Superfly® Trials, Lab Analyses & Literature Review

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Introduction

Natural ecosystems produce some of the most productive soils in the world without the need for synthetic fertilisers. These ecosystems are typically highly populated with insects, which rapidly convert organic material into insect castings (frass). Frass has many unique properties that fertilise and stimulate the soil food web, which in turn feeds and protects the plants.

Modern agricultural soils tend to lack the quantities of frass found in wild ecosystems, and therefore have reduced biodiversity, soil health and overall productivity.

Bardee uses black soldier fly larvae (BSFL) to convert food waste into high-value insect protein for pet and stock feed, and an organically certified fertiliser and soil conditioner, called Superfly®, which is ready for use in farms across Australia.

A recent Australian paper analysing the market potential for black soldier fly fertiliser products and found "just one large-scale producer in Australia—Bardee, located in Victoria who sell BSF frass produced from food waste as Superfly® Organic Fertiliser in either pelletised or fine granular forms.[1]

In this report we review the major benefits of Superfly® for agricultural applications and discuss the mechanisms in which Superfly® achieves these benefits. This review draws from Bardee's own lab analysis and real-world trials of Superfly®, as well as peer reviewed scientific papers discussing insect frass.

[1] Dempster, F., Subroy, V., Harold, T. & Kragt, M. (2022). Market potential for Black Soldier Fly fertiliser products. The University of Western Australia.

About the Author

Alexander Arnold is a global expert in industrial-scale black soldier fly larvae (BSFL) rearing, production and product development. Alex grew up in a farming family and is passionate about increasing agricultural efficiency and sustainability through the application of biological systems and precision technology.

Alex is the Chief Technical Officer and co-founder of Bardee, established in 2019 to transform food waste into organic certified fertiliser and soil enhancer, and protein for pet and animal feed.

Alex is a published academic in the CSIRO Journal of Botany and has led Bardee through the University of Melbourne startup accelerator program, MAP, and Startmate accelerator, and secured over $7m of venture capital funding.

Alexander Arnold
Co-founder & CTO

BSc, Hons (Gen), MAgrSc

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Superfly®
Improves Soil Health & Resilience

Qualitative analysis of Superfly® microbial life

Vegetable bed trial in Mornington Peninsula, Victoria

One of the clearest examples of Superfly® improving soil health and resilience is shown in a field trial conducted on a large, commercial vegetable farm on the Mornington Peninsula, Victoria. The photos in figure 1 show the difference in winter soil activity in rows of leeks 50 days after treatment, with no treatment, composted poultry manure, and Superfly®.

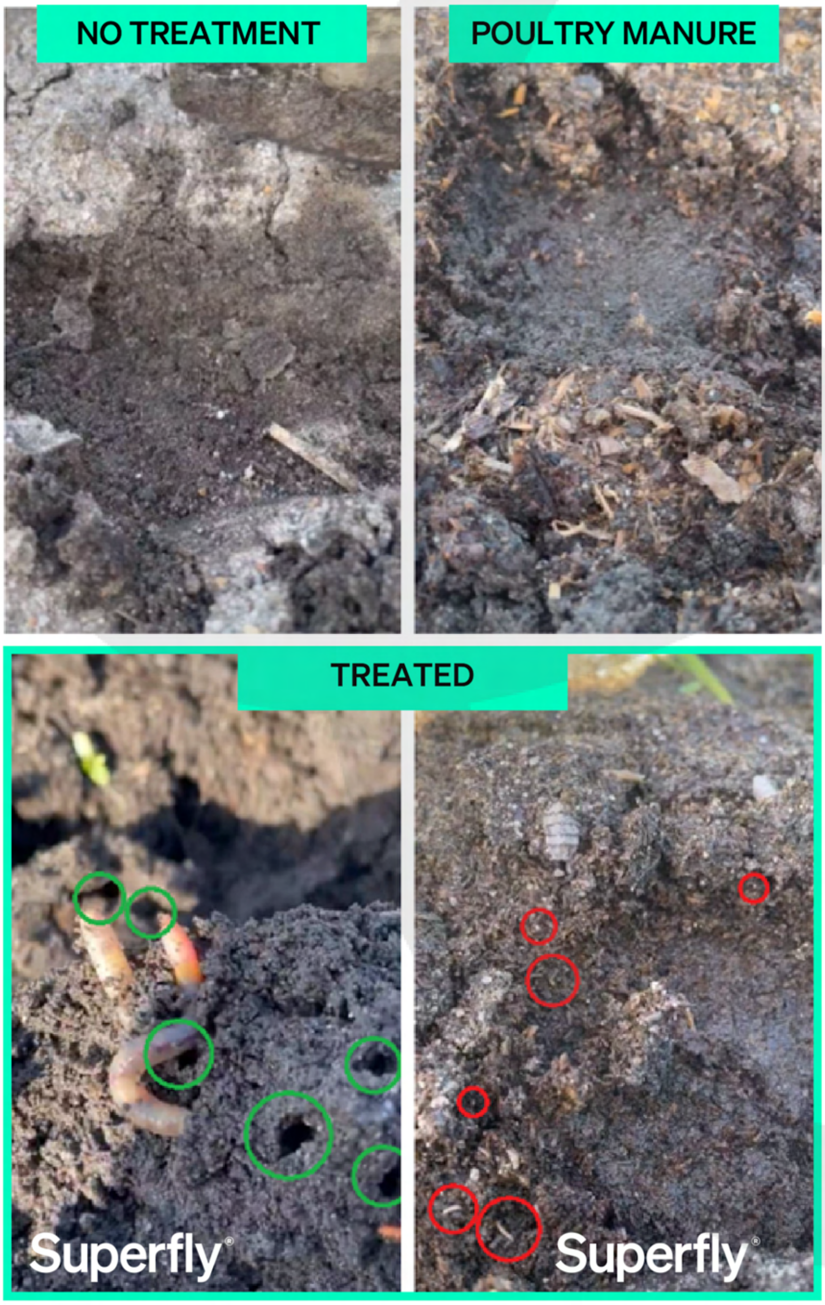

Figure 1

Top left = no treatment.

Top right = composted poultry manure 50 days after application.

Bottom Left = Superfly 50 days after application; wormholes circled in green.

Bottom right = Superfly 50 days after application; macroinvertebrates circled in red (this image is a still frame of a video, which allowed us to identify macroinvertebrates through their movement).

Superfly® biological activity under the microscope

To demonstrate the diversity and abundance of microbes in Superfly®, Bardee observed the pellets under microscopes. Figure 2 shows the abundance and diversity of fungal activity when Superfly® pellets are re-wet to simulate what would happen to the pellets when applied to an irrigated or rainfed field or paddock.

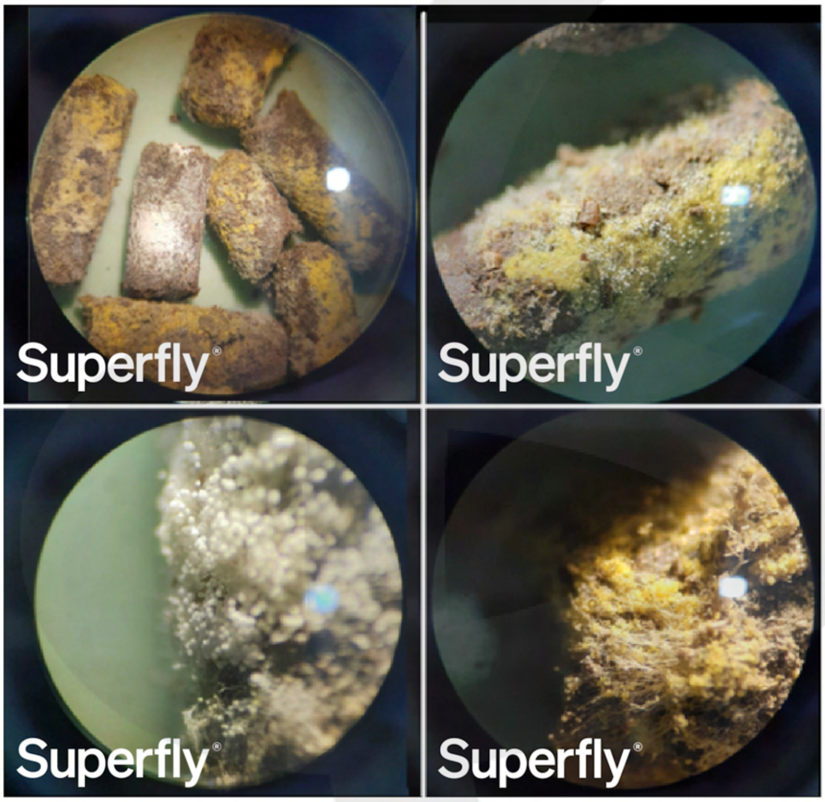

Figure 2

Superfly pellets were misted with distilled water and left to incubate at room temperature for 3 days in a sealed sterile petri dish before being observed with a stereo microscope.

Top left = several 6mm diameter pellets.

Top right = one Superfly pellet with fungi fruiting bodies present.

Bottom left and bottom right = close-ups of the side of Superfly pellets showing many fruiting bodies present

Image of Superfly fertiliser and soil enhancer pellets (left) and fine granule (right).

The improvement in soil health seen in figure 1 is the product of multiple positive qualities of Superfly® working simultaneously; namely the beneficial microbes, chitin, organic matter and nutrients. Soil structure was also distinctly improved with the application of Superfly®.

Good soil health is largely due to high levels of diversity and abundance of soil biology. Typically this can be seen with the naked eye with increased worm and macroinvertebrate activity, as seen in figure 1; however, microbial life is also key to soil health and resilience.

Soil bacteria and fungi facilitate the cycling of nutrients between plant-available and organic states, releasing nutrients to plants as plants require; this results in an overall increase in nutrient uptake and reduced nutrient runoff.

Soil fungi also help bind soil particles together, improving soil structure and preventing soil erosion. Abundant and diverse soil microbiology helps outcompete pest microbes that would otherwise cause significant damage to crops.

Additionally, Healthy soil microbiology is fundamental to the soil food web as it breaks down organic matter from larger life forms and serves as a food source for beneficial insects and worms, further building up organic matter and soil carbon for improved water-holding capacity and drought resilience.

Figure 3 shows the abundance and diversity of both fungi and bacteria; various kinds of fungi hyphae are visible as long strands, as well as budding fungi. Abundant levels of protozoa could also be seen moving around the microscope slide.

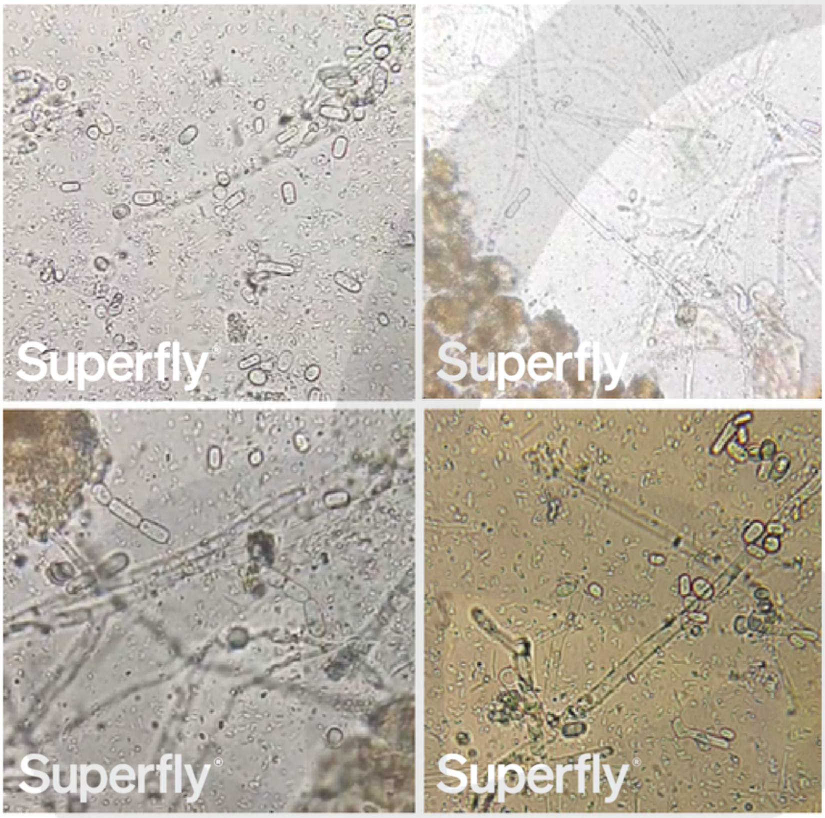

Figure 3

Various photos of dissolved pellets from figure 2 viewed under a compound microscope at 500x magnification.

The larger circular structures are budding fungi and the long strands and fungi hyphae (some are coenocytic and some are septate). The smaller, fainter structures are bacterial cells.

Larger beneficial macroinvertebrate organisms are also present in Superfly®, such as soil mites. These mites are very different from other infamous mites such as spider mites and red-legged earth mites; soil mites are completely harmless to plants.

