoforce 1kg/ha	0.4 ± 1.0 (n=10)	Nil
Mycoforce 2kg/ha	0.3± 0.9 (n=10)	Nil
Mycoforce 4kg/ha	0.5 ± 1.6 (n=10)	Nil
Mycoforce 8kg/ha	0.6 ± 1.9 (n=10)	Nil

n=number of replicate boxes

BOX TRIAL #13.

Table 30. Total numbers of stablefly (SF) developing from celery crop residues treated with either Bioprime™, Digester™, CaCN (Perlka®) or Mycoforce and left under overhead irrigation (n=number of replicate boxes).

Treatment	Total Number of SF	% SF reduction
Control	7.6 ± 6.0 (n=10)	
Bioprime 150L/ha	1.8 ± 4.0 (n=10)	76.3
Digester 12L/ha	6.5 ± 9.1 (n=10)	14.5
Digester 24L/ha	4.3 ± 3.4 (n=10)	43.5
Mycoforce	9.3 ± 13.4 (n=10)	Nil
CaCN	1.3 ± 1.8 (n=10)	82.9

BOX TRIAL #14.

Table 31. Total numbers of stablefly (SF) developing from celery crop residues treated with either Bioprime^{TM}, Digester^{TM}, CaCN (Perlka®) or Mycoforce and left without further overhead irrigation (n=number of replicate boxes).

Treatment	Total Number of SF	% SF reduction
Control	No Flies developed	
Bioprime 150L/ha	No Flies developed	NA
Digester 12L/ha	No Flies developed	NA
Digester 24L/ha	No Flies developed	NA
Mycoforce	No Flies developed	NA
CaCN	No Flies developed	NA

10 replicate boxes per treatment; NA=Not Applicable

Table 32. Summary of Box Trial Results carried out between 2012-2014 on various treatments to reduce stablefly development (from best to worst).

Treatment	Impact on Stableflies (SF)
CaCN (Perlka®) 0.25-1t/ha	Reduced SF by up to 85%
Diatomaceous Earth (0.1t/ha)	Reduced SF by up to 60%
Mycoforce® (3 fungal species) (1-4kg/ha)	Reduced SF by up to 55%
Lime 2.5-5t/ha	Reduced SF by up to 50%
Lime Sand 5t/ha	Reduced SF by up to 50%
Digester 4-12L/ha	All reduced SF by up to 50%
Bioprime 25-150L/ha	From increased to up to 40% reduction
Pre-Compost	Increased SF by over 100%

Appendix 6. Treatment of Stablefly Larvae with Entomopathogenic Fungi

The opportunity arose to test entomopathogenic fungi against stablefly larvae that were already active in field vegetable crop residues in contrast to treating residues prior to stablefly laying eggs on the residues. A centre-pivot irrigated celery crop was found to have high numbers of stablefly larvae in an area previously harvested and left undisturbed where the celery root stumps remaining in the ground provided an ideal environment for the larvae to develop (Figure 8).

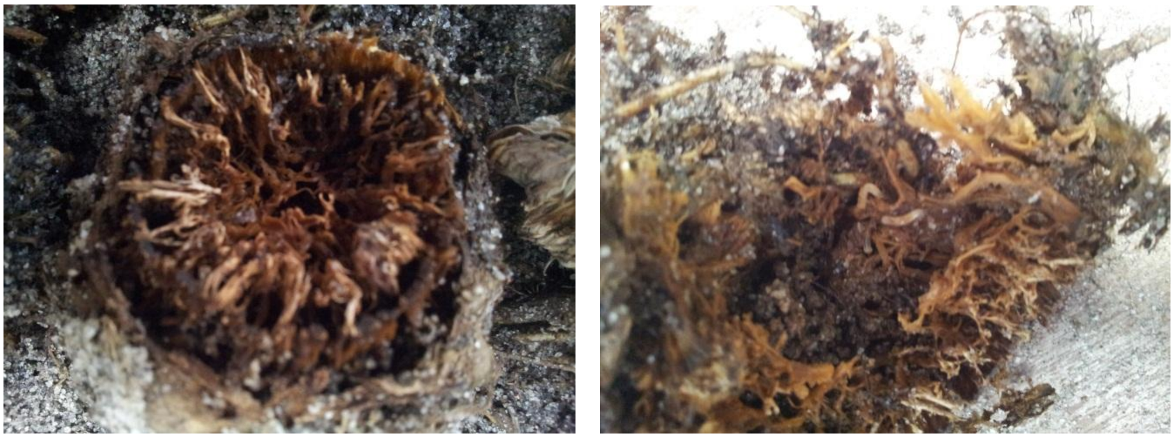


Figure 8. A rotting celery root stump (L) with active stablefly larvae (R).

A total of 180 individual root stumps with active stablefly larvae were sampled on 18th January, 2013 and treated with different rates of Mycoforce®, which contains the 3 entomopathogenic fungi *M.anisopliae, V.lecanii* and *B.bassiana*. A total of 45 celery root stumps were treated with Mycoforce® at either 1kg/ha equivalent (4mL spray solution/stump), 2kg/ha equivalent (8mL spray solution/stump), or 4kg/ha equivalent (16mL spray solution/stump) in the field with freshly extracted root stumps. Control (untreated) root stumps (n=45) were treated with water.

The treated celery stumps were placed in boxes (n=9 stumps/box) on a 5cm bed of sand after treatment and then transferred individually into circular plastic containers with a mesh lid to allow for any adult stableflies to develop for later counting and identification (Figure 9).

Figure 9. Celery root stumps (L) transferred into individual containers and covered with mesh to measure adult stablefly emergence from each celery root stump (R).

The adult fly emergence data (Table 33) indicates that the entomopathogenic fungi only resulted in a 15-20% reduction in stablefly emergence even when applied at 4X the recommended rate of 1kg/ha. This fits in with most other published literature where entomopathogenic fungi do not exert much impact on stablefly larvae and/or pupae (see Moraes *et al*. 2008).

RESULTS

Table 33. Total numbers of stablefly (SF) developing from rotting celery root stumps (with active stable fly larvae) treated with Mycoforce® at either 1kg, 2kg or 4kg equivalent.

