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BIOLOGICAL SYSTEMS

Advances in our understanding of the disease biocontrol potential & enhancement of *Trichoderma harzianum* strain T22 (Trianum®)

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Advances in our understanding of the disease biocontrol potential & enhancement of *Trichoderma harzianum* strain T22 (Trianum®)

My primary aims were to:

Evaluate new solutions to enhance the disease biocontrol activities of *Trichoderma harzianum* T22
Assess the potential of the biostimulant Vidi Parva and the pelleted product Vidi Funda as growth promotors of T22
Re-evaluate the growth stimulatory effects of T22 in *Tagetes patula*
Evaluate the performance of T22 in HONS propagation and production trials
Review the modes of action of *Trichoderma harzianum* T22

TRICHODERMA HARZIANUM (T22) MYCOLOGY

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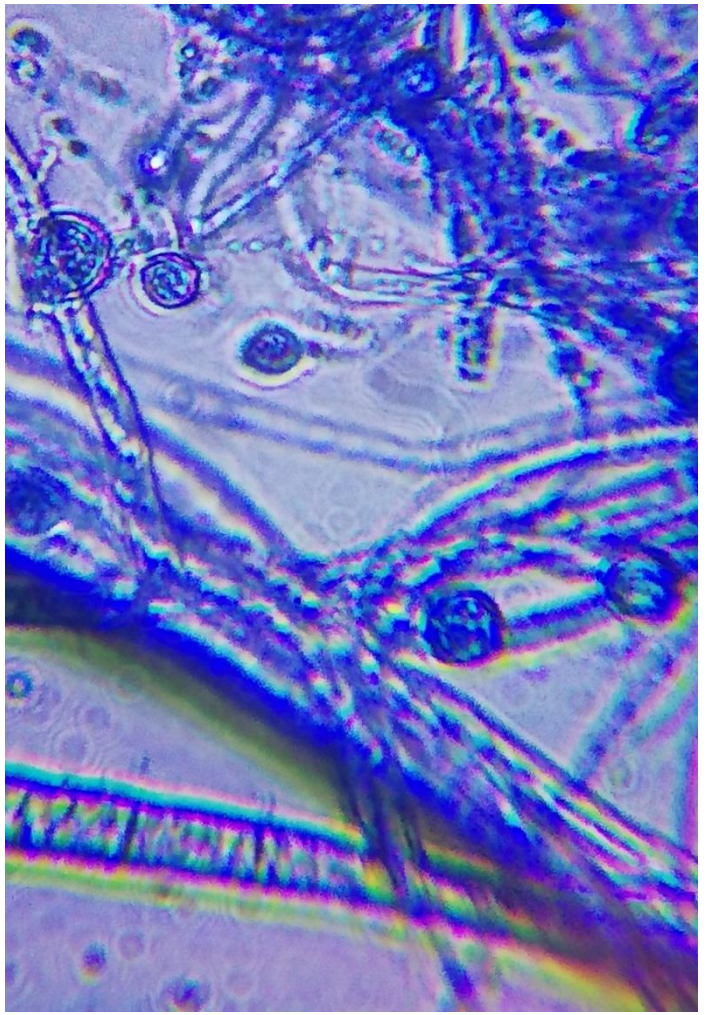
Mycelium

Conidiospores

Chlamydospores



TRIANUM-P (& M149)