As the name suggests, soil mites live in the soil, however, they are also commonly found in compost piles. Similar to earthworms, soil mites do not eat living plant tissue and instead only eat decaying organic matter and help build healthy soils and cycle nutrients into a plant-available format. Some soil mites even predate other mite species and nematodes.

Chitin provides unique benefits to soil health

Chitin is a molecule found in the exoskeletons of insects. As BSFL grow they shed their exoskeletons 5 times, meaning that Superfly® contains the chitin from all the exoskeletons shed as the larvae grow whilst they consume food waste.

Natural soils in healthy ecosystems rarely suffer from widespread pathogenic fungi and nematodes that agricultural soils are commonly susceptible to. Natural soils with rich insect life naturally contain higher levels of insect frass and chitin, and would therefore have large populations of chitin-digesting microbes. These microbes are able to control populations of pathogenic fungi and nematodes by feeding on the chitin in fungi cell walls and in nematode eggshells. However, since most agricultural systems lack the insect activity of natural ecosystems, agricultural soils have low levels of microbes that feed on chitin. This means that without chitin, agricultural soils lack the ability to prevent pathogenic nematodes and soil fungi from causing significant damage to crops.

The benefits of chitin in agricultural soil have been extensively studied for decades. Historically, the only commercially available source of chitin as a soil amendment has been produced from crustacean shells, which serve to mimic the effect that insect chitin would have in the soil. Despite the well-documented benefits of chitin in soil (which are discussed in detail later), the adoption of applying it to agricultural soils has not been widespread due to its high costs.

Superfly® not only contains actual insect chitin but is also significantly more economically viable than crustacean shells as a source of chitin.

Importantly, Superfly® already contains a large population of beneficial chitin-digesting microbes, meaning Superfly® not only feeds and grows any (probably small) populations of chitin-digesting microbes already in the soil but also inoculates the soil with a significant population of new chitin-digesting microbes to give Superfly®-amended soils a rapid head-start at preventing outbreaks of soil-borne pathogenic fungi and nematodes.

Image of Bardee's black soldier fly exoskeletons included in Superfly fertiliser and soil enhancer.

Quantitative laboratory analysis of Superfly® microbial life

Colony Forming Units Per gram in Superfly®

A quantitative analysis of the abundance of microbial life in Superfly® was undertaken by (NATA-certified) Symbio Laboratories. Results showed that Superfly® had 240 million colony forming units per gram (CFU/g), making it several orders of magnitude higher in microbial life than standard compost, see figure 4.

This result helps explain some of the results we are seeing with the increased soil activity in figure 1, since these colony forming microbes form the basis of the soil food web.

Image of Bardee scientists in one of Bardee's research labs looking at microbial diversity.

Bardee

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CERTIFICATE OF ANALYSIS

ABN: 82 079 645 015

Certificate Number	M868603 [R00]	Page	1/1
Client	Beyond Ag	Registering Laboratory	Melbourne
Contact	Alex Arnold	Contact	Customer Service Team
Address	333 Exhibition Street Melbourne VIC 3000	Address	Unit 36/640-680 Geelong Rd, Brooklyn, VIC 3012
		Email	admin@symbiolabs.com.au
Telephone	0431 630 058	Telephone	1300 703 166
Order Number	---	Date Samples Received	22/01/2020
Job Description	Frass	Date Analysis Commenced	22/01/2020
Client Job Reference	---	Issue Date	26/01/2020
No. of Samples Registered	1	Receipt Temperature (°C)	21
Priority	Normal	Storage Temperature (°C)	21

NATA

Accreditation No: 2455 Accredited for compliance with ISO/IEC 17025 - Testing

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Definitions

| <: Less Than | >: Greater Than | RP: Result Pending | ~: Estimated | MPN: Most Probable Number | CFU: Colony Forming Units | ---: Not Received/Not Requested | | ^ Subcontracted Analysis| NA:Not Applicable| [NT]:Not Tested| LOR:Level of Reporting| TBA:To Be Advised| ND:Not Detected| * Test not covered by NATA scope of accreditation | # Result derived from a calculation and includes results equal to or greater than the LOR | IH: Inconsistent results possibly caused by sample homogeneity

Authorised By

Name	Position	Accreditation Category
Kashif Ahmed	National Microbiology Manager	Environmental and Food Microbiology

Sample Information - *Client/Sampler Supplied*

Sample ID	Sample Description	Sample Matrix
M868603/1	Beyond Ag Frass 21/1/20 400g	Other - Frass

Analytical Results

Compound/Analyte	Method	LOR	Units	M868603/1
Standard Plate Count	M2.5 - AOAC 990.12	100	CFU/g	240000000

Analysis Location

All in-house analysis was completed by Symbio Laboratories - Melbourne.

Report Comments

Sampling was conducted by the customer and results pertain only to the samples submitted. Responsibility for representative sampling rests with customer.

Figure 4

Symbio Laboratories analysis of Superfly, showing a standard plate could of 240 million colony forming units per gram (CFU/gram)

Microbial diversity in Superfly®

The diversity of the microbes present in Superfly® were also thoroughly analysed by independent commercial laboratories. Superfly® was analysed by Microbiology Laboratories Australia (see figure 5) and was found to have good amounts of key beneficial bacteria and was particularly high in protozoa.

Protozoa
Protozoa help regulate soil bacteria populations and mineralise organic nutrients so they become available to plants. Microbiology Laboratories Australia commented that protozoa often appear after composts have aged for some time, and their presence in Superfly® may be indicative of maturity.

Mycorrhizal fungi
Superfly® was shown to contain mycorrhizal fungi, which have a mutually beneficial symbiotic relationship with many plant roots. Mycorrhizal fungi provide the plant with minerals and water in exchange for sugars from the host plant. This means that Superfly® has the potential to inoculate soils with mycorrhizal fungi, which could further improve plant health by increasing the amount of plant-available water and fertiliser efficiency.

Actinomycetes
Importantly, Superfly® recorded an abundance of Actinomycetes, an order of bacteria that are responsible for characteristically "earthy" smell of freshly turned healthy soil. Actinomycetes break down crop residues, cycle organic matter into plant-available nutrients; inhibit the growth of several plant pathogens in the rhizosphere, fix atmospheric nitrogen into soils, and produce many extracellular enzymes which are conducive to crop

production.[2] An academic paper showed that the application of chitin in soil greatly increased the actinomycete population by 24-34 fold compared to the untreated control[3].

General microbial diversity

Generally, since Superfly® is produced from the castings of Bardee's black soldier fly larvae that have been fed food waste that contains a lot of plant matter, the specific species of microbes that are abundant in Superfly® will be those that are very capable of processing crop residues and roots left in the soil after a harvest or grazing.

The microbes in Superfly® also had good results for the amount of nutrients they contained, particularly nitrogen, calcium, magnesium and carbon. This is important since it is the microbes themselves that help cycle nutrients between organic and plant-available states and convert the carbon in plant roots and crop residues into soil carbon.

Group	Biomass (mg/kg)	
	Yours	Guide
Bacteria		
Actinomycetes	1.819	1.000
Gram positive	10.016	4.000
Gram negative	12.029	11.000
Methane oxidisers	48.288	0.500
Sulphur reducers	0.000	< 0.005
Eukaryotes		
Protozoa	5.647	1.300
Mycorrhizal fungi (including VAM)	0.356	10.000

Nutrients held in microbes	Concentration (mg/kg)	
	Yours	Guide
Nitrogen (N)	4.115	3.450
Phosphorus (P)	1.009	1.500
Potassium (K)	0.336	0.500
Sulphur (S)	0.336	0.500
Calcium (Ca)	0.336	0.250
Magnesium (Mg)	0.336	0.250
Carbon (C)	16.523	22.688

Poor Fair Good
Key

Figure 5

Summary of the key results from a Microbiology Laboratories Australia, Microbe Wise analysis on Superfly

[2] Bhatti AA, Haq S, Bhat RA. Actinomycetes benefaction role in soil and plant health. Microb Pathog. 2017 Oct;111:458-467. doi: 10.1016/j.micpath.2017.09.036. Epub 2017 Sep 18. PMID: 28923606.
[3] El Hadrami, Abdel & Adam, Lorne & Hadrami, Ismail & Daayf, Fouad. (2010). Chitosan in Plant Protection. Marine drugs. 8. 968-87. 10.3390/md8040968.

Bardee

Dr Mary Cole from AgPath, an independent agricultural pathology laboratory, said the following about Superfly®:

"The product [Superfly®] will activate when in soil at field moisture. This product has a fungal to bacterial ratio that is useful for healthy plant growth – approx. 5:1. Many fungi e.g. Aspergillus species and bacteria such as Flavobacterium and Pseudomonas species can use chitin as a nitrogen and carbon source.

Together with the increased organic matter from the bulk, this product will have a positive benefit in encouraging soil health, diversity and resulting plant health. There is enormous diversity of bacterial species and fungal species.

The exoskeleton material together with microbial populations have a stimulatory effect on plant growth when mixed in soil. Extra organic matter from the product increases the activity of soil biology leading to the obvious results seen in the field."

Superfly® is free from pathogens

Pathogens, including Salmonella, Listeria, E.coli and Legionella have never been detected in Superfly®, despite repeated testing by independent laboratories with NATA-certified methods over multiple years. This is primarily due to the high quality of the food waste fed to Bardee's larvae, which consists of food manufacturing byproducts or food intended for human consumption.

Furthermore, BSFL are very capable of significantly reducing populations of microbial pathogens which may be present in the food waste,[4] this is because of their ability to naturally produce novel antibiotic compounds which are effective against a broad range of microorganisms.[5] Furthermore, as an additional step during the production of Superfly®, the insect castings are thermophylically composted in accordance with the Organic Standard, reaching >55°C for at least 3 days, further ensuring the safety of Superfly®. See figures 6 and 7 for examples of laboratory reports of pathogen testing on Superfly®.

[4] Erickson MC, Islam M, Sheppard C, Liao J, Doyle MP. Reduction of Escherichia coli O157:H7 and Salmonella enterica serovar Enteritidis in chicken manure by larvae of the black soldier fly. J Food Prot. 2004 Apr;67(4):685-90. doi: 10.4315/0362-028x-67.4.685. PMID: 15083719.

[5] Park, Soon-Ik & Chang, Byung & Yoe, Sung. (2014). Detection of antimicrobial substances from larvae of the black soldier fly, Hermetia illucens (Diptera: Stratiomyidae). Entomological Research. 44. 10.1111/1748-5967.12050.

Image of Bardee's food waste decontamination processing.

Image of Bardee's food waste post-decontamination.

Image of Bardee's black soldier fly larvae fed food waste with no additional water.

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CERTIFICATE OF ANALYSIS

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ABN: 82 079 645 015

Certificate Number	M1172962 [R00]	Page	1/1
Client	Bardee	Registering Laboratory	Melbourne
Contact	Kaya Moore	Contact	Customer Service Team
Address	333 Exhibition Street Melbourne VIC 3000	Address	Unit 36/640-680 Geelong Rd, Brooklyn, VIC 3012
		Email	admin@symbiolabs.com.au
Telephone	0431 630 058	Telephone	1300 703 166
Order Number	---	Date Samples Received	08/07/2022
Job Description	Other - Frass	Date Analysis Commenced	09/07/2022
Client Job Reference	---	Issue Date	18/07/2022
No. of Samples Registered	1 | Sampler: Customer	Receipt Temperature (°C)	16
Priority	Normal	Storage Temperature (°C)	21

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Definitions

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Authorised By

Name	Position	Accreditation Category
Sarah Batticciotto	Laboratory Manager – Microbiology	Environmental and Food Microbiology

Sample Information - *Client/Sampler Supplied*

Sample ID	Sample Description	Remarks
M1172962/1	Fine (compost)	

Analytical Results

Compound/Analyte	Method	LOR	Units	M1172962/1
Escherichia coli	M8.8 - AOAC 991.14	10	CFU/g	<10
Salmonella spp.	M16.10 - Food Biotech 29	ND	/25g	ND
Listeria spp.	M13.10SPP - Food Biotech 29	ND	/25g	ND

Analysis Location

All in-house analysis was completed by Symbio Laboratories - Melbourne.

Figure 6

Sybio Laboratories results for pathogen testing on Superfly. For E.coli the lowest score that can be recorded is <10 CFU/g, meaning that there was no E.coli detected or if it was, it was less than 10 CFU/g. ND = not detected

Symbio LABORATORIES

CERTIFICATE OF ANALYSIS

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ABN: 82 079 645 015

Certificate Number	M1200629 [R00]	Page	1/1
Client	Bardee	Registering Laboratory	Melbourne
Contact	Kaya Moore	Contact	Customer Service Team
Address	333 Exhibition Street Melbourne VIC 3000	Address	36 Business Park Drive Ravenhall VIC 3023
		Email	admin@symbiolabs.com.au
Telephone	0431 630 058	Telephone	1300 703 166
Order Number	FR220921	Date Samples Received	21/09/2022
Job Description	Soils - Compost	Date Analysis Commenced	22/09/2022
Client Job Reference	---	Issue Date	29/09/2022
No. of Samples Registered	1 | Sampler: Customer	Receipt Temperature (°C)	21
Priority	Normal	Storage Temperature (°C)	21

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Authorised By

Name	Position	Accreditation Category
Sarah Batticciotto	Laboratory Manager – Microbiology	Environmental and Food Microbiology

Sample Information - *Client/Sampler Supplied*

Sample ID	Sample Description	Remarks
M1200629/1	FR220921A - Fine (compost)	

Analytical Results

Compound/Analyte	Method	LOR	Units	M1200629/1
Legionella not L.pneumophila	M11.0 - AS/NZS 3896	10	CFU/mL	<10
Legionella pneumophila SG1	M11.0 - AS/NZS 3896	10	CFU/mL	<10
Legionella pneumophila SG2-15	M11.0 - AS/NZS 3896	10	CFU/mL	<10
Total Legionella Count	M11.0 - AS/NZS 3896	10	CFU/mL	<10

Analysis Location

All in-house analysis was completed by Symbio Laboratories - Melbourne.