Treatment	Mean Number of SF/stump	% SF reduction
Control	43.7	
Mycoforce (1kg/ha)	49.6	Nil
Mycoforce (2kg/ha)	35.8	18.1
Mycoforce (4kg/ha)	36.3	16.9

45 root stumps/treatment

Appendix 7. Impact of Organic Matter Bio-accelerants on Soil Organic Matter Fractions and Marketable Yield

Soil Organic Matter: Measurements were taken of the organic matter fractions in soil before and after application of organic matter bio-accelerants Digester^{TM} or Bioprime^{TM}. There was no significant difference in the levels of organic matter in the top 15cm of soil when the vegetable crop residues (freshly mulched) were treated with an organic matter bio-accelerant (either Bioprime^{TM} or Digester^{TM}). Both the 2-5mm and greater than 5mm fractions of the soil had organic matter content assessed and there was no evidence (based on measurements across 3 field trials – lettuce, cabbage, and cauliflower residues) that a single application of organic matter bio-accelerants to the post-harvest residues (freshly mulched) did in fact accelerate the decomposition of the organic matter present in the soil (see Figure 10 (lettuce), Figure 11 (cabbage), Figure 12 (broccoli) and Figure 13 (cauliflower)) respectively. Similarly, there was no evidence that two applications of organic matter bio-accelerants to a growing vegetable crop (see Figure 14 (cabbage and celery)) accelerated the breakdown of organic matter fractions in the soil when measured 8-10 days after harvest. The organic matter bio-accelerants were applied at the 3 leaf stage of cabbage and the 4.5 leaf stage of celery, then again 6 weeks later (half way point prior to harvest).

Marketable Yield: There was no measurable difference in in marketable head yields (kg/head) of crops that organic matter bio-accelerants were applied to once (at early growth) or twice in split applications (early and mid-growth) of both cabbages (Green (cv Beverly Hills), Red (cv Red Queen) and Savoy (cv Melissa)) or celery (cv Tango) (see Table 34).

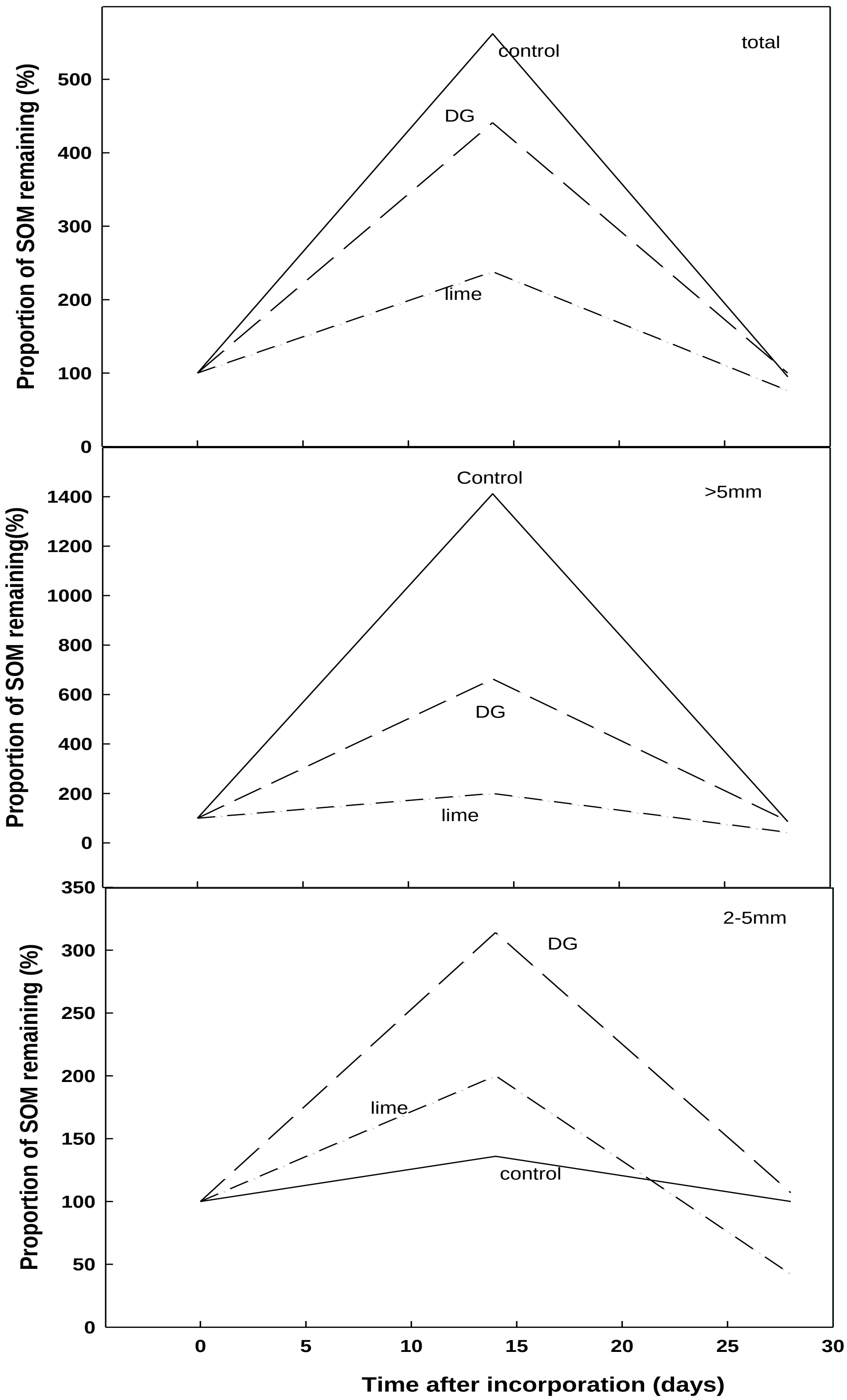


Figure 10. Soil organic matter (SOM) Total, 2-5mm and >5mm fractions levels in soils versus time after incorporation of lettuce residues treated with the organic matter bio-accelarant Digester^{TM}=DG. Bogdanich Farms, Cowalla Rd, Gingin, February 2013.