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Powdered formulation

Pack size 500g

Diluted in water

USING TRIANUM TO CREATE A DISEASE-SUPPRESSIVE SUBSTRATE

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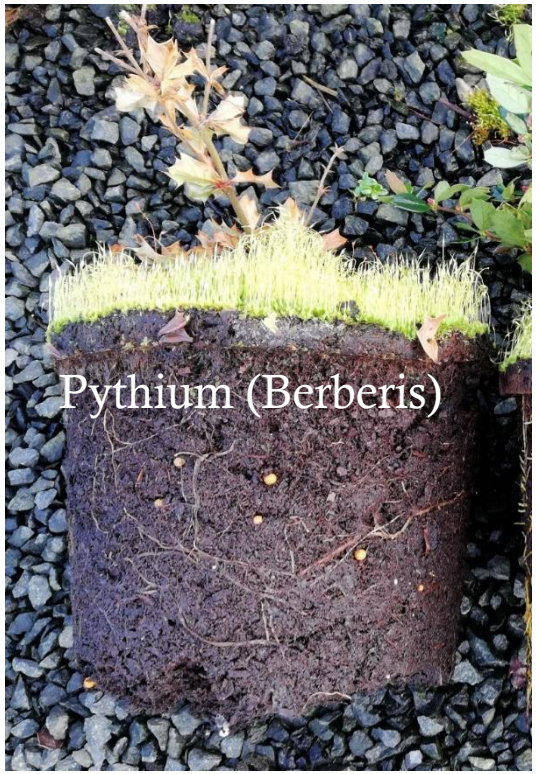
- The label states that T22 should be used preventatively
- Susceptible pathogens listed on-label are Rhizoctonia, Fusarium & Pythium



T22 may also have an effect against other soil-borne plant pathogens including:

Cylindrocarpon

Phytophthora

Armillaria

Penicillium

Sclerotinia

Thielaviopsis

T22 PRODUCTION – LIQUID CULTURE

Stainless steel fermenters

Fermenter biomass

Quality control

MODES OF ACTION

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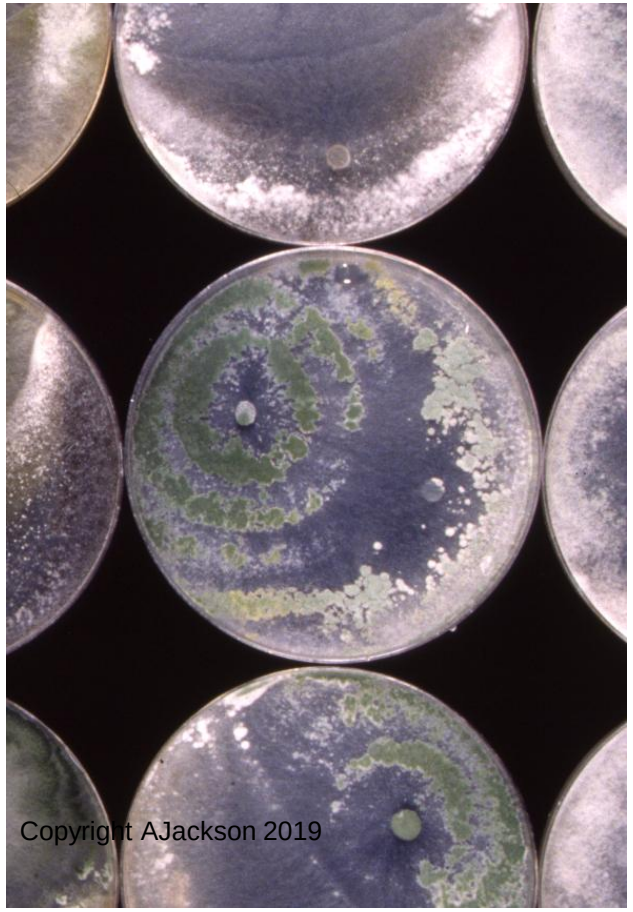
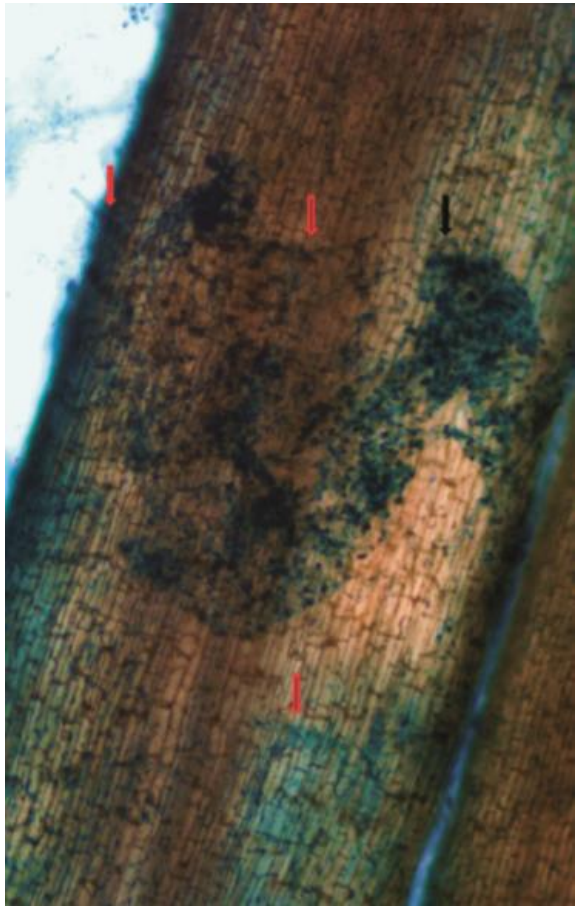
Competition (Jackson, 1990)