Figure 7

Symbio Laboratories results for pathogen testing on Superfly. The lowest score that can be recorded is <10 CFU/g, meaning that there was no E.coli detected or if it was, it was less than 10 CFU/g.

Superfly® is free from chemical residues and heavy metals

Superfly® is well within the safe limits of heavy metals (table 1) and chemical residues (figure 8 and table 2).

This is because of the high quality of the food waste input, consisting only of material that was destined for human consumption or was a by-product of food production. This means Bardee's food waste inputs will have extremely low levels of heavy metals and will have already met the withholding period requirements to control for chemical residues and have passed the chemical residue standards required to be fed to people.

To help further reduce the levels of chemical residues, the food waste is fed to Bardee's BSFL, and then the frass is thermophylically composted in accordance with ACO standards. Finally, Superfly® is an organically certified product and Bardee's production methods meet the strict standards for an organic product to guarantee it is free from harmful levels of chemical residues (figure 9 and figure 10).

	Product Name: Product Type: Manufacturing Site: Application: Test Applicable:	**Sample 1** Loose Frass Fertiliser BA CA-PACK-020	***Guideline*** *AS4454:2012* *Composted Product*
Parameter	**Method Reference**	**M0063/1**	
Total Cadmium (mg/kg)	Rayment & Lyons 2011 - 17C1 Aqua Regia	<0.5	*< 1 Cd*
Total Lead (mg/kg)		<1	*< 150 Pb*
Total Arsenic (mg/kg)		<2	*< 20 As*
Total Chromium (mg/kg)		3.89	*< 100 Cr*
Total Nickel (mg/kg)		3.11	*< 60 Ni*
Total Mercury (mg/kg)		<0.1	*< 1 Hg*
Total Silver (mg/kg)		<1	..

Table 1

Heavy metal analysis results conducted by Environmental Analysis Laboratory (EAL) as part of Bardee's regular testing of Superfly to ensure product safety. The results for heavy metal concentrations are well below the requirements of AS4454:2012 Compost Product for all heavy metals. Where are '<' is used, this indicates that the concentration was below the limit of reporting (LOR)

Figure 8

Chemical residue analysis results conducted by Environmental Analysis Laboratory (EAL) as part of Bardee's regular testing of Superfly to ensure product safety.

Superfly tested negative (<LOR) for 22 of the 25 crop residue classes tested for the standard multi-pesticide residue scan; the remaining 3 residues were detected at levels of less than 1/10 the maximum residue limit (MRL) as shown in table 2.

ANALYTE	METHOD REFERENCE	FR220908A
	Job No.	N2736/1
AMPA (mg.kg)**	*c*	<0.01 (not detected)
Glufosinate (mg/kg)**	*c*	<0.01 (not detected)
Glyphosate (mg/kg)**	*c*	<0.01 (not detected)
MULTI-PESTICIDE RESIDUE SCAN		
ORGANOPHOSPHATES (OPs)		
Diazinon (mg/kg)	*a*	*<LOR (not detected)*
Other OPs (mg/kg)	*a*	*<LOR (not detected)*
HERBICIDES		
Chlorthal Dimethyl (mg/kg)	*a*	*<LOR (not detected)*
Other Herbicides (mg/kg)	*a*	*<LOR (not detected)*
PHENOLS		
Phenyl-Phenol (mg/kg)	*a*	*<LOR (not detected)*
Other Phenolics (mg/kg)	*a*	*<LOR (not detected)*
CARBAMATES		
Carbaryl (mg/kg)	*a*	*<LOR (not detected)*
Other Carbamates (mg/kg)	*a*	*<LOR (not detected)*
ACARICIDES		
Bifenazate(mg/kg)	*a*	*<LOR (not detected)*
Other Acarides (mg/kg)	*a*	*<LOR (not detected)*
SYNTHETIC PYRETHROIDS (SPs)		
Permethrin (mg/kg)	*a*	*<LOR (not detected)*
Other SP's (mg/kg)	*a*	*<LOR (not detected)*
ORGANOCHLORINES (OCs)		
Dieldrin (mg/kg)	*a*	*<LOR (not detected)*
DDD/ DDE/ DDT (mg/kg)	*a*	*<LOR (not detected)*
Other OCs (mg/kg)	*a*	*<LOR (not detected)*
FUNGICIDES		
Fludioxinil (mg/kg)	*a*	**0.14**
Imazalil (mg/kg)	*a*	**0.49**
Thiabendazole (mg/kg)	*a*	**0.17**
Other Fungicides (mg/kg)	*a*	*<LOR (not detected)*

OTHER RESIDUES		
Thiamethoxam (mg/kg)	a	<LOR (not detected)
Other Residues (mg/kg)	a	<LOR (not detected)

METHODS REFERENCE

a. Analysis sub-contracted - Symbio report no. B1199827

NOTES:

1. <LOR = Less Than LIMIT OF REPORTING
2. All services undertaken by EAL are covered by the EAL Laboratory Services Terms and Conditions (refer SCU.edu.au/eal/t&cs or on request).
3. .. Denotes no limits set or available
4. Analysis conducted between sample arrival date and reporting date.
5. ** NATA accreditation does not cover the performance of this service.
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Table 2

MRL the three fungicides detected in Superfly for various food commodities according to the Agricultural and Veterinary Chemicals Code (MRL Standard) Instrument 2019.

Of the three chemical residues found in Superfly, they are all many fold lower concentrations than what is required for human consumption; furthermore, of the very low concentrations of Fludioxonil, Imazalil, and Thiabendazole present in Superfly, it is very unlikely that any residues would be transferred to crops if Superfly were applied to agricultural soil.

COMPOUND	FOOD	MRL (mg/kg)
Fludioxonil		
FS 0240	Apricot	10
FI 0326	Avocado	2
FB 0018	Berries and other small fruits {except grapes}	5
VA 0035	Bulb vegetables {except Fennel, bulb; Onion, bulb}	3
TN 0664	Chestnuts	1
FC 0001	Citrus fruits	10
VP 0526	Common bean (pods and/or immature seeds)	0.7
VC 0424	Cucumber	0.5
DF 0269	Dried grapes (=currants, raisins and sultanas)	5
Imazalil		
FC 0001	Citrus fruits	10
VC 0046	Melons, except watermelon	10
VO 0450	Mushrooms	1
FP 0009	Pome fruits	5
VR 0589	Potato	5
Thiabendazole		
FP 0226	Apple	10
FI 0327	Banana	3
FC 0001	Citrus fruits	10
VO 0450	Mushrooms	0.5
FP 0230	Pear	10
VR 0589	Potato	5

Registered Product Certificate

ACO CERTIFICATION LTD.

CERTIFIED OPERATION

Bardee

Unit 6, 45 Bunnett Street
Sunshine North Victoria 3020 AUSTRALIA

Certification Number: **13303**

SCOPE OF CERTIFICATION
Processor
Allowed Inputs

FACILITIES

Unit 6, 45 Bunnett Street Sunshine North VIC 3020 AUSTRALIA

PRODUCTS
SEE ANNEX

STANDARDS
The above named operator has been assessed by ACO Certification Ltd. The products listed on this certificate have been reviewed as compliant with the requirements set out in the Australian Certified Organic Standard 2021v.1. This certificate is not a legal or commercial document and may not be used as such. This certificate remains the property of ACO Certification Ltd and remains valid until the expiry date or until surrendered, suspended or decertified.

The Common Seal of

Authorised by:
Kate Allan
General Manager - Certification

Place & Date of Issue:
Brisbane, Australia
07 December 2021

Valid Until 31 Dec 2023
C-12444-2021

Figure 9

Bardee's ACO Registered Product Certificate as a processor and for allowed inputs. Certification number: 13303. ACO certified products are listed in the annex (Figure 10)

ACO CERTIFICATION LTD.

Company name and address:
Bardee
Unit 6, 45 Bunnett Street Sunshine North Victoria 3020, AUSTRALIA
Certification number: **13303**
Type of operation: **Processor Allowed Inputs**

ANNEX: Certified product listing Date of issue: **7 Dec 2021**

The certificate and annex remains property of ACO Certification Ltd and can be withdrawn in case of changes or termination. This annex is only valid when presented with a valid certificate number corresponding with the following number: *C-12444-2021*

Product name	Group	Services	Brands
Superfly Fertiliser	Crop Fertilisers and Soil Amendments	Allowed Inputs	

Figure 10

Annex to Bardee's ACO certification. Superfly is organically certified as an allowed input for crop fertilisers and soil amendments.

Superfly® Enhances Growth

In addition to improving soil health, Superfly® increases overall plant productivity by combining plant biostimulants and nutrients to stimulate and fuel growth. The germination, rooting, flowering and general productivity gains of applying Superfly® can be seen in the various trials below. The overall benefits to plant growth can be clearly seen in a pasture trial conducted earlier this year (figure 12 & 13), where it more than doubled pasture growth weight.

Superfly® improves overall plant growth

Pasture trial in Goulburn Region, Victoria

Method

During Autumn 2022 Bardee conducted a field trial of Superfly® on an established cattle pasture in the Goulburn region, Victoria.

Superfly® was spread with a conventional PTO-powered compost spreader (figure 11). While filling the compost spreader, some Superfly® was spilled onto the ground; the subsequent effects on the grass growth in that area can be seen in figure 15. The paddock chosen for the trial had an even, slight slope falling from East to West, with windbreak of tall gums towards the Northern boundary (figure 11). Treatments strips were marked with star pickets, ran North-South, were 45 metre-long and were 5 metre-wide (which is as wide as what the compost spreader was set to throw the Superfly®). The control strip still had the tractor and spreader drive over them, but did not have Superfly® applied.

Figure 11

Left = Superfly is being spread with a conventional PTO-powered twin disc compost spreader.

Right = the trial site with a slight incline towards the East.

Application rates were measured by calculating the volume of material that was used in each treatment strip by measuring the depth and width of material left in the hopper. The following application rates were tested: 2.60, 3.25, 4.27 and 6.27 T/Ha. Cattle were moved off this paddock on 22/07/22, Superfly® was spread 2 days later on 24/7/22 and results were measured 90 days later on 22/10/22 and repeated on 23/10/22. Being a relatively high elevation and the trial beginning in Winter, growth was relatively mild in the region during most of this period, with the majority of growth occurring in the final weeks.

Pasture growth was measured by using a lawn mower (Masport 650) with an attached grass catcher to mow and collect clippings from a straight strip along the treatment strips (figure 12). Pasture clippings were weighed by placing the clippings into a large bucket suspended on a set of bag scales. The mower cutting height was set to 71mm to best simulate the collection of the pasture that would have been grazed before the cattle would be moved to another paddock to avoid overgrazing. The effect of Superfly® on pasture growth was measured as a '% yield increase compared to the control yield', where 'yield' was the biomass collected in the mower grass catcher bag for each treatment.

Results

Superfly® had a very noticeable effect on the growth of the pasture. Figures 12 and 13 show a visually obvious difference in the height of the pasture when treated with Superfly®. The largest amount of growth was observed at the highest level of fertilisation at 6.27 T/Ha, which showed 130% increase in pasture biomass collected compared to the control (Figure 14).

However, the most efficient application rate tested was 3.25 T/Ha, which showed a 100% growth increase during the trial period. The theoretical most efficient application rate is likely to be somewhere between 3.25 and 2.60 T/Ha.

The sudden increase in pasture growth between 2.60 and 3.25 T/Ha could be due to the specific soil of the trial site, which may have been deficient in specific nutrients which were not made available to the pasture unless higher rates of Superfly® were applied; Superfly® may have different effects on other soil types, depending on their nutrient levels. It is also worth noting that the data was collected after only a few weeks after the growing season started, and it is very likely that the effect of Superfly® would be more pronounced if the trial were extended.

Figure 12

The path of the lawn mower left along the lengths of the trial strips.

The left side was treated with Superfly, the right is the control. Both paths were cut with the mower blades set at 71mm.

Figure 13

The paths created by the mower along the trial strips show the difference in pasture growth when Superfly is applied (top), compared to the control (bottom).

Both paths were cut with the mower blades set at 71mm.