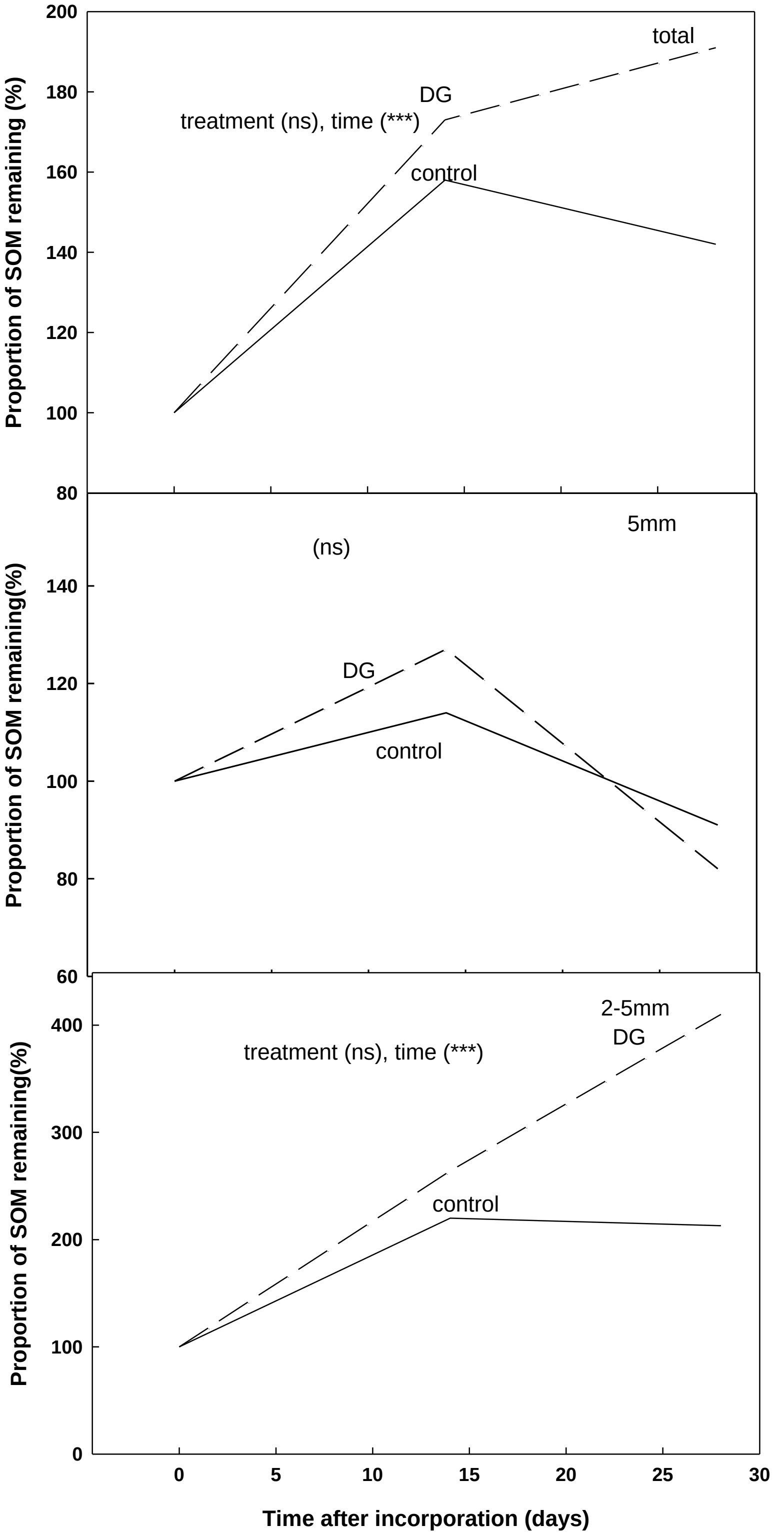


Figure 11. Mean soil organic matter (SOM) Total, 2-5mm and >5mm fractions in soils versus time after incorporation of cabbage residues treated with the organic matter bio-accelarant Digester™=DG. Bogdanich Farms, Chitna Rd, Gingin, March 2013.

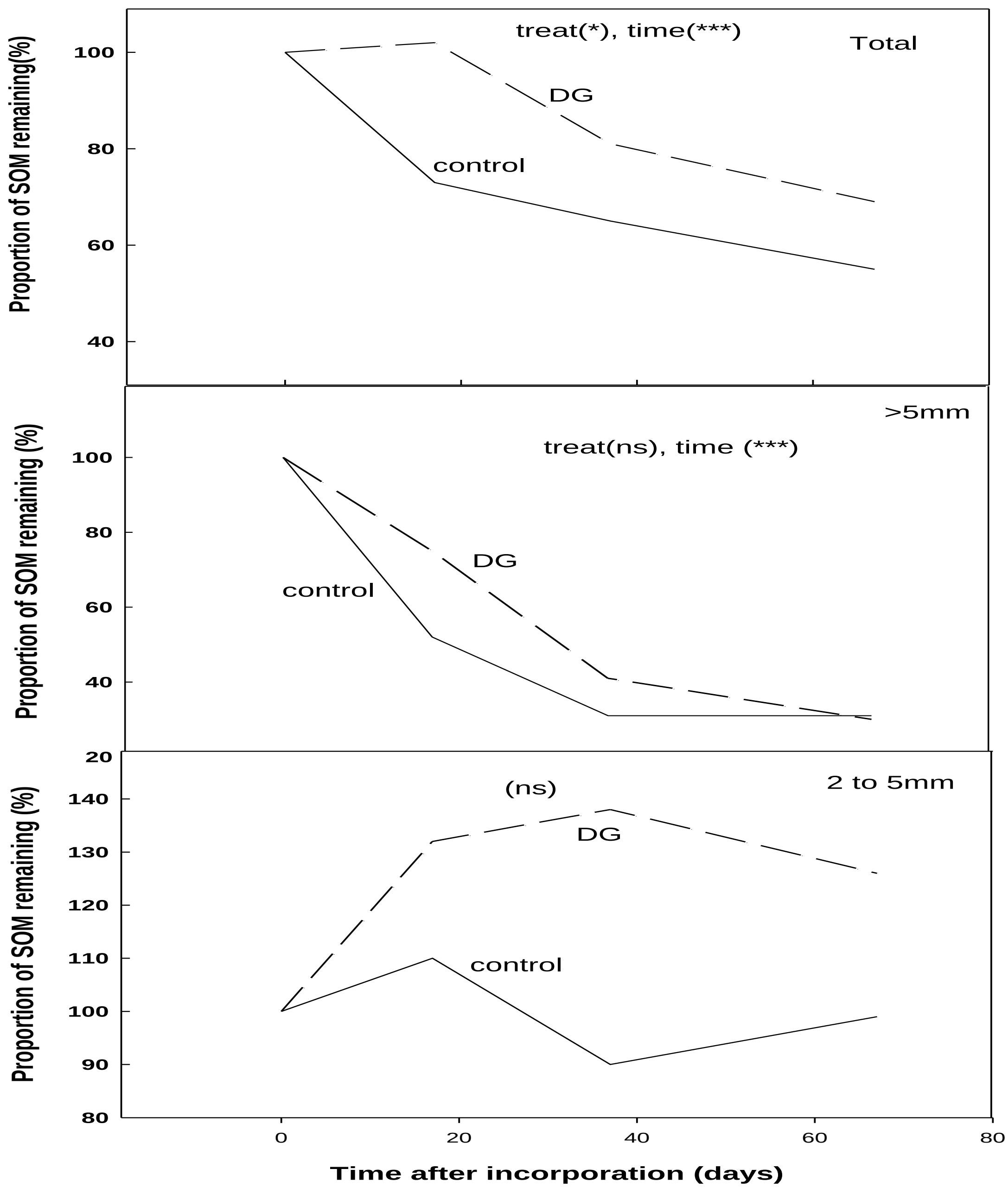


Figure 12. Mean soil organic matter (SOM) Total, 2-5mm an d>5mm fractions in soils versus time after incorporation of broccoli residues treated with the organic matter bio-accelarant Digester^{TM}=DG. Monte & Sons, Sappers Rd, Gingin, April 2013.