Rhizosphere competence (Sofo *et al*, 2010)



Secondary metabolite production

Hydroxy methyl anthraquinone

Di hydroxy methyl anthraquinone

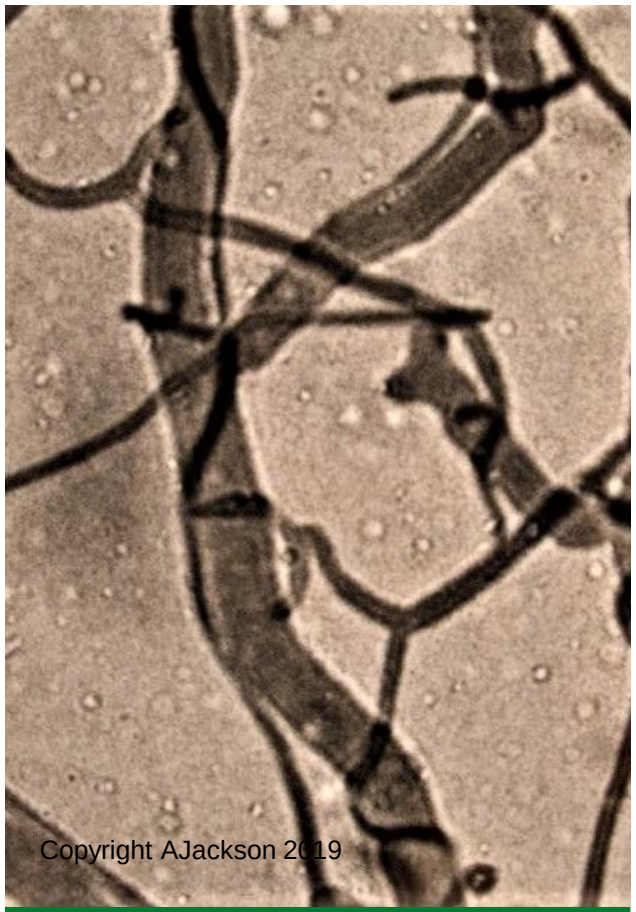
T22 azaphilone

Harzianolide

harzianopyridone

MODES OF ACTION (JACKSON, 1990)