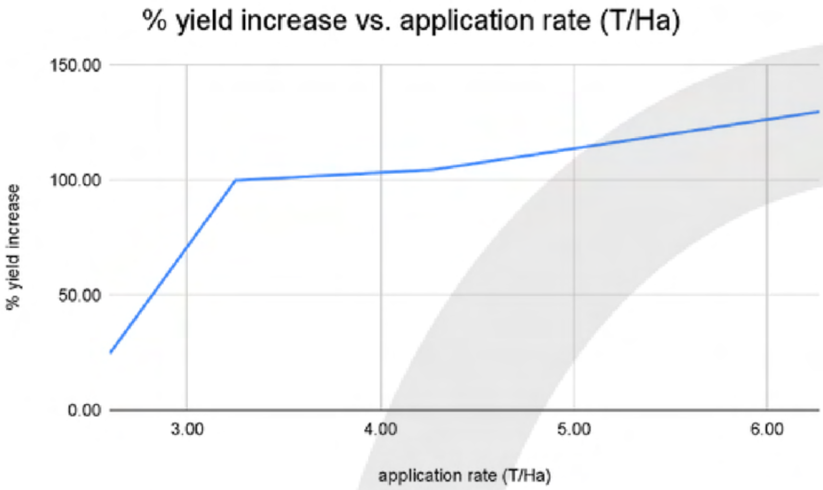

Figure 14

The % yield increase compared to the control yield at different application rates. This graph shows the average of the results from the two days of collecting pasture growth data.

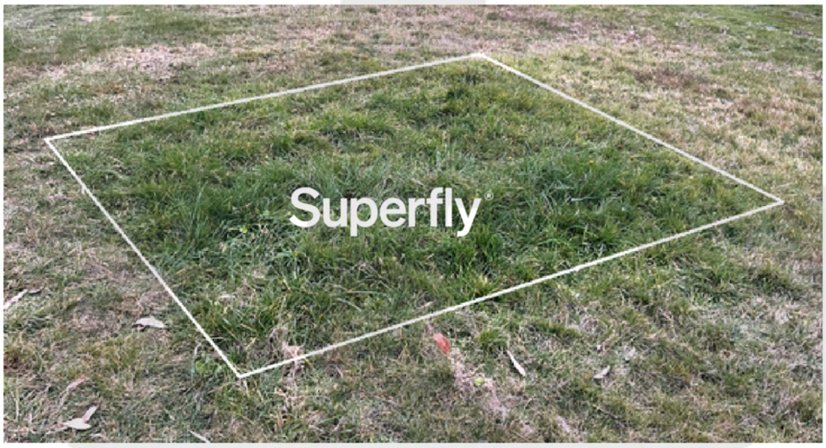

Figure 15

The white rectangle outlines where the compost spreader was positioned when it was being loaded with Superfly from a bulk bag. During this process, some Superlfly was spilled on the ground. All the grass in this photo was heavily grazed by cattle some time later, however, there was a very obvious increase in grass growth in the area where the Superfly was spilled. This shows that Superfly has a long lasting effect on yield, beyond a single crop. Quantitative data was not collected here.

Maize trial

Successful applications of BSFL frass have also been shown in published public science journals. A paper titled "Exploring Black Soldier Fly Frass as Novel Fertilizer for Improved Growth, Yield, and Nitrogen Use Efficiency of Maize Under Field Conditions" compared the effect of BSFL frass to conventional organic fertiliser and urea. Both frass the the conventional organic fertiliser (COF) were applied at the rates of 0, 2.5, 5, and 7.5 T/Ha, and 0, 30, 60, and 100 kg nitrogen (N) per hectare; urea was also applied at 0, 30, 60 and 100 kg N per hectare.

Maize grown in plots treated with frass had the highest yields, tallest plants and highest chlorophyll concentrations. Plots treated with 7.5 T/Ha of frass had 14% higher grain yields and 23% higher nitrogen update than plots treated with a similar rate of the COF. There was a 27% and 7% increase in grain yields in plots treated with 100 kg N ha-1 of frass compared to those treated with equivalent rates of COF and urea, respectively. Likewise, application of frass at 100 kg N/Ha increased maize N uptake by 76% and 29% compared to COF and urea, respectively. The agronomic nitrogen use efficiency (NUE) of maize treated with 2.5 T/Ha of frass was 2.4 times higher than the value achieved using an equivalent rate of COF. Application rates of 2.5 T/Ha and 30 kg N/Ha of frass were found to be effective in improving maize yield, while double rates of COF were required .

[6] Beesigamukama D, Mochoge B, Korir NK, Fiaboe KKM, Nakimbugwe D, Khamis FM, Subramanian S,Dubois T, Musyoka MW, Ekesi S, Kelemu S and Tanga CM (2020) Exploring Black Soldier Fly Frass as Novel Fertilizer for Improved Growth, Yield, and Nitrogen Use Efficiency of Maize Under Field Conditions. Front. Plant Sci. 11:574592. doi: 10.3389/fpls.2020.574592

Superfly® contains growth promoting compounds (biostimulants)

The ability for frass to consistently increase yield is disproportionate to the amount of NPK it contains; and while Superfly® can increase soil health and biodiversity for long-term benefits, the significant improvements seen in a relatively short timeframe in the above trials are also attributable to the biostimulants present in frass.

According to the European Biostimulants Industry Council (EBIC), the definition of biostimulants is “substances and/or microorganisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress and crop quality“. Biostimulants are not nutritive but instead activate natural plant biochemical and hormonal pathways to affect plant metabolic functions and physiology in numerous ways.

Biostimulants have been shown to increase overall growth, as seen in the above trials, however, biostimulants can also accelerate rooting, stimulate germination and increase flowering. There are multiple types of biostimulants within Superfly®, namely chitin from the BSFL exoskeletons, humic acid and specific microbes[7].

> “The exoskeleton material together with microbial populations have a stimulatory effect on plant growth when mixed in soil”

Dr Mary Cole, Plant Pathologist

[7] Ivã Guidini Lopes, Jean WH Yong, Cecilia Lalander, Frass derived from black soldier fly larvae treatment of biodegradable wastes. A critical review and future perspectives, Waste Management, Volume 142, 2022, Pages 65-76, ISSN 0956-053X

Chitin

Natural soils found in wild ecosystems are amongst the most productive in the world, and do not depend on fertiliser inputs. These soils are rich in insect frass due to the large numbers of insects in a healthy ecosystem. Cultivated land tends to have significantly fewer insects than wild ecosystems, and consequently has much less insect frass in the soil. In a similar way to flowering plants co-evolving with insect pollinators, plants have evolved around the presence of insect frass and the molecules it contains, such as chitin.

Plants, and even other soil life can detect the presence of chitin in the soil and can respond in numerous beneficial ways for plant health, making chitin a plant biostimulant. [8] When chitin is in the soil it will break down through biological and chemical processes into chitosan, another biomolecule with many beneficial plant biostimulant effects.

Chitin amplifies the benefits of mycorrhizal fungi

In addition to Superfly® already containing mycorrhizal fungi (figure 5), the chitin in Superfly® also positively interacts with mycorrhizal fungi. It was found that chitosan addition amplified the benefits of mycorrhizal inoculation in strawberry with Glomus sp.; specifically increased growth, fruit yield and a delay of the onset of powdery mildew[9].

[8] Sharp, Russell G. 2013. "A Review of the Applications of Chitin and Its Derivatives in Agriculture to Modify Plant-Microbial Interactions and Improve Crop Yields" Agronomy 3, no. 4: 757-793. https://doi.org/10.3390/agronomy3040757
[9] Lowe, A.; Rafferty-McArdle, S.M.; Cassells, A.C. Effects of AMF- and PGPR-root inoculation and a foliar chitosan spray in single and combined treatments on powdery mildew disease in strawberry. Agric. Food Sci. 2012, 21, 28–38.

Chitin stimulates root nodulation in legumes

For nitrogen fixing root nodules on legumes to develop, first the Rhizobium bacteria must produce a chitin-based signal molecule that is detected by the legume. It has been shown that the addition of chitin derivatives can induce nodulation in alfalfa (Medicago sativa)[10].

Chitin promotes plant growth

Chitin has been found to directly increase plant growth in a range of crops even at very low concentrations (10mg/L). The growth promoting benefits of chitin are independent of chitin's effects on pest and disease control, which are discussed later. Significant

improvements in growth from chitin and chitosan have been show in daikon radishes (Raphanus sativus)[11], cabbage (Brassica oleracea)[12], soybean sprouts[13], sweet basil[14], grapevine[15], chillis[16], as well as ornamental crops, such as Gerbera[17] and Dendrobium orchids[18].

[10] Staehelin, C.; Schultze, M.; Tokuyasu, K.; Poinsot, V.; Promé, J.C.; Kondorosi, E.; Kondorosi, A. N-deacetylation of Sinorhizobium meliloti Nod factors increases their stability in the Medicago sativa rhizosphere and decreases their biological activity. Mol. Plant-Microbe Interact. 2000, 13, 72–79.
[11] Tsugita, T.; Takahashi, K.; Muraoka, T.; Fukui, H. The application of chitin/chitosan for agriculture (in Japanese). In Proceedings of Special Session of the 7th Symposium on Chitin and Chitosan; Japanese Society for Chitin and Chitosan: Fukui, Japan, 1993; pp. 21–22.
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[13] Lee, Y.S.; Kim, Y.H.; Kim, S.B. Changes in the respiration, growth, and vitamin C content of soybean sprouts in response to chitosan of different molecular weights. HortScience 2005, 40, 1333–1335.
[14] Kim, H.J.; Chen, F.; Wang, X.; Rajapakse, N.C. Effect of chitosan on the biological properties of sweet basil (Ocimum basilicum L.). J. Agric. Food Chem. 2005, 53, 3696–3701.
[15] Ait Barka, E.; Eullaffroy, P.; Clément, C.; Vernet, G. Chitosan improves development, and protects Vitis vinifera L. against Botrytis cinerea. Plant Cell Rep. 2004, 22, 608–614.
[16] Chookhongkha, N. & Miyagawa, S. & Jirakiattikul, Yaowapha & Photchanachai, Songsin. (2012). Chilli growth and seed productivity as affected by chitosan. International Conference on Agriculture Technology and Food Sciences (ICATFS' 2012). Nov. 17-18, 2012. 146-149.
[17] Wanichpongpan, P; Suriyachan, K; Chandrkrachang, S. Effects of Chitosan on the growth of Gerbera flower plant (Gerbera jamesonii). In Chitin and Chitosan in Life Science, Proceedings of the Eighth International Chitin and Chitosan Conference and Fourth Asia Pacific Chitin and Chitosan Symposium, Yamaguchi, Japan, 21–23 September 2000; Uragami, T., Kurita, K., Fukamizo, T., Eds.; pp.198–201
[18] Chandrkrachang, S. The applications of chitin in agriculture in Thailand. Adv. Chitin Sci. 2002, 5, 458–462.

The growth stimulant effects of chitin were even noticed when plants were grown in sterile conditions, showing that chitin stimulates plant growth independently of its beneficial effects on promoting the activity of beneficial bacteria and fungi[19][20]. One possible mechanism for chitin increasing plant growth directly is that chitin has been found to significantly increase the size of chloroplasts (the organelle responsible for photosynthesis), and increase silica uptake[21].

Chitin promotes flowering

In addition to increasing growth and beneficial microbial activity, chitin has also been shown to modify flower development. In concentrations as low as 1ppm, chitin and chitosan have been shown to significantly increase number of flowers, induce earlier flowering[22][23][24], and produce deeper petal pigmentation[25]. These effects of Superfly® on flowering can be seen on a rosebed trial in figure 16 and figure 17.

[19] Pornpeanpakdee, P.; Pichyangkura, R.; Chadchawan, S.; Limpanavech, P. Chitosan effects on Dendrobium 'Eiskul' Protocorm-like body production. In Proceedings of the 31st Congress on Science and Technology of Thailand, Nakornrachaseema, Thailand, 18–20 October 2005; pp. 1–3.

[20] Nahar, S.J.; Kazuhiko, S.; Haque, S.M. Effect of Polysaccharides Including Elicitors on Organogenesis in Protocorm-like Body (PLB) of Cymbidium insigne in vitro. J. Agric. Sci. Technol. 2012, 2, 1029–1033.

[21] Patchra Limpanavech, Subhalai Chaiyasuta, Ranitha Vongpromek, Rath Pichyangkura, Chumpol Khunwasi, Supachitra Chadchawan, Pongtharin Lotrakul, Reungwit Bunjongrat, Anchalee Chaidee, Thapana Bangyeekhun, Chitosan effects on floral production, gene expression, and anatomical changes in the Dendrobium orchid, Scientia Horticulturae, Volume 116, Issue 1, 2008, Pages 65-72

[22] Patchra Limpanavech, Subhalai Chaiyasuta, Ranitha Vongpromek, Rath Pichyangkura, Chumpol Khunwasi, Supachitra Chadchawan, Pongtharin Lotrakul, Reungwit Bunjongrat, Anchalee Chaidee, Thapana Bangyeekhun, Chitosan effects on floral production, gene expression, and anatomical changes in the Dendrobium orchid, Scientia Horticulturae, Volume 116, Issue 1, 2008, Pages 65-72

[23] Utsunomiya, N.; Kinai, H. Effect of chitosan-oligosaccharides soil conditioner on the growth of passionfruit. J. Jpn. Soc. Hortic. Sci. 1994, 64, 176–177.