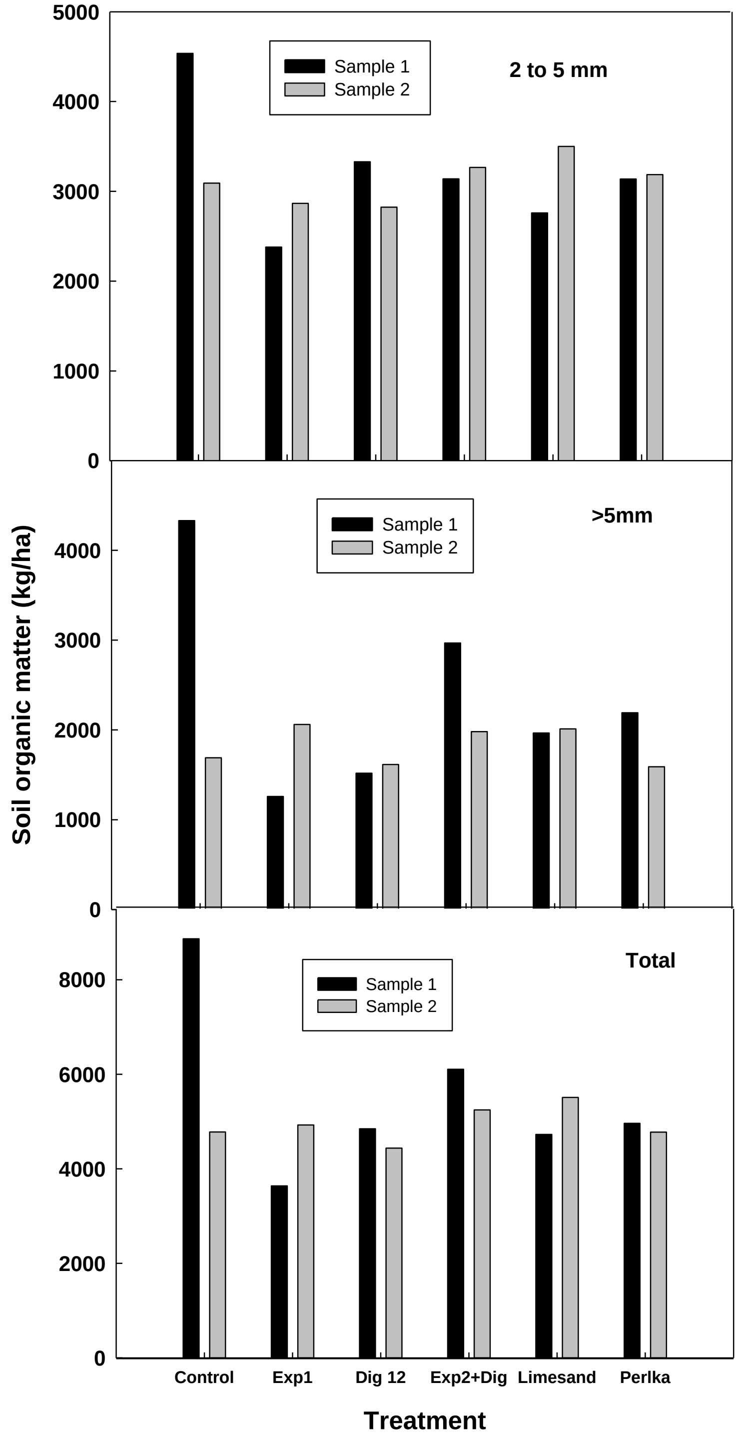


Figure 13. Soil organic matter (kg/ha) in 2 to 5, >5mm and total fractions of top soil (0-15cm) at 2 sampling times after various treatments to cauliflower residues (cv Liberty) after harvest and high speed mulching. Dig 12 = Digester^{TM} @ 12L/ha. Exp1=Bioprime @ 100L/ha. Exp2+Dig2=Bioprime + Digester^{TM}, Lime sand @ 2t/ha, Perlka® @ 0.5t/ha. Harvest was on 28/10/13, treatments applied on 30/10/13 and soil sampled on 19/11/13 (sample 1) and 11/12/13 (sample 2) for organic matter analysis. There was no significant differences between treatments at each sample time and in each soil fraction (P<0.05)