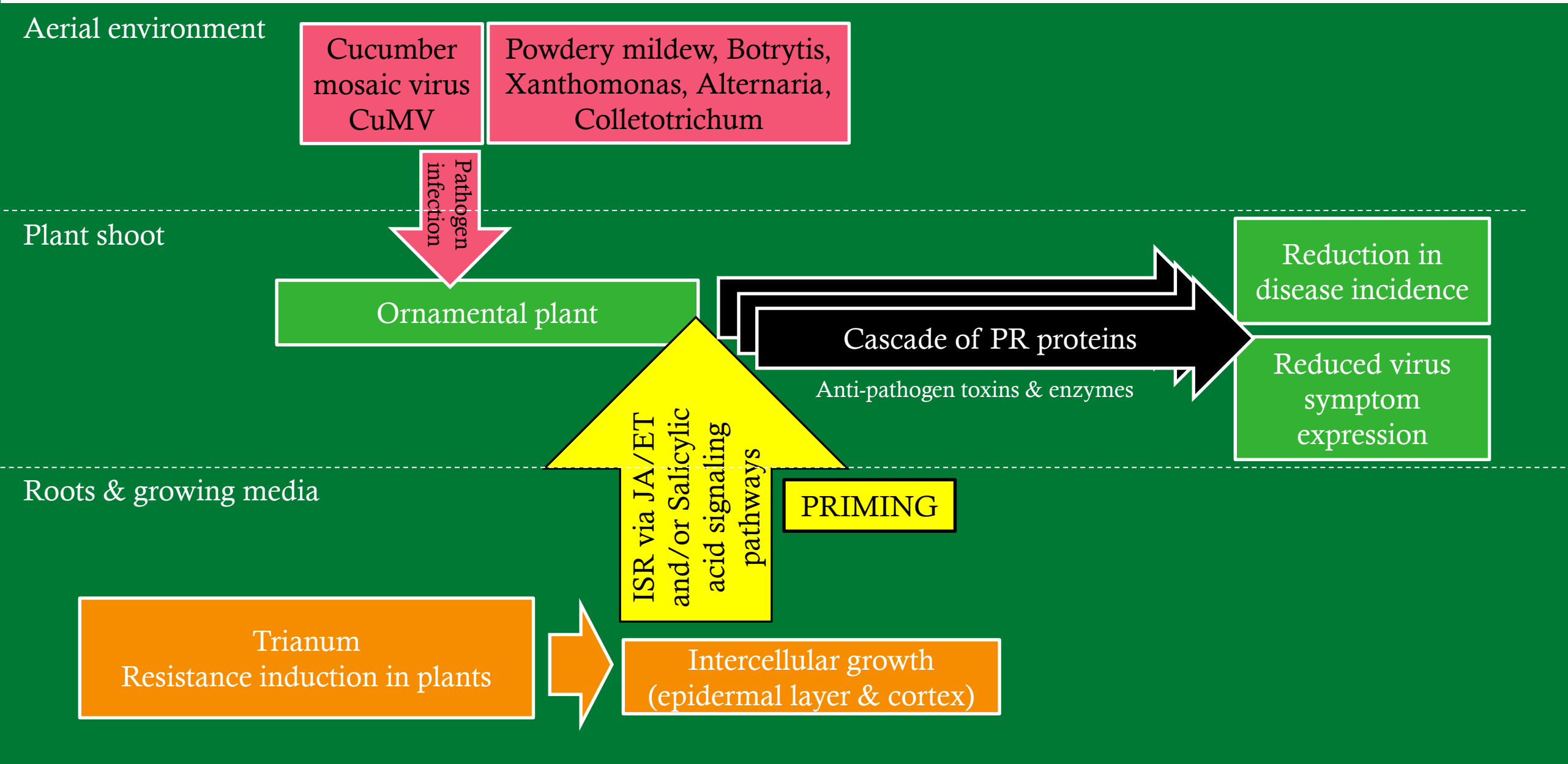
Granulosis



Branched & directed growth

Mycoparasitism

TRIANUM (T22) RESISTANCE INDUCTION IN PLANTS (MODE OF ACTION)



VIDI PARVA & T22 GROWTH EXPERIMENT

Experiment to determine if the plant biostimulant Vidi Parva can benefit the growth of T22 (in static liquid culture)