[24] Ohta, K.; Tanguchi, A.; Konishi, N.; Hosoki, T. Chitosan treatment affects plant growth and flower quality in Eustoma grandiflorum. HortScience 1999, 34, 233–234.

[25] Uddin, A.F.M.J.; Hashimoto, F.; Shimiza, K.; Sakata, Y. Monosaccharides and chitosan sensing in bud growth and petal pigmentation in Eustoma grandiflorum (Raf.) Shinn. Sci. Hortic. 2004, 100, 127–138.

Chitin increases germination of seeds

The germination of seeds has been shown to be improved in a range of crops following chitin-based treatments including maize[26] and wheat[27]. In these studies, chitosan accelerated germination and/or increased the percentage of seeds germinating.

Image of Superfly bulka bags at a Bardee facility.

[26] Guan, Y.J.; Hu, J.; Wang, X.J.; Shao, C.X. Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. J. Zhejiang Univ. Sci. B. 2009, 10, 427–433.
[27] Bhaskara Reddy, M.V.; Arul, J.; Angers, P.; Couture, L. Chitosan treatment of wheat seeds induces resistance to Fusarium graminearum and improves seed quality. J. Agric. Food Chem. 1999, 47, 1208–1216.

Microbes

Superfly® contains an abundance of diverse microbial life which help to build soil resilience and cycle nutrients to become plant-available, however, some of the microbes in Superfly® also act as biostimulants, or plant growth-promoting microorganisms (PGPM) [28].

BSFL frass contains multiple species of Lactobacillus, Actinomycetes and Bacillus; these microbes produce phytohormones with beneficial traits for plant, such as auxins (promotes growth and increases drought tolerance), cytokinins (stimulate root exudation), jasmonic acid (induce salinity stress tolerance), gibberellins (increases seed germination, enhance nutritional metabolites, regulates endogenous phytohormones, induce thermotolerance), among others [29] [30] [31]. Additionally, the bacterial species Bacillus subtillus can digest chitin and it has been found that the addition of chitin increases the amount of auxin that B. subtillus produces, which further increases seed germination [32] [33]. The effect of Superfly® on germination can be seen in a grass seed germination trial in figure 18.

[28] Gold, M., Von Allmen, F., Zurbrügg, C., Zhang, J., Mathys, A., 2020b. Identification of bacteria in two food waste black soldier fly larvae rearing residues. Front. Microbiol. 11, 582867.
[29] Raman, J.; Kim, J.-S.; Choi, K.R.; Eun, H.; Yang, D.; Ko, Y.-J.; Kim, S.-J. Application of Lactic Acid Bacteria (LAB) in Sustainable Agriculture: Advantages and Limitations. Int. J. Mol. Sci. 2022, 23, 7784.
[30] Poveda, J., Gonz´alez-Andr´es, F., 2021. Bacillus as a source of phytohormones for use in agriculture. Appl. Microbiol. Biot. 105 (23), 8629–8645.
[31] Aamir, Mohd & Singh, Sushil & Zeyad, Mohammad & Upadhyay, Ram. (2021). Actinomycetes as biostimulants and their application in agricultural practices.
[32] Manjula, K.; Podile, A.R. Increase in seedling emergence and dry weight of pigeon pea in the field with chitin-supplemented formulations of Bacillus subtilis AF 1. World J. Microbiol. Biotechnol. 2005, 21, 1057–1062.
[33] Malfanova, N.; Kamilova, F.; Validov, S.; Shcherbakov, A.; Chebotar, V.; Tikhonovich, I.; Lugtenberg, B. Characterization of Bacillus subtilis HC8, a novel plant-beneficial endophytic strain from giant hogweed. Microb. Biotechnol. 2011, 4, 523–532.

Humic acid

In a similar way to earthworm castings, Superfly® is fundamentally made from matured, decomposed organic matter, meaning that it contains humic acids. Humic acids interact with soil microorganisms to release several biostimulants like auxins and cytokinins which are critical plant hormones controlling cell division, plant development, overall growth and root development[34].

The effect of Superfly® on root development can be seen in several field and pot trials on tomatoes (figure 19) and on turf (figure 20 and figure 21).

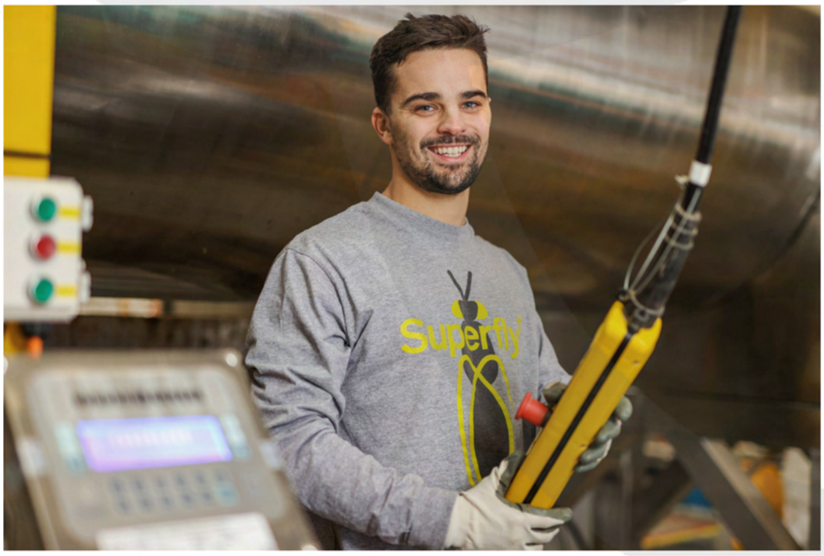

Image of Bardee processing line.

[34] Maite Olaetxea, David De Hita, C. Andrés Garcia, Marta Fuentes, Roberto Baigorri, Verónica Mora, Maria Garnica, Oscar Urrutia, Javier Erro, Angel M Zamarreño, Ricardo L. Berbara, José Maria Garcia-Mina, Hypothetical framework integrating the main mechanisms involved in the promoting action of rhizospheric humic substances on plant root- and shoot- growth, Applied Soil Ecology, Volume 123, 2018, Pages 521-537, ISSN 0929-1393, https://doi.org/10.1016/j.apsoil.2017.06.007.

Rose flowering trial in Melbourne, Victoria

Method

In April 2021 one rose bed was treated with two handfuls (approximately 100g) of Superfly® above the root zone of each rose bush (treated bed), the other rose bed was left untreated (control); at this stage both beds looked very similar. Both rose beds have the same variety of rose (Rose Parole, Hybrid Tea), were planted at the same time with the same method and in the same kind of poor quality, heavy clay soil mulched with black tambark.

Neither rose bed received any irrigation during these 12 months and relied solely on rainfall. Both rose beds were pruned around the same time in August 2022. Deadheading was done every two weeks throughout the flowering period.

Results

Photos were taken on 22/10/21, 24/10/22, 7/11/22 (figure 1). In both 2021 and 2022 the roses in the treated bed flowered approximately 5 weeks before the untreated rose bed, had noticeably more flowers, had a longer flowering period and had significantly greater vegetative growth than the control rose bushes, see figures 1 and 2. The treated rose bushes also had a noticeably deeper, richer petal colour and a stronger scent. The flowers of the treated bed developed consistently and were of a high quality, as shown in figure 13.

The visibly obvious improvement in flowering and overall growth in 2022, despite not having received Superfly® for over 12 months, shows the long-term, multicrop benefits of Superfly®. These long-term benefits cannot be explained by NPK alone and are due to Superfly improving soil health and containing plant biostimulants.

It was also noticed that the treated bed had significantly more mushrooms sprouting in Spring; which is a strong indication for a higher level of soil biological activity.

Figure 16:

Left column of photos are of the treated rose bed, right column of photos are of the control bed.

Top row of photos was taken on 22/10/21: flowering is well underway in the treated bed before any flowers have bloomed in the control bed.

Middle row of photos was taken on 24/10/22: the treated bed already has a mixture of spent flowers, active flowers and buds about to burst before the untreated bed has any flowers.

Bottom row of photos was taken 7/11/22: the treated bed is in full bloom, while the control bed is only starting to bloom.

Vegetative stem growth is visibly greater in the treated bed at all stages, with a far greater number of water shoots (reddish stems and leaves).

Figure 17

Left photo is of the treated bed, right photo is of the control bed. At this camera angle, the difference in overall rose bush size and development is very apparent. The fire hydrants are for scale reference.

Figure 18

Both photos are of roses from the treated bed. Flower development is healthy and petal colour is deep and rich, producing overall high-quality flowers.

Grass seed germination trials

Method

On 13/09/22 lawn seed (Garden Basics, All Purpose) was sown equally onto a two 1 sqm areas (as per packet instructions); the areas were prepared for sowing by aerating the low quality compacted heavy clay soil with a garden fork and spreading fertiliser-free garden soil (Garden Basics Soil Mix) and soil wetting agent (Hortico Soil Wetter) as per packet instructions. One of the two areas also had Superfly® spread at a rate of 25g per square metre (treated area); the other area did not have Superfly® added (control area). At this stage, both areas looked very similar. Both areas were watered in with equal amounts of tap water and watered each day for the following two weeks with a watering can to keep the seedbed damp; after two weeks the lawn was only watered with rain, which was ample for keeping the seedbed damp.

Results

Photos were taken on 29/09/22 (day 16) and 24/10/22 (day 41), see figure 19. The germination rate and growth rate of the seedlings were visibly greater in the treated area than in the control area, suggesting Superfly® has a very low level of phytotoxicity. The seeds in the treated area germinated earlier, and had a higher germination success rate, and the subsequent seedlings grew faster and with a noticeably darker colour, indicating greater nitrogen uptake, and possibly larger chloroplast growth. These obvious improvements in germination and growth rate are the results of the combined benefits of Superfly's plant biostimulants and nutrients improving both soil and plant health.

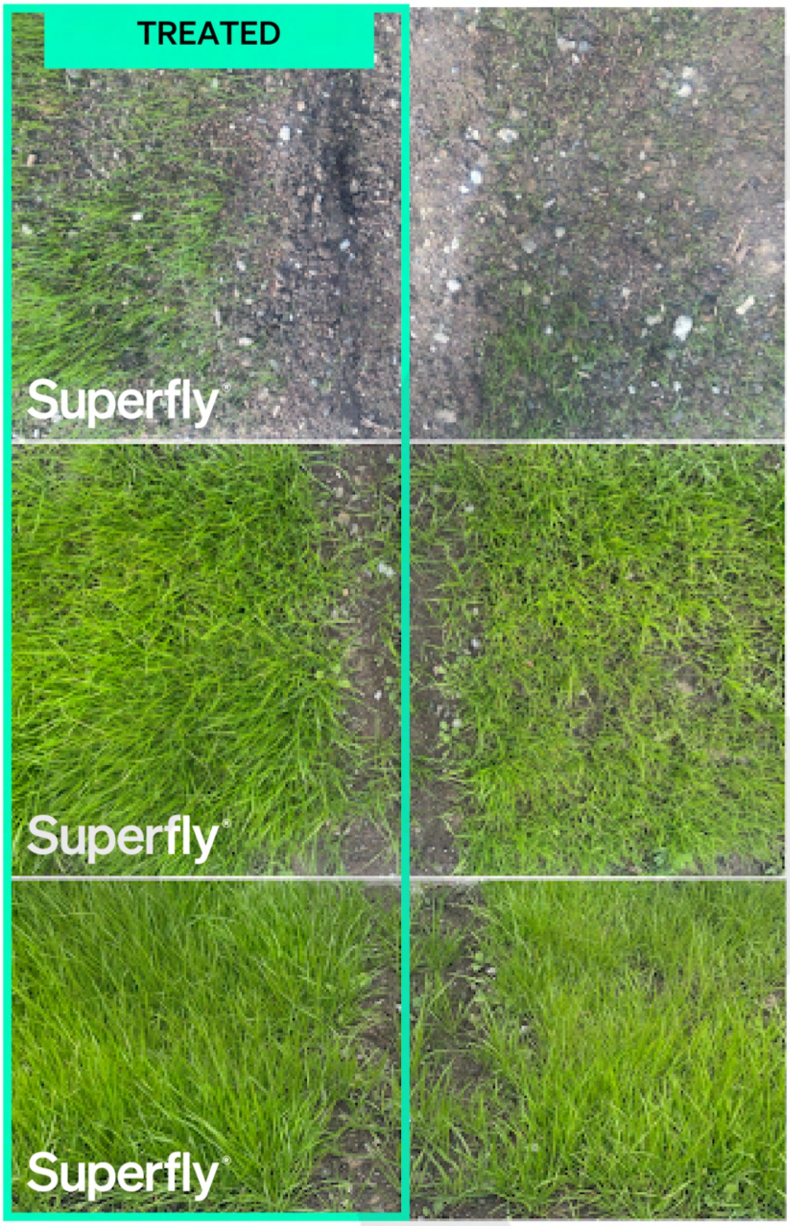

Figure 19

Left column of photos are of the treated area, the right column of photos are of the control area.

Top row of photos was taken on 29/09/21 (day 16): germination rate and speed is noticeably higher in the treated area than the control area.

Middle row and bottom row of photos were all taken on 24/10/22 (day 41): seedling growth rate is visibly greater in the treated area than the control area.