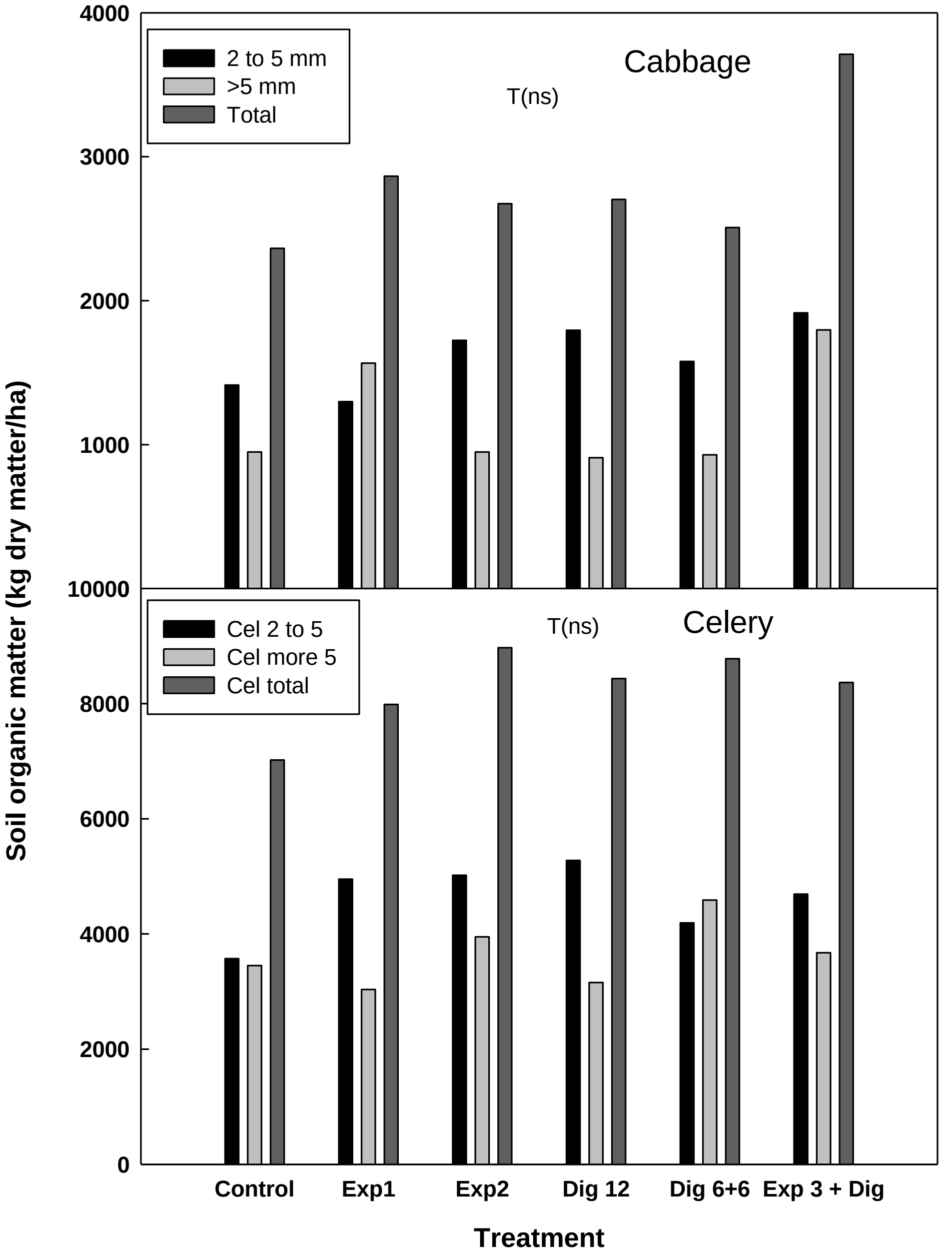


Figure 14. Soil organic matter (kg/ha) in 2 to 5, >5mm and total fractions of top soil (0-15cm) at 2 sampling times after harvest following various treatments in the growing crop of both cabbage and celery. Bioprime^{TM} (Exp1 @ 100L/ha)) and Digester^{TM} (Dig 12L/ha) were applied at 3 leaf stage of cabbage (7/11/13) or 4.5 leaf stage of celery as was the first of the split treatments (Dig 6+6L/ha) and Exp3 (Bioprime) + Digester and 2nd application 6 weeks later (20/12/13 for cabbage and 22/1/14 for celery). Cabbage (cv Beverly Hills) trial at Bogdanich Farms, Chitna Rd and celery (cv Tango) trial at Bogdanich Farms, Lancelin Road. The cabbage and celery were sown on the 6/11/13 and 6/12/13 and harvested on 18/2/14 and 12/3/14 respectively. Soil samples collected for OM analysis on 26/2/14 (cabbage) and 26/3/14(celery). There was no significant difference between treatments in each crop and in each soil fraction (P<0.05)

Marketable Yield after application of Organic Matter Bio-accelerants

Table 34. Marketable head yields (kg/head) of Green (cv Beverly Hills), Red (cv Red Queen) and Savoy (cv Melissa) cabbage plus celery (cv Tango) after applications of the bio-accelarants Digester™ or Bioprime® once (early growth) or twice in split applications (early and mid-growth)* in 2014.

Treatment(L/ha)	Head yield (kg/head)			
	Cabbage			Celery
	Green	Red	Savoy	
Cntrol	1.83	1.03	1.12	1.65
Digester-once 12L	1.78	1.02	1.07	1.69
Digester- (6L+6L)	1.74	1.02	1.11	1.72
Bioprime- once 150L	1.85	1.05	1.19	1.63
Bioprime (75L+75L)	1.78	0.96	1.14	1.61
LSD	0.086	0.062	0.097	0.13
P<0.05	ns	ns	Ns	ns

*The first application of bio-accelerant was at 3 to 4 leaf (Cabbages) or 4.5 leaf (Celery) stage in all treatments and the second application 6 weeks later in the split treatments.

Appendix 8. Reducing the Impact of Stablefly on Livestock:

Modification of an existing Cattle walk-through Fly Trap against stablefly

As shown in Figure 15, the original buffalo fly walk through trap is simply a darkened tunnel with an area either side down the middle being the only area where light is allowed to enter the trap (see red arrows in Figure 15 Left).

Figure 15. The original cattle walk-through fly trap design from QLD for catching buffalo flies off cattle. The RHS picture is the side view indicating the lightened window areas which the adult buffalo flies are attracted to when coming off the cattle as they walk through the darkened tunnel.

With the help of DSY Engineering in Muchea, the original buffalo fly walk through trap design from QLD (Figure 15) has undergone several transformations as we monitor 1) the ease with which the cattle walk through the trap, 2) how readily the stable flies come off the animals lower legs, underbelly and shoulders, 3) how well stable flies are attracted to the lighted areas on the side of the trap, and finally 4) how are stable flies kept within the lighted area to then ultimately die from heat and lack of water. The first trap produced by DSY Engineering is shown in Figure 16. The Perspex side windows proved highly problematic and even though UV-resistant perspex was used, this material buckled severely in the summer heat.

Figure 16. The first cattle walk-through fly trap design made by DSY Engineering (Muchae, WA) for catching stablefly off cattle placed in the field (Gingin).

In the second modification to the trap, the side Perspex window was replaced with a semi-circular window lower down to the legs and underbelly of the cattle, where most stablefly bite and annoy the animals (Figure 17). The black ceiling in the trap acts as a heat sink and stablefly leaving the cattle should be attracted to the side windows.

Figure 17. The second cattle walk-through fly trap design modified by DSY Engineering for catching stableflies off cattle placed in the field (Gingin).

The most recent modifications included replacing the semi-circular Perspex windows (which make moving and handling the trap quite difficult) with bolt on Perspex windows with reduced lighting that only comes in from the top of the window; this was done to help concentrate the light and better draw stableflies from inside the tunnel trap into this area for capture (see Figure 18). In the third version of the trap, we installed black rubber curtains to help brush stableflies off the cattle as they walk through the tunnel (see Figure 19). In addition, the reduced opening to the light window area on the sides of the traps should focus the light and prevent most stable flies from exiting the trap once in the side windows. This 3rd version of the trap is back in the paddock after a lot of engineering and observations on the behaviour of stable flies inside the trap has been helped by very enthusiastic cattle growers.

Figure 18. The third cattle walk-through fly trap design placed alongside the previously modified trap (LHS) for catching stableflies off cattle and placed in the field (Gingin).

Figure 19. The third cattle walk-through fly trap design with more concentrated (narrow) light entrance into the side window for capture (L) and the rubber strips on the inside of the cattle trap to help physically remove stableflies from the animals (R).