<u>Vidi Parva is a 100% natural plant based biostimulant</u>

80% seaweed extract, 20% amino acids

Cold compressed seaweed extract from *Ascophyllum nodosum*

High % L-tryptophan, precursor amino acid in the production of auxins

Commercial propagation rate: 1ml/m^2 in 2 litres of water (0.05%)

VIDI PARVA & T22 GROWTH EXPERIMENT

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Experiment to determine if the plant biostimulant Vidi Parva can benefit the growth of T22 (in static liquid culture)

A static culture experiment was repeated on three separate occasions

Test dilutions (% v/v) : 5, 0.05, $5x10^{-4}$, $4x10^{-6}$, $5x10^{-8}$, $5x10^{-10}$, $5x10^{-12}$

0.05g Trianum-P was added to each bottle

Visual estimate of growth was made daily over 7 days (18-21^{o}C)

Parva dilution	2DAT	3DAT	4DAT	6DAT	7DAT
5%	0	10	10	12	14
0.05	**50**	**75**	**80**	**85**	**85**
$5x10^{-4}$	2	5	10	15	16
$5x10^{-6}$	2	5	7	12	12
$5X10^{-8}$	2	5	5	10	10
$5X10^{-10}$	1	3	4	7	7
$5X10^{-12}$	2	7	7	7	7
Water	1	5	7	6	7

- The 0.05% Vidi Parva solution produced the highest quantity of T22 biomass
- Therefore Vidi Parva might benefit the growth of T22 when applied at 0.05% in nursery plant production

PLANT ROOT DEVELOPMENT & PROTECTION

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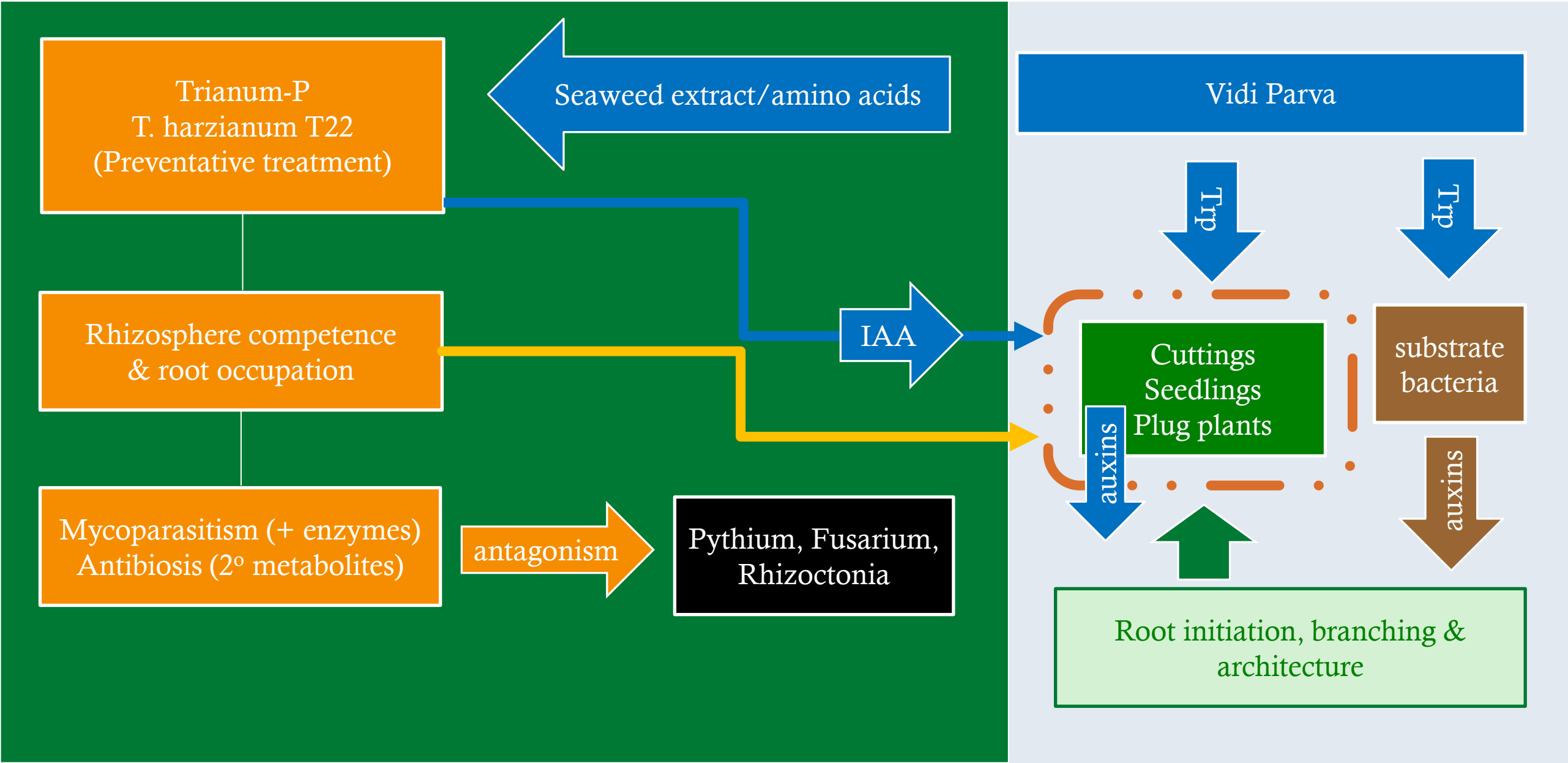