The top and middle rows of photos are taken from above to show germination rate and seedling density differences; the bottom row of photos is taken from a more horizontal angle to show seedling height difference.

Tomato root growth trial

Methods

Tomato seeds (Solanum lycopersicum, Siberian) were germinated in a germination tray for 24 days in germination media. For day 0-10 the tray had a lid cover and was kept at 25 °C and 75-85% humidity. For day 10-24 the tray had the lid removed and was kept in a grow tent at 18-21 °C and 80-90% humidity with grow lights 40cm above plants (18 hours light : 6 hours dark). On day 24 the seedlings were 8cm tall and had their first leaves; the seedlings were transferred to individual 10x10cm pots and had fertiliser added, the seedlings were kept at 20 °C and 80 - 90% humidity with grow lights 60 cm above plants (18 hours light : 6 hours dark) until day 42. Seedlings were watered at day 24 and 35 with 100mL each. Fertiliser treatments at day 24 included Superfly® at 25, 50 and 100 g/sqm, the market leader at 150 g/sqm, and a negative control; there were 5 replicates per treatment. Observations were taken on day 42.

On day 42 (22/09/20), the three seedlings with the highest growth rates for each treatment were selected (a total of 21seedlings), uprooted and were washed in a bucket of cool water until the root mass was mostly cleaned of soil and debris. Seedlings were then photographed in the office studio.

Results
Seedlings treated with Superfly® had longer and denser roots and a higher average growth rate than both the control and market leader (figure 20). All Superfly® treatments had higher growth rates than the control and market leader. Seedlings treated with Superfly® at 100 g/sqm, had an average growth rate of 4.2 mm/day, while the market leader and the control had an average growth rate of 3.1 and 3.6 mm/day (respectively).

Root growth was noticeably greater in all Superfly® treatments when compared to the control and market leader. It is unclear why the market leader performed worse than the control; the packet instructions were strictly followed. Interestingly, despite all Superfly® treatments producing significantly greater root growth, there was not a significant difference in root growth between the Superfly® treatments, with the 25g/sqm Superfly® treatment appearing to have the greatest root growth.

Root growth was noticeably greater in all Superfly® treatments when compared to the control and market leader.

The significant effect of Superfly® on the root development, regardless of application rate, is very likely due to the biostimulant effects of the microbes, humic acid and chitin, which are known to have profound effects even at very low concentrations. Chitin in partilcular increases activity of plant growth promoting microbes, such as B. subtilus, which digest chitin and produce plant hormones such as auxin, which stimulates root growth.

Figure 20

A = Control,
B = Superfly at 25 g/sqm,
C = Superfly at 50 g/sqm,
D = Superfly at 100 g/sqm,
E = the market leader at the suggested application rate (150g/sqm).

E is noticeably darker green, which suggests increased nitrogen uptake, however, it also had significantly more nitrogen applied.

The larger roots in B, C and D (Superfly) will help the plant become more drought tolerant, and be able to uptake more nutrients to support further growth and eventual flowering and fruiting.

Bardee

Golf tee construction trial

Method

Superfly® was used in the construction of the 13th tee at the Riversdale Golf Club (Victoria). The tee construction site was fully drained and capped with 300mm Medium Top Dressing Sand (figure 21). Superfly® was spread with shovels over the area and then York raked through as a soil amendment, the area was then solid turfed in late May with Santa Ana couch grass (figure 21).

Results

Despite the expectation that the turf would be dormant until after winter, the superintendent was surprised that the turf grew roots into the substrate two weeks before winter began. Although not overly scientific, these results corroborate with other trials of Superfly® where there has been an obvious increase in root growth compared to negative controls and when compared to market leading organic fertilisers.

This trend of Supefly promoting root growth is very likely due to the biosimulant effects of Superfly®, which both contains plant growth promoting microbes (figure 5), and also contains insect chitin, which stimulates existing beneficial bacteria, like B. subtilus, which produces the root growth promoting plant hormone, auxin.

Figure 21

The photo on the left is on day 1 (28/05/22 - end of Autumn), after Superfly was York-raked into the sand, but before the turf was laid late that week. The photo on the right is on day 100 (05/09/22 - start of Spring) after the turf was establishing over winter.

Bardee™

"The laying of the couch grass was far too late in the season to really take, our thoughts were that we would let the couch sit through the winter dormancy and hopefully jump out the ground come spring. We were a little surprised to see that Santa Ana still managed to drop some roots down two weeks prior to the commencement of Winter. The area has been left through the winter months though is ready to go once the soil temperatures improve."

Travis Scott
Course Superintendent
Riversdale Golf Club

These comments suggest that Superfly® is either able to stimulate root growth much faster than other fertilisers, or it is able to stimulate root growth at lower soil temperatures.

Buffalo turf root development trial

Method

On 13/09/22 turf (Sir Walter DNA Certified Soft Leaf Buffalo Turf Slab) was laid onto a two 1 sqm areas; the areas were prepared for turf by aerating the low quality compacted heavy clay soil with a garden fork and spreading fertiliser-free garden soil (Garden Basics Soil Mix) and soil wetting agent (Hortico Soil Wetter) as per packet instructions. One of the two areas also had Superfly®spread at a rate of 25g per square metre (treated turf); the other area did not have Superfly® added (control turf). At this stage, both turfed areas looked very similar. Both turfed areas were watered in with equal amounts of tap water and watered each day for the following two weeks with a watering can to keep the turf and topsoil damp; after two weeks the turf was only watered with rain, which was ample for keeping the turf and topsoil damp.

Results

On day 41 (24/10/22) the turf was lifted at the corners and photos were taken of the root development against a steel ruler (figure 22). Root growth in the treated turf was noticeably more abundant and longer; these results are consistent with those other trials (figure 20).

The soil also appeared darker, indicating greater water retention, organic matter and more biological activity; these results are consistent with the Superfly® trial on leek beds at a Mornington Peninsular vegetable farm in figure 1, where soil health was visibly improved.

These results can be explained by Superfly® containing the high levels of organic matter (68.4%) (table 3), an abundance of plant growth promoting microbes (figure 4 and figure 5), and chitin, which stimulates existing plant growth promoting microbes already in the soil.

Figure 22

Image on the left has been treated with Superfly.

The image on the right is untreated. Both photos were taken on 24/10/22, 41 days after the turf was laid on 13/09/22.

Superfly® is a source of plant nutrients

In addition to the beneficial microbes and other plant biostimulants, Superfly® also contains a high level of plant nutrients (table 3). Compared to other organic manure based fertilisers (table 4), Superfly® has significantly greater nitrogen and phosphorus, and has far more calcium and magnesium. Furthermore, Superfly® has a full micronutrient profile, with high levels of boron, zinc, iron, silicon, molybdenum, manganese, and the macronutrient, sulphur.

Superfly® has a neutral pH and a high cation exchange capacity, which combined with the high levels of biodiverse plant growth promoting microbes and biostimulants such as chitin, increases nutrient uptake and increases fertiliser efficiency.

This rich and balanced nutrient profile is achieved because Bardee's BSFL are fed a diverse diet of pre-consumer food waste from a wide range of sources. This makes Superfly® different to other manure-based fertilisers, and even other insect frass products; other manure-based fertilisers are produced by animals that are usually fed very specific, narrow diets with only a few ingredients, such as pasture (cow manure), chicken feed (poultry manure) or spent grain (other insect frass). Given that Superfly® is produced by processing many types of food waste, it is unsurprising that it contains all the nutrients required for healthy plant growth.

The food waste streams used in the production of Superfly® meet Bardee's HACCP certification standards and approved-supplier program. The food waste streams are visibly inspected and any packaging removed before they are processed, scored, labelled, and blended to achieve nutritionally balanced and consistent insect feed recipes to optimise larval and frass quality.

The resulting frass is then sieved and thermophylically composted according to the standards required for Superfly's organic certification (figure 9). The end result is a highly consistent, high quality Superfly® product, in terms of microbiological, physical and chemical properties. Superfly's production methods
allow for traceability back to the food waste streams that contributed to each specific batch of Superfly® product.

Not all insect frass fertilisers are created equal. Researchers reviewing the efficacy of insect frass as an organic fertiliser suggest that some poor plant growth results could be attributable to a lack of stability in the frass[35]. While these poorly performing frass products may contain adequate nutrients, their lack of stability results in plant toxicity and results in poor plant health[36].

While other insect frass products may be regarded as unstable fertiliser products, Superfly® undergoes thermophylic composting, which matures and stabilises the frass by breaking down organic matter and mineralising plant nutrients, making them readily available for plant uptake[37][38] . The effect of composting frass before soil application has been directly tested on Brassica rapa; it was found that uncomposted frass stunted growth, while the same frass when composted improved growth[39]. The thermophilic composting process also produces humic compounds. These substances contribute to several soil fertility parameters by regulating soil acidity, improving the cation exchange capacity, increasing the water holding capacity, improving the uptake of nutrients and stimulating plant growth[40][41][42].

[35] Ivã Guidini Lopes, Jean WH Yong, Cecilia Lalander, Frass derived from black soldier fly larvae treatment of biodegradable wastes. A critical review and future perspectives, Waste Management, Volume 142, 2022, Pages 65-76,
[36] Setti, L., Francia, E., Pulvirenti, A., Gigliano, S., Zaccardelli, M., Pane, C., Caradonia, F.,Bortolini, S., Maistrello, L., Ronga, D., 2019. Use of black soldier fly (Hermetia illucens (L.), Diptera: Stratiomyidae) larvae processing residue in peat-based growing media. Waste. Manage. 95, 278–288.
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[42] Conselvan, G.B., Fuentes, D., Merchant, A., Peggion, C., Francioso, O., Carletti, P., 2018. Effects of humic substances and indole-3-acetic acid on Arabidopsis sugar and amino acid metabolic profile. Plant Soil 426 (1-2), 17–32

Table 3

Independent laboratory analysis results on the plant nutrient analysis of frass. Environmental Analysis Laboratory (EAL), Southern Cross University. Bardee has been regularly analysing Superfly over the last 2 years and the nutrient levels have been highly consistent.

		Sample 1	Guideline
	Product Name:	Loose Frass	
	Product Type:	Fertiliser	
	Manufacturing Site:	BA	AS4454:2012
	Manufactured Date:	4/08/2021	Composted Product
	Application:	--	
	Test Applicable:	CA-PACK-020	
Parameter	**Method Reference**	**M0063/1**	
Moisture Content (%)	**Inhouse S2 (105°C)	25	> 25
pH	Rayment & Lyons 2011 - 4A1 (1:5 Water)	7.52	> 5
Electrical Conductivity (dS/m)	Rayment & Lyons 2011 - 3A1 (1:5 Water)	8.12	< 10
Total Carbon (%)	Inhouse S4a (LECO Trumac Analyser)	40.3	≥ 20
Total Nitrogen (%)		3.45	≥ 0.8
Carbon/Nitrogen Ratio	**Calculation - Total Carbon/Total Nitrogen	12	--
Estimated Organic Matter (% OM)	**Calculation - Total Carbon x 1.7	68.4	--
Total Calcium (%)	Rayment & Lyons 2011 - 17C1 Aqua Regia	8.22	--
Total Magnesium (%)		0.71	--
Total Potassium (%)		2.04	--
Total Sodium (%)		0.52	< 1 Na
Total Sulphur (%)		0.42	--
Total Phosphorus (%)	Rayment & Lyons 2011 - 17C1 Aqua Regia	1.83	≤ 0.1 P
Total Zinc (mg/kg)	Rayment & Lyons 2011 - 17C1 Aqua Regia	320	< 300 Zn
Total Manganese (mg/kg)		425	--
Total Iron (mg/kg)		1,580	--
Total Copper (mg/kg)		45.2	< 150 Cu
Total Boron (mg/kg)		14.6	< 100 B
Total Silicon (mg/kg)		688	--
Total Aluminium (mg/kg)		824	--
Total Molybdenum (mg/kg)	Rayment & Lyons 2011 - 17C1 Aqua Regia	1.94	--
Total Cobalt (mg/kg)		1.19	--
Total Selenium (mg/kg)		<1	< 5 Se
Nitrogen/Sulphur Ratio	**Calculation - Total Nitrogen/Total Sulfur	8.2	--
Nitrogen/Phosphorus Ratio	**Calculation - Total Nitrogen/Total Phosphorus	1.9	--
Nitrogen/Potassium Ratio	**Calculation - Total Nitrogen/Total Potassium	1.7	--