Appendix 9. Stablefly Population Monitoring (Case Study)

A trapping grid of 25 stablefly monitoring traps were set up within a 2.5km^2 Case Study area along Caraban Road and within the western half of the Woodridge Estate in the Shire of Gingin. Each of the 25 monitoring traps were serviced weekly to record the local stablefly population (Figure 20). Trapping and monitoring was done over a period of 34 weeks, with the first trapping week of data on the 8th October, 2014 and the final week of data collected on the 27th May, 2015

Figure 20. Location of the 25 Stablefly Monitoring traps placed throughout the Caraban Road area of Gingin, with a large commercial vegetable producer to the east of the Woodridge Rural Residential Estate and the Moore River to the west, where several livestock producers are located.

Stable Fly Monitoring Traps: The standard trap used to catch adult stablefly was the Broce Alsynite Cylinder Trap (Figure 21 Left) (Broce 1988; Taylor et al. 2013). Light refracted from the Alsynite material attracts the stableflies and a clear sleeve coated with a thin film of non-drying glue on the outside of the cylinder captures the stablefly once they land on the film (Figure 21 Right).

Every week, each trap was serviced in the same order, i.e., the outer film was removed and replaced on the trap and all stable flies caught on the sticky film were counted and recorded. Occasionally traps were damaged (storms, vehicles, vandalism) and no data was recorded for that trap over the previous week, but this was usually no more than 1 or 2 traps at most each week.

Some of the replacement sticky films (purchased from Olson Products Inc. (US)) varied in their level of "stickiness" and stableflies were observed landing on the sticky film and then being capable of flying away. Although this was not ideal, this problem was not noted until several weeks into the monitoring period. Although the traps may have not caught all the stableflies they "could" have done, the numbers of stableflies caught on each trap were relative to each other in each weekly trapping period. Sticky films were not put out across traps from more than one different batch (box of 250 films).

Figure 21. Broce traps for adult stablefly population monitoring at time of placement (L) and several days later with adult stablefly caught on the sticky outer film (R).

Stable Fly Population Data

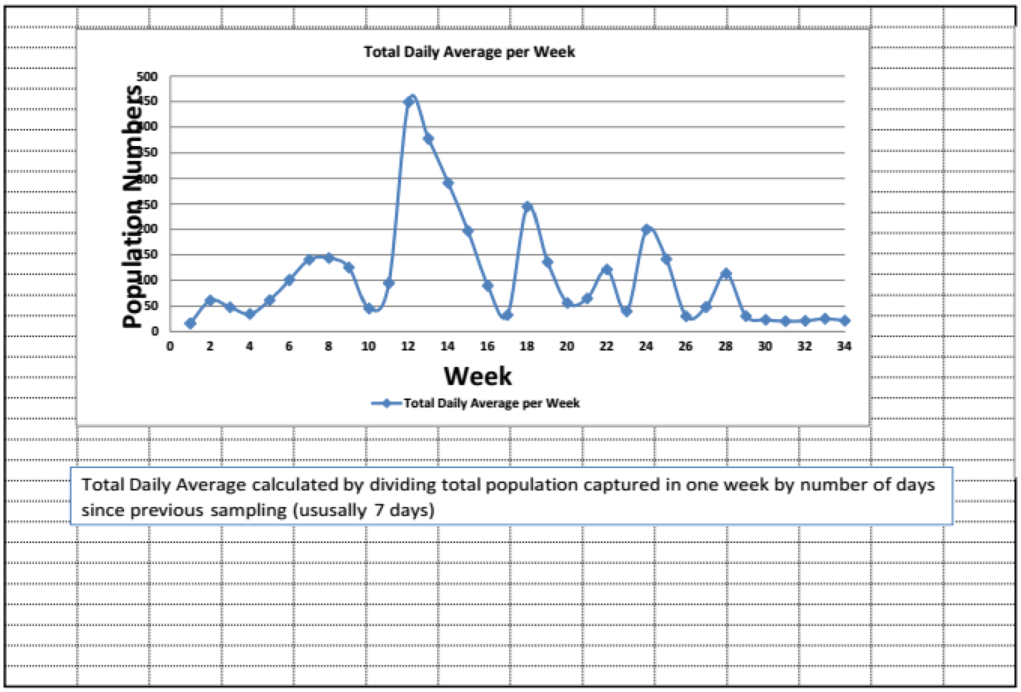


Figure 22. A plot of the mean number of stableflies trapped per week over the entire 25 traps during the 34 weeks of monitoring.

Figure 22 shows that there were distinct peaks in the stablefly local population the first being around week 8 (26th Nov), with the next and most significant peak being weeks 12-14 (late December to early

January) then some further peaks at week 18 (4th Feb), 22 (4th March), 24 (18th March) and 28 (15th April). Numbers dropped dramatically by week 29 (22nd April) and stayed consistently low for the following 5 weeks up to late May.

This pattern of a small initial population peak in late spring, followed by a large peak through the Christmas/New Year period, and then a series of smaller declining peaks from late summer into autumn is what is typically experienced and reflected in the severity and frequency of stablefly complaints by livestock owners and nearby residents with animals. The biggest peak in stablefly numbers in late December coincides with the most amount of harvested vegetable crop residues being produced in the time leading up to Christmas/New Year when demand for fresh salad vegetables is at its highest.

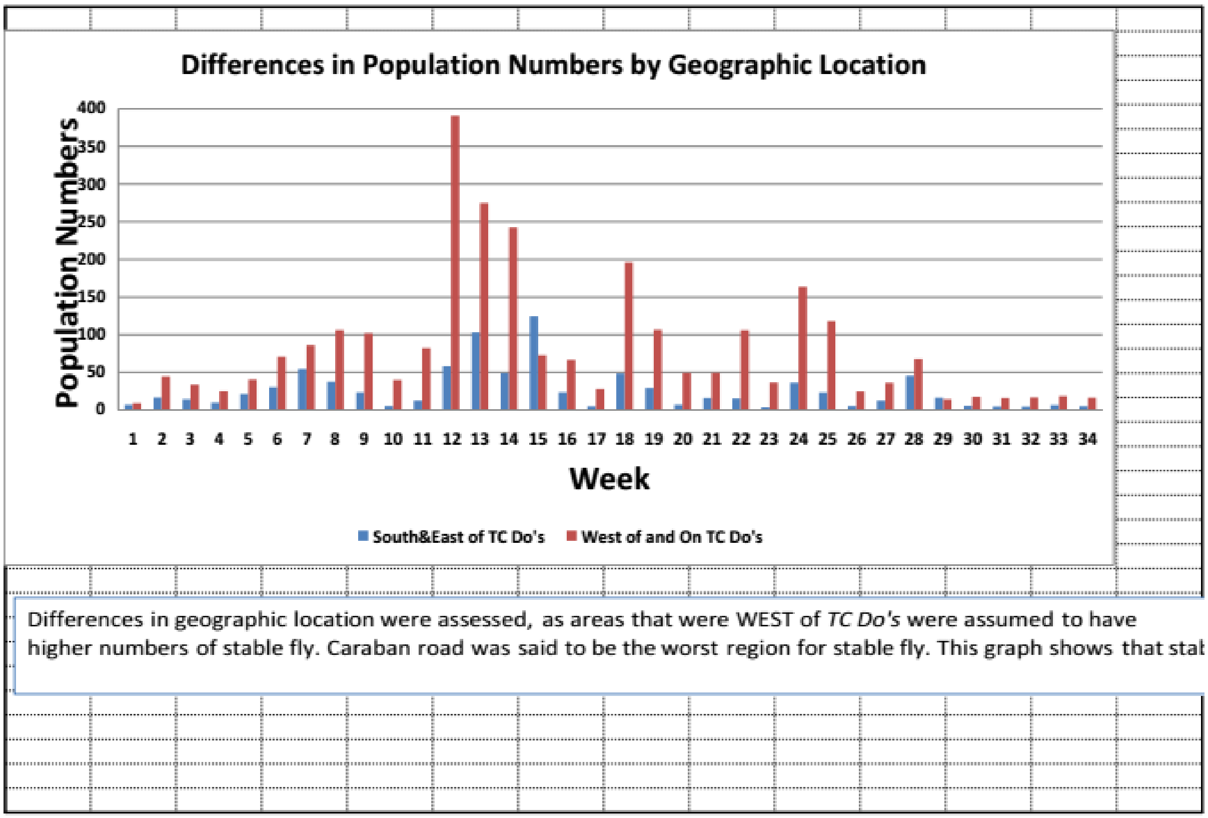


Figure 23. The mean number of stableflies trapped per week in traps set either west (red bars) or south and east (blue bars) of the large commercial vegetable growing operation on Caraban Road during the 34 weeks of monitoring.

Figure 23 highlights that the movement of stable flies from the large commercial vegetable growing operation is predominantly west of the vegetable operation, as newly emerged adult stable flies disperse after coming out of the soil using the prevailing winds. During the summer months in and around Perth, the winds are predominantly in an easterly direction and quite strong first thing in the morning (when flies typically emerge from the ground). This would explain why most of the flies are found west of the vegetable growing operation (source of the flies) and found on livestock properties nearby (sink for the flies).

Appendix 10. Stablefly mass trapping systems employed during the period of stablefly population monitoring

Coth-Treated Target Traps: A series of stablefly capturing and removal systems were employed during the summer of 2015 to reduce the impact of this fly on cattle in properties close-by to commercial vegetable production operations in the Shire of Gingin. These included cloth-treated target traps (Figure 24) and sticky white boards (Figure 25). Both these traps were the simplest in terms of construction and exposure. Cloth-treated target traps were identified Foil & Younger (2006) as an effective means of leaving a semi-permanent stablefly killing trap in the field. The traps consist of exposing a square meter of white or blue cloth that has been treated with a 0.1% solution of the insecticide lambda-cyhalothrin (pyrethroid). The cloth is simply dipped in the insecticide solution and allowed to air-dry on a flat surface prior to erecting in the trap. When placed out in the field, these cloth traps, which provide a big, visual target that attract stable flies to land on them, are capable of killing stable flies for up to 3 months after exposure (Hogsette *et al.* 2008).

Figure 24. Cloth treated target traps placed near livestock yards to attract and kill stableflies. The cloth was dipped in a 0.1% solution of lambda-cyhalothrin to kill stableflies that land on the material for up to 3 months in the field.

Sticky Whiteboards: In addition to the cloth-treated target traps, white boards of various materials (eg corflute, polycarbonate boards, melamine, aligloss, roof panels) were placed perpendicular to the ground and painted with a thin film of non-drying glue (Stik-em®, equivalent to Tac-gel® or Tanglefoot® (Figure 25). These white boards attracted stableflies to them, which become stuck in the non-drying glue when they land on the board, where they remain and die. This trapping system however, requires that the traps, once covered in stableflies, need to be regularly serviced. This involves scraping off the dead flies and old glue, then repainting the boards with a new layer of glue. The Stik-em® glue must be first heated to make it less viscous, so that it can be more easily painted onto the boards.

Figure 25. Sticky white board panels with stableflies captured on the non-drying glue applied to the surface of either a polycarbonate board (L) or a corflute board (R).

A series of 10 white boards were put out along Caraban Road in December 2014 followed by cloth treated target traps (n=20) exposed during late January to early February 2015 (weeks 16-18 of trapping period). The peaks in numbers of stablefly were ameliorated after exposure of the 20 cloth-treated target traps, which remain active in killing any stableflies that land on them for up to 3 months under field conditions (UV and rain).

Appendix 11. Establishing a Laboratory Colony of Stableflies.

A stablefly colony has been successfully established at DAFWA (South Perth) with a steady adult population of around 200 individuals being maintained at 25°C with 14h light: 10h dark photoperiod. Daily blood meals are provided with citrated pigs blood (non-coagulating) presented at 40°C on a circular sponge. This has proved extremely effective and quite simple to employ with feeding the stableflies. Egg laying (oviposition) sources provided to the flies include both aged grass clippings and horse manure. After eggs have been laid these are then reared on a standard medium of 1 part wheatbran: 1 part sawdust: 2 parts water, which is mixed and left to age for 7 days prior to use. Stablefly eggs are simply placed onto the larval medium and sprayed with a fine mist of water and moist paper towel spread over the eggs. More recently, aged carrot top waste has been used to extract eggs from the colony as horse manure has to be of a particular age to elicit stable fly oviposition and was too variable in it's response from adult stableflies.

After larval development is complete, the stablefly pupae are extracted by water flotation of the larval medium, and then air drying the collected pupae. Most of the pupae are isolated and collected clean by placing a moist cloth on a shelf positioned at the end of the rearing tray where most of the wandering larvae collect on this cloth and can be easily gathered. Also, known pupal age can be determined by daily examination of larvae turning to pupae. Modifications to the rearing protocol of late have used plastic take-away containers with carrot top harvested waste being moistened and aged for a week prior to exposure to stableflies for them to oviposit on. This medium also enables the stable fly larvae to readily develop in the ageing carrot tops and pupate in the container. Freshly preserved adult SF have been preserved in RNAlater and sent to Oxitec (UK) for use in the development of a RIDL strain of Stable Fly as part of HG 13035 (*Novel genetic control of the biting fly, Stomoxys calcitrans*).

Appendix 12. Report on travel by Dr David Cook to present at the 59th Livestock Insect Workers Conference in Boston, Massachusetts (US) on 11-14th July, 2015

Dr David Cook gave an oral presentation at the 59th Livestock Insect Workers Conference (LIWC) held in Boston (11th -14th July, 2015) entitled "Managing stablefly development from vegetable crop residues in Western Australia".