Soluble Calcium (mg/kg)	**Inhouse S10 - Morgan 1	3,645
Soluble Magnesium (mg/kg)		2,737
Soluble Potassium (mg/kg)		13,328
Soluble Phosphorus (mg/kg)		3,627
Phosphorus (mg/kg P)	**Rayment & Lyons 2011 - 9E2 (Bray 1)	557
	**Rayment & Lyons 2011 - 9B2 (Colwell)	5,314
	**Inhouse S3A (Bray 2)	3,896
Nitrate Nitrogen (mg/kg N)	**Inhouse S37 (KCl)	11.2
Ammonium Nitrogen (mg/kg N)		2,030
Sulfur (mg/kg S)		1,609
pH	Rayment & Lyons 2011 - 4A1 (1:5 Water)	7.52
Electrical Conductivity (dS/m)	Rayment & Lyons 2011 - 3A1 (1:5 Water)	8.115
Estimated Organic Matter (% OM)	**Calculation - Total Carbon x 1.7	68.4
Exchangeable Calcium (cmol$_+$/kg)	Rayment & Lyons 2011 - 15D3 (Ammonium Acetate)	19.8
Exchangeable Calcium (kg/ha)		8,907
Exchangeable Calcium (mg/kg)		3,977
Exchangeable Magnesium (cmol$_+$/kg)		5.30
Exchangeable Magnesium (kg/ha)		1,443
Exchangeable Magnesium (mg/kg)		644
Exchangeable Potassium (cmol$_+$/kg)		10.0
Exchangeable Potassium (kg/ha)		8,763
Exchangeable Potassium (mg/kg)		3,912
Exchangeable Sodium (cmol$_+$/kg)		4.53
Exchangeable Sodium (kg/ha)		2,333
Exchangeable Sodium (mg/kg)		1,041
Exchangeable Aluminium (cmol$_+$/kg)	**Inhouse S37 (KCl)	0.11
Exchangeable Aluminium (kg/ha)		22.1
Exchangeable Aluminium (mg/kg)		9.86
Exchangeable Hydrogen (cmol$_+$/kg)	**Rayment & Lyons 2011 - 15G1 (Acidity Titration)	<0.01
Exchangeable Hydrogen (kg/ha)		<1
Exchangeable Hydrogen (mg/kg)		<1
Effective Cation Exchange Capacity (ECEC) (cmol$_+$/kg)	**Calculation - Sum of Ca,Mg,K,Na,Al,H (cmol$_+$/kg)	39.79
Calcium (%)	**Base Saturation Calculations - Cation cmol$_+$/kg / ECEC x 100	49.9
Magnesium (%)		13.3
Potassium (%)		25.1
Sodium - ESP (%)		11.4
Aluminium (%)		0.28
Hydrogen		0.00
Calcium/Magnesium Ratio	**Calculation - Calcium / Magnesium (cmol$_+$/kg)	3.7

Zinc (mg/kg)	Rayment & Lyons 2011 - 12A1 (DTPA)	119
Manganese (mg/kg)		82.1
Iron (mg/kg)		126
Copper (mg/kg)		15.6
Boron (mg/kg)	**Rayment & Lyons 2011 - 12C2 (Hot $CaCl_2$)	9.51
Silicon (mg/kg Si)	**Inhouse S11 (Hot CaCl2)	161
Total Carbon (%)	Inhouse S4a (LECO Trumac Analyser)	40.3
Total Nitrogen (%)		3.45
Carbon/Nitrogen Ratio	**Calculation - Total Carbon/Total Nitrogen	12

Table 4

Analysis of organic manures. Taken from Different organic manure sources and NPK fertilizer on soil chemical properties, growth, yield and quality of okra[43].

Manure	Organic C (%)	N (%)	C: N	P (%)	K (%)	Ca (%)	Mg (%)
Rabbit manure	30.1a	1.01e	29.8a	0.54b	1.95d	1.15d	0.40c
Cow dung	26.5b	1.86d	14.24b	0.82a	2.11c	1.01d	0.51b
Poultry manure	17.8e	2.91a	6.12e	0.84a	3.79a	3.34a	0.64a
Green manure	23.6c	2.51b	9.40d	0.52b	3.04b	3.01b	0.10d
Pig manure	20.1d	2.16c	9.77c	0.80a	2.16c	1.45c	0.54b

[43] Adekiya, A.O., Ejue, W.S., Olayanju, A. et al. Different organic manure sources and NPK fertilizer on soil chemical properties, growth, yield and quality of okra. Sci Rep 10, 16083 (2020).

Superfly®
Boosts natural defences

Bardee

> “Considering the beneficial effects of chitin against fungal pathogens and other pests, and the presence of chitin in the frass from BSF composting, it is likely that this product displays beneficial effects in the soil–plant system, in relation to pathogen control.”

Lopes et al 2020
Frass Derived From Black Soldier Fly Larvae Treatment of Biodegradable Wastes. A Critical Review and Future Perspectives

Superfly® is a unique fertiliser and soil amendment product in that it contains insect chitin (a structural molecule in insect exoskeletons) and chitosan (a kind of molecule produced from enzymatic or alkaline reaction with chitin). These molecules are in Superfly® as a consequence of the BSFL shedding their exoskeleton as they consume food waste and grow, and the microbes in Superfly® digesting the chitin, converting some of it into chitosan. It has been shown in scientific literature that both chitin and chitosan can provide natural protection against agricultural pests by either affecting soil biology, or affecting the plants directly[44].

[44] Sharp, Russell G. 2013. "A Review of the Applications of Chitin and Its Derivatives in Agriculture to Modify Plant-Microbial Interactions and Improve Crop Yields" Agronomy 3, no. 4: 757-793. https://doi.org/10.3390/agronomy3040757

“Part of the effect observed by chitosan on the reduction of soilborne pathogens comes from the fact that it enhances plant defence responses. The other part is linked to the fact that this biopolymer is composed of polysaccharides that stimulate the activity of beneficial microorganisms in the soil such as Bacillus, fluorescent Pseudomonas, actinomycetes, mycorrhiza and rhizobacteria. This alters the microbial equilibrium in the rhizosphere disadvantaging plant pathogens. Beneficial organisms, on the other hand, are able to outcompete them through mechanisms such as parasitism, antibiosis, and induced resistance[45].”

Hadrami et. al. 2010
Chitosan in Plant Protection

[45] El Hadrami A, Adam LR, El Hadrami I, Daayf F. Chitosan in plant protection. Mar Drugs. 2010 Mar 30;8(4):968-87. doi: 10.3390/md8040968. PMID: 20479963; PMCID: PMC2866471

Many plants are able to detect the presence of very low concentrations of chitin in their environment and activate defence mechanisms to protect themselves from insects, fungi, nematodes[46]. This plant response to chitin can be successfully triggered by chitin-based treatments which mimic the compounds that a plant would normally respond to when being attacked by chitin-containing organisms[47]. Plant cell membranes contain chitin-specific receptors, which are known to activate defence mechanisms[48].

Once a plant has detected chitin, it has been found that their defence mechanisms can include the production of various molecules like phytoalexins [49], phenolics[50], chitinase[51] and terpenes[52], which act as toxins to the attacking organism by puncturing cells, disrupting metabolism or preventing reproduction of the pathogen. Overall, chitin and chitosan reduce the negative impact of diseases on yield and quality of crops.

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[47] Sharp, Russell G. 2013. "A Review of the Applications of Chitin and Its Derivatives in Agriculture to Modify Plant-Microbial Interactions and Improve Crop Yields" Agronomy 3, no. 4: 757-793. https://doi.org/10.3390/agronomy3040757

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Fungus

Direct control

Studies have repeatedly shown that chitosan can directly control fungal diseases in multiple crops, particularly fusarium[53], (which infects tomato, tobacco, legumes, cucurbits, cotton, sweet potato and banana plants), and grey mould[54][55], (which infects many fruits and vegetables such as wine grapes and broccoli, and can also infect broadacre crops such as lentils). Microscope analysis shows that chitosan disrupts pathogenic fungal development[56]. Significant reductions in fusarium were observed in treatments of chitosan below 1 mg/ml[57].

Indirect control

Chitin can also help control pathogenic fungi by activating the plant's natural defence mechanisms. Many plants have physical structures in their cells that are able to detect the presence of pathogenic fungi from the chitin in the fungi cell walls. Once the plant detects the chitin it stimulates specific biochemical pathways within the plant that activate various plant defences against fungal pathogen infection. Some fungi, however, are able block the host plant's ability to detect the chitin, preventing the plant from defending itself from the fungus[58]. Therefore, by applying chitin directly to the soil with Superfly®, plants may be able to respond to the presence of chitin in Superfly® before pathogenic fungi can mask their own presence, facilitating the activation of the plant's fungus defence mechanisms and minimising damage to the plant.

[53] Rabea, E.I.; El Badawy, M.T.; Stevens, C.V.; Smagghe, G.; Steurbaut, W. Chitosan as antimicrobial agent: Applications and mode of action. Biomacromolecules 2003, 4, 1457–1465.
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Additionally, chitin can feed and stimulate chitinase production in beneficial chitin-eating fungal and bacterial species, such as those in the fungi genera Trichoderma[59]. Trichoderma species are of particular interest as they have been shown to be highly effective at controlling many kinds of pathogenic fungi, including those that cause crown rot and fairway patch [60][61][62]. Trichoderma is commercially available as a biopesticide in a number of products[63], however, Superfly® may be a more cost-effective way of being able to obtain the benefits of this genera since Superfly® could grow the Trichoderma population already growing within the soil, as well as potentially inoculate the soil with new Trichoderma species.

Another widely used biopesticide in agriculture is Bacillus subtilis[64], which secretes chitinases into its environment[65]. It has been shown that adding chitin to a B. subtilis environment improved its growth rate and fungicidal control against Fusarium wilt in pigeon pea and crown rot in peanuts caused by Aspergillus niger[66], and improved its effectiveness against powdery mildew in strawberry[67].

[59] Sivan, A.; Chet, I. Degradation of fungal cell walls by lytic enzymes of Trichoderma haryianam. J. Gen. Microbiol. 1989, 135, 675–682.
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Insects

While it has been shown that at high enough concentrations, chitosan can be directly toxic to insects[68], chitin and chitosan can be effective at protecting plants from insect damage at much lower concentrations through indirect pathways. As plants and insects co-evolved over millennia, many complex interactions developed, beyond those involving flowers and pollinators.

Once plants detect chitin with highly specialised sub-cellular structures, many species have been shown to produce chitinase, an enzyme that digests chitin[69]. This means that when insects eat these plants, the insects will be consuming enzymes that digest their internal membranes, damaging the insect in return.

Furthermore, chitin has been shown to elicit a lignification response in many plants, including wheat[70]; this process reinforces cell walls and makes it more difficult for insects, particularly those with small mouthparts to penetrate the plant's outer cells, such as aphids, thrips and scale. This means that Superfly® can be applied to the soil to stimulate plant defence mechanisms so that the plant is prepared for an insect attack, thereby reducing revenue loss associated with damaged crop yield and quality and reducing operating costs associated with spraying insecticides.

Chitin and chitinase have also been shown to amplify the effect of other popular biological means of controlling pest insects. The bacterium Bacillus thurigensis is the most widely used biopesticide worldwide[71]. B. thuringiensis produces the insecticidal Cry-protein toxin that activates in the alkaline conditions of the guts of the insects that consume it. Not only can chitin be used as a food source for B. thuringiensis[72], but chitinase has been shown to increase the penetration of B. thurigensis and improve its lethality against crop pest insects[73].

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Purchasing commercial enzymes such as chitinase is relatively expensive, however, if Superfly® is applied, it could activate chitinase-producing biochemical pathways within the plant; if this plant is also treated with B. thurigensis then it is possible to achieve an economically efficient means of amplifying the effect of the insecticidal Cry-protein.

Nematodes

There are many studies showing that chitin is an effective means at controlling pathogenic nematode species, such as peanut root knot nematode (Meloidogyne arenaria)[74] [75] and the soybean cyst nematode (Heterodera glycines) [76]. Chitin is a key component in the nematode eggshells[77].

When chitin-rich material, such as Superfly®, is added to the soil it feeds organisms that are able to digest chitin, such as Flavobacterium and Pseudomonas and increases the population size of these chitinolytic microorganisms. These bacteria are not only important for product plant growth promoting compounds and cycling nutrients, but also help control pathogenic nematodes by feeding on the chitin in nematode eggshells[78][79][80].

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Bacteria

Direct actions

Superfly® may have a means of directly controlling pathogenic bacteria populations. Research has shown that extract made from BSFL was effective at controlling all 6 strains of plant-pathogenic bacteria tested. The extract was made from ground whole BSFL and was found to be effective in concentrations as low as 50 mg/mL[81]. This may not be surprising, considering that BSFL are very often the dominant species in their micro microenvironment, outcompeting all other species, whether they contain chitin or otherwise. It has been shown that BSFL are very capable at controlling the bacterial species in their environment by producing and secreting potent antibacterial peptides into their surroundings[82][83]. Given the findings of the above research, it is probable that Superfly® would also contain such antibacterial substances and is capable of directly controlling some plant pathogen bacteria.

Indirect action

Superfly® can also help to control plant pathogen soilborne bacteria by generally increasing microbial biodiversity within the soil. Superfly® is produced via an insects digestive system and so will contain microbial species that are not common in agricultural soils that do not have high levels of insect activity. When Superfly® is applied to soils, the increased biodiversity and biological soil activity (see figure 1) will make it more difficult for soilborne plant pathogen bacteria to thrive as they would be competing against many other species for the resources they require to grow to population levels that would cause significant crop damage.