The presentation was really well received and generated a lot of interest from people attending the conference, most notably from those in Costa Rica, Brazil and the US.

Most notable was the parallels between the stablefly situation in WA and that in Costa Rica

Pineapple production is increasing at a phenomenal rate in Costa Rica and is set to become the country's biggest export commodity in the next few years (worth around $1b US p.a.).

Pineapple residues left after harvest are creating huge stablefly outbreaks in Costa Rica

The 200t/ha of post-harvest residue are ideal for stablefly to develop in numbers >2,000/m^2.

This has created a huge stablefly problem, which is having a devastating effect on the local cattle industry, with SF numbers exceeding 1,000/animal often being observed.

The pineapple producers do not believe they are breeding stableflies, but are producing what they call "mosca de la piña" or "the pineapple fly".

Current management of the pineapple residues involves treating the area with high rates of diflubenzuron prior to chopping the residues and leaving on the surface.

Producers are typically spending >$2,000/ha to control stablefly (insecticides and traps).

The Cost Rican government has deployed authorised inspectors to visit pineapple producers. Detection of <u>any</u> stablefly larvae results in sanctions for that producer such as not being allowed to grow pineapples in a prescribed area for a minimum of 6 months.

Dr David Cook left a copy of the Stablefly Training Manual (48pp) that he produced for training Stablefly Compliance Officers in WA for use by those involved in the Costa Rican situation.

Social discord is so great over stablefly that meetings on this issue have to be held at separate times between pineapple producers and livestock owners to prevent conflict.

The conference delegates working on stablefly were particularly interested in our proposed research into determining the depth at which residues need to be buried to prevent stablefly access as well as the timing of the burial after harvest (HG 15002).

The researchers and government agencies of Costa Rica and Brazil saw the simplicity of such an option and were keen to implement this management option. Both countries wasted to be kept up to date on the findings and future research.

An excellent overview of genetic control of ectoparasite dipteran livestock pests was given by Max Scott (North Carolina State University), who runs a mass rearing facility in Panama for releasing New World Screwworm fly (*Cochliomyia hominivorax*) for SIT releases. New and novel technologies have removed females from the production system (saving on rearing costs) at the embryonic stage, where rearing up to 100 million flies/week is a costly production system. This technology is transferrable to other dipteran livestock pests.

Collaborative experiments were discussed between Dr Cook and both Dr David Taylor and Dr Kristina Friesen (University of Nebraska, Lincoln) with regards to stablefly depth of burial experiments, and stablefly development rates between the US and Australian populations of stablefly.

Several presentations were given on horn fly impacts on cattle (called buffalo fly in Australia) notably Bradley Mullens (University of California, Riverside) and Brandon Smythe (New Mexico State University) where the weaning weight of cattle affected by horn flies was significantly greater in herds regularly treated with insecticides. The impact of repellents was again very transient, with 1-3 days reduction in horn fly numbers on cattle post-treatment with geraniol oil, and better control associated with the use of straight-chain fatty acids against the fly.

Douglas Ross (Bayer) talked about a new cattle insecticidal ear tag called Tolfenpro®, which contain the novel compound tolfenpyrad, which is a pyrazole insecticide and works by inhibiting the mitochondrial electron transport system. This compound has never been used in Australia before, so stable fly populations would not have previously been exposed to this class of chemical and they would represent a novel option for livestock owners to use on cattle against SF. Bayer were agreeable to supplying Tolfenpro® ear tags to us for testing on cattle around Perth.

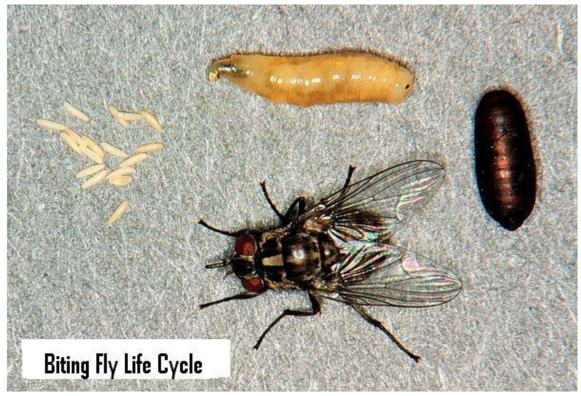
Dr Georgina Bingham from Vestergaard produce insecticide impregnated mesh screens to protect livestock from biting flies. Called ZeroFly® Screen, it is the first insecticide-incorporated screen that keeps livestock healthy and productive by reducing the impact of nuisance and biting flies, for example tsetse flies and stable flies. The insecticide continuously refreshes itself on the surface of the yarns and includes UV protection to extend durability and effectiveness for several years, if properly maintained. Flying insects do not see the mesh as they move in towards an animal and hence land on the mesh and acquire a dose of insecticide. Dr Bingham is happy to supply insecticide impregnated mesh to Australia for testing against stableflies.

Jennifer Williams from MGK Insect Control Solutions was happy to supply new Insect Growth Regulators (IGR's) for testing against stableflies. These IGR's have long term residual in the soil against soil dwelling life history stages of insect pests.

Emeritus Professor Gary Mullen from the Department of Entomology Plant Pathology at the Auburn University, Alabama approached Dr Cook about using images presented in my talk in the new 3rd edition of *Medical and Veterinary Entomology* textbook (Academic Press).

Discussions were held with Dr Phil Kaufman who has previously published work on stable flies and insecticidal control strategies as well as trapping methodologies and Dr Jeffrey Meyer who previously worked on stable flies in dairies including population dynamics using different trapping methodologies as well as chemical and biological control strategies over 20 years and now is the Technical and Business Development Director for Triveritas USA (Animal Health Solutions Company).

Appendix 13. Flyer advertising a grower discussion night on stablefly in April, 2012



WE WANT TO HEAR FROM YOU!

The biting or stable fly problem has remained with us for too long

We have ideas and long term solutions that we know will help

But, we really need **your** input and knowledge on how workable they will be on farm

WHERE: Bogdanich Farms, cnr Gingin Brook Rd and Cowalla Rd

WHEN: Thurs 19th April at 6pm

We look forward to seeing and hearing from you

David Cook
Entomologist, UWA

Jim Turley
Executive Officer

Ian McPharlin
DAFWA

Appendix 14. The predatory staphylinid beetle found consuming stablefly eggs in rotting celery crop residues on a commercial vegetable farm in Lancelin, 120km north of Perth.

Figure 26. Staphylinid beetle of the genus Aleochara, which have been found around the world predating on filth flies including stable flies (L) and the staphylinid beetle found in rotting celery crop residues north of Perth feeding on fly eggs in the laboratory (R).