[81] How to cite this article:Kwan Ho Park, Kyu Won Kwak, Sung Hee Nam, Ji Young Choi, Seok Hyun Lee, Hong Geun Kim, Seong Hyun Kim. Antibacterial activity of larval extract from the black soldier fly Hermetia illucens (Diptera: Stratiomyidae) against plant pathogens. J Entomol Zool Stud 2015;3(5):176-179.
[82] Qiaolin Liu, Jeffery K. Tomberlin, Jeff A. Brady, Michelle R. Sanford, Ziniu Yu, Black Soldier Fly (Diptera: Stratiomyidae) Larvae Reduce Escherichia coli in Dairy Manure, Environmental Entomology, Volume 37, Issue 6, 1 December 2008, Pages 1525–1530
[83] Park, S.-I., Chang, B.S. and Yoe, S.M. (2014), Antimicrobial activity of H. illucens. Entomological Research, 44: 58-64.

Wound healing

Chitin detection has also been found to activate plant biochemical pathways leading to the formation of multiple physical barriers to protect against pest and pathogen attack. These defences includes the deposition of callose[84], which acts like a temporary cell wall where a plant is physically damaged, and lignin[85], which accumulates at plant wound sites, creating a waterproof barrier that confers plant protection by preventing pathogen infiltration, making the plant cell less accessible to cell wall degradation. These physical barriers protect the plant from insect attack by making it harder for insects to penetrate plant tissues and accelerate the sealing of wounds to help prevent secondary microbial infections starting or spreading in the plant.

Viruses

Chitosan has been shown to be effective at improving plant defences against viruses. It is thought that viral infection is disrupted by chitosan, inducing a resistance to plant viruses [86] . Chitosan is unlikely to be directly toxic to viruses and it is supposed that chitosan modifies the plant's response to viruses and disrupts the ability for viruses to enter plant cells[87][88].

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[88]Chirkov, S.N. The antiviral activity of chitosan (review). Appl. Biochem. Microbiol. 2002, 38, 1–8.

Superfly® Increases Water Holding Capacity

Bardee

Superfly® is able to significantly increase the water holding capacity of soil through both direct and indirect means. Being very high in both carbon (40.3%) and organic matter (68.4%) (table 3), Superfly® applications will have an immediate and direct improvement on soil water holding capacity, as seen in figure 1. Additionally, Superfly® is able to indirectly increase soil water holding capacity by inoculating soil with new soil microbes and stimulating existing soil microbes. This increase microbial soil activity will help decompose organic matter, such as crop residues and build up soil organic matter and soil carbon

In addition to increasing soil water holding capacity, Superfly® may also be able to significantly increase water use efficiency due to the chitin it contains. A published academic study on lettuce showed that when is applied to the soil at concentrations of 2g/kg it profoundly reduces the amount of water required over a 4 week growing period from 715 mL to 560 mL (21.67% reduction in water per plant), while increase lettuce leaf area from 480 cm2 to 877 cm2 (82.70% increase in yield per plant)[89]. This resulted in a water use efficiency improvement from 29.41 L of water per kg of lettuce shoots, to 13.69 L of water per kg of lettuce shoots (53.45% reduction in water required per kg of yield). Additionally, chitin-treated lettuces had better looking leaves with visually less leaf chlorosis (yellow leaves from insufficient chlorophyll); this could also be due to chitin's documented ability to improve chloroplast development[90]. It is highly likely that chitin could have had a significant effect on the lettuce growth at a much lower concentration than 2g/kg, since this was the lowest concentration tested and yet had the most significant effect on both water use efficiency and yield.

"Chitin improves plant performance against water supply limitations"

Lin et. al. 2020
Effects of Betaine and Chitin on Water Use Efficiency in Lettuce (Lactuca sativa var. capitata)

[89] Lin, F., Lin, K., Wu, C., Chang, Y., Lin, K., & Wu, C. (2020). Effects of Betaine and Chitin on Water Use Efficiency in Lettuce (Lactuca sativa var. capitata), HortScience horts, 55(1), 89-95.
[90] Patchra Limpanavech, Subhalai Chaiyasuta, Ranitha Vongpromek, Rath Pichyangkura, Chumpol Khunwasi, Supachitra Chadchawan, Pongtharin Lotrakul, Reungwit Bunjongrat, Anchalee Chaidee, Thapana Bangyeekhun, Chitosan effects on floral production, gene expression, and anatomical changes in the Dendrobium orchid, Scientia Horticulturae, Volume 116, Issue 1, 2008, Pages 65-72

Superfly®
Suitable for all soil types

Superfly® is a general-purpose fertiliser and soil amendment with many positive traits that make it suitable for all soil types and crops.

Slow release organic NPK
Good carbon:nitrogen for some immediate mineralisation followed by continued slow release of nitrogen. Longterm nitrogen release from Superfly® could be supplemented by improved soil microbial activity increasing the availability existing previously inaccessible nitrogen in the soil though increased nutrient cycling, or from fixing atmospheric nitrogen via increased legume root modulation[91] or higher populations of free-living nitrogen-fixing microbes (e.g. actinomycetes)[92][93].

Micronutrient profile
Has a far more diverse micronutrient profile than synthetic fertilisers, which typically only focus on NPK, and will be more balanced than other animal manure fertilisers which are usually the results of animals being fed a very narrow range of dietary ingredients. Micronutrient deficits can be as important as deficits in NPK, and unless these micronutrients are being replaced in the soil over time they will become a limiting factor and crop yields will fall short of their potential. Applying Superfly® to soil will address most micronutrient deficits and help prevent them from occurring into the future.

Good Cation Exchange Capacity (CEC)
Being high in organic matter (68.4%), Superfly® unsurprisingly has a high CEC (39.79 cmol/kg). This means that Superfly® helps the soil store more cations (like potassium, magnesium, and calcium, which Superfly® also supplies in its nutrient profile) and helps crops actually uptake these cations, increasing the overall fertility of the soil. Over time, the microbes and microbial stimulants in Superfly® will aid in the creation of more organic matter and humus, further increasing CEC.

[91] Staehelin, C.; Schultze, M.; Tokuyasu, K.; Poinsot, V.; Promé, J.C.; Kondorosi, E.; Kondorosi, A. N-deacetylation of Sinorhizobium meliloti Nod factors increases their stability in the Medicago sativa rhizosphere and decreases their biological activity. Mol. Plant-Microbe Interact. 2000, 13, 72–79.
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[93] El Hadrami, Abdel & Adam, Lorne & Hadrami, Ismail & Daayf, Fouad. (2010). Chitosan in Plant Protection. Marine drugs. 8. 968-87. 10.3390/md8040968.

Reduces risk of salinity

Superfly® has a low salt concentration, especially compared other organic manures, like poultry manure, which has an average EC of 13.8 dS/m[94], compared to Superly's 8.3 dS/m (table 3). Additionally, the organic matter in Superfly® is able to buffer the effects of salinity on soil fertility.

Mitigates erosion damage

The added organic matter, microbes, and microbe stimulants in Superfly® will help stabilise erosion-prone soils. Organic matter and soil microbes bind soil particles into larger, interlinked aggregates with pores in between aggregates; this improves soil structure, and allows water to infiltrate into the soil, reducing the amount of free flowing surface water. Improving soil structure with Superfly® will be particularly effective at mitigating damage caused by many different kinds of erosion, including: sheet, rill, gully, water, scalding, and wind erosion.

[94] Al-Turki, Ahmad & El-Hadidy, Yasser & Alromian, Fahad. (2013). ASSESSMENT OF CHEMICAL PROPERTIES OF LOCALLY COMPOSTS PRODUCED IN SAUDI ARABIA COMPOSTS LOCALLY PRODUCED. International Journal of Current Research. 5.

Superfly® prevents weed growth

Ammonium nitrogen source to control weeds

Although both nitrate and ammonium are both rich sources of plant-available nitrogen, these different forms of nitrogen favour different kinds of plants. Generally, weeds thrive on nitrates, whereas most other plants require higher levels of ammonium[95]. As shown in table 3, Superfly® contains 181 times more ammonium than nitrate. The reason there is so much more ammonium than nitrate in Superfly® is due to the specific microbes it contains, which likely prevent nitrifying bacteria from converting ammonium into nitrate. The effects of Superfly® preventing weed growth while favouring desired plant growth can be seen in figure 24 and figure 25 below.

Figure 24

The effect of Superfly at facilitating desirable pasture species to outcompete weed species.

Left photograph = an area of untreated permanent pasture.

Right photograph = an area of permanent pasture that has been treated with Superfly.

Both photographs are taken on the same day in the same paddock, 90 days after Superfly was spread on the area in the right photo.

These images are taken from the pasture trial discussed earlier in the paper.

[95] Ingham, E. (2019) Weed Suppression Using the Soil Food Web | Soil Food Web School, Dr. Elaine's Soil Food Web School, https://www.youtube.com/watch?v=pRIXUdhGptU&t=108s

Figure 25

The impact on weed development from areas treated with different fertilisers.

Left photograph = an area treated with Superfly.

Right photograph = an area treated with industry leading specialist lawn fertiliser.

This photograph was taken only a couple of weeks after fertiliser application and so the effect on the turf growth was not yet apparent, however, there was a significant difference between treatments regarding the amount of weeds that had developed in that short time.

Weed development in the Superfly-treated area are far fewer and significantly less developed. This is likely due to the Superly very strongly favouring the production of ammonium, compared to nitrates, with the latter generally being the favoured nitrogen source for weeds.

Superfly® is effective in conjunction with synthetic fertilisers

Despite Superfly® being organically certified, it works very well in conjunction with synthetic fertilisers on non-organic farming systems (see the kale trial below).

Kale trial with Superfly® and synthetic fertilisers

Method

Superfly® was applied in late May 2021 to a 40 sqm bed of kale seedlings in a commercial farm near Bairnsdale, Victoria, at a rate of 375g per square metre. These kale seedlings were also treated with the regular synthetic NPK fertiliser formulated for the farm. Kale beds on either side had the same kale seedlings, and were treated with the same amount of NPK fertiliser, but were not treated with Superfly®. Synthetic pesticides were used during this trial according to normal farming operations.

Results

Quantitative measurements were not taken for this trial, however, the effect of Superfly® on kale growth rate was visibly obvious after 38 days (Figure 26). The kale grower commented that the treated bed was "more lush than the beds either side"

Similar results have also been observed in published scientific literature in trials on tomatoes, kale and French beans. This study tested NPK fertiliser, composted BSFL frass, and other commercial organic fertiliser, by themselves, and in combination. Generally, it was found that a combination of composted frass and NPK fertilisers delivered the best results in terms of growth, crop yield, N uptake and nutrient use efficiency, in comparison to unfertilized soil and soil fertilised exclusively with frass. However, the sole application of composted BSF larvae frass also resulted in positive results for the three species tested[96]. These outcomes are also consistent with the maize trial discussed earlier in this paper, where it was shown that BSFL frass significantly improved nitrogen use efficiency and overall growth.

[96] Anyega, A.O., Korir, N.K., Beesigamukama, D., Changeh, G.J., Nkoba, K., Subramanian, S., Van Loon, J.J.A., Dicke, M., Tanga, C.M., 2021. Black soldier fly-composted organic fertilizer enhances growth, yield, and nutrient quality of three key vegetable crops in Sub-Saharan Africa. Front. Plant Sci. 12, 680312.

The successful results of applying Superfly® in conjunction with synthetic fertilisers is most likely explained by a combination of Superfly's qualities working synergistically with the supply of immediately available inorganic NPK from the synthetic fertiliser. The microbial diversity and abundance in Superfly® could help cycle the NPK nutrients so that they are released more slowly to the plant as the plant required; in the meantime, these nutrients are stored in the soil in less soluble, less volatile organic molecules; minimising fertiliser losses to the environment via runoff and evaporation. The high CEC from Superfly® could also help more nutrients be stored in the soil, further minimising NPK losses to the environment. Additionally, the mycorrhizae in Superfly® could help the plant access more nutrients at any point in time. Finally, the plant growth promoting effects of chitin and Superfly® microbes could help develop root development and vegetative growth, as shown in other Superfly® field trials and academic studies discussed above.

Figure 26

The effect of Superfly on kale growth rate in a non-organically certified farm operation.

Middle bed is treated with Superfly (in addition to NPK fertiliser and synthetic pesticides).

The left and beds are treated with NPK fertiliser and synthetic pesticides, but are not treated with Superfly.

Carbon credits

In a landmark decision by the Australian Government Clean Energy Regulator, bardee was the first insect technology company in the world approved for a carbon credit project. The project avoids up to 50,000 tonnes of CO_2e emission per annum by diverting food waste from landfills.

Farmers using Superfly® fertiliser and soil enhancer are also able to claim credits for carbon sequestration and biodiversity.

Australian Government
Clean Energy Regulator

EMISSIONS REDUCTION FUND

Declaration of an Emissions Reduction Fund project

I, Anjuka Jayasooriya, delegate of the Clean Energy Regulator, declare under subsection 27(2) of *the Carbon Credits (Carbon Farming Initiative) Act 2011* (the Act) that Bardee (the project) is a registered project for the purposes of the Act.

The participant for the project is Beyond Ag Pty Ltd, with ABN 34 633 351 452

The crediting period for the project is 7 years, commencing on 4 October 2022.

Anjuka Jayasooriya
Manager (A/g)
Waste and Energy Assessment Section
Emissions Reduction Fund Branch

4 October 2022

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Superfly® Trials, Lab Analyses & Literature Review

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