VIDI FUNDA & T22 GROWTH EXPERIMENT

Experiment to determine if the soil conditioner Vidi Funda can benefit the growth of T22 (in commercial growing media)

<u>Vidi Funda is a soil conditioner and organic fertiliser</u>

Pelleted product (2.5 x 4-7mm) based on plant material

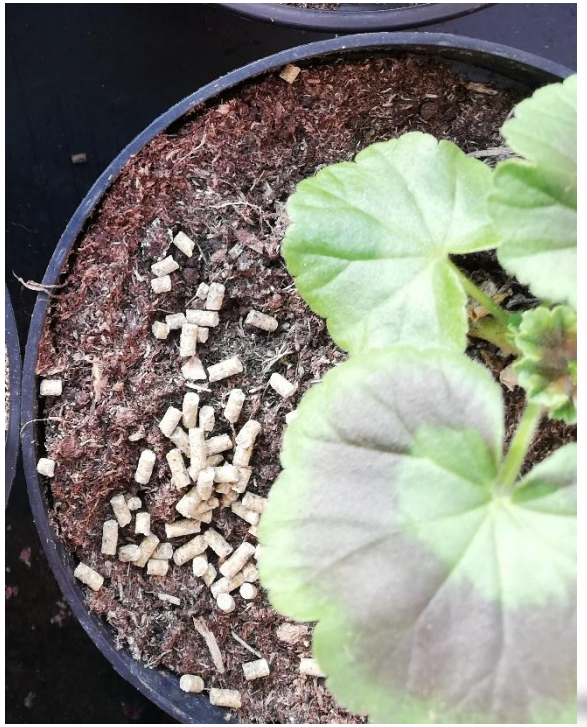
Breaks down over 4-12 weeks

Contains N, P & K in the ratio 7:2:4

Growing media incorporation: 0.5-2kg/m^3

Top dressing/pots: <10L=2-5g; 10-50L=15g; >50L=20g

Soil application: 100-400kg/ha



VIDI FUNDA & T22 GROWTH EXPERIMENT

Experiment to determine if the soil conditioner Vidi Funda can benefit the growth of T22 (in commercial growing media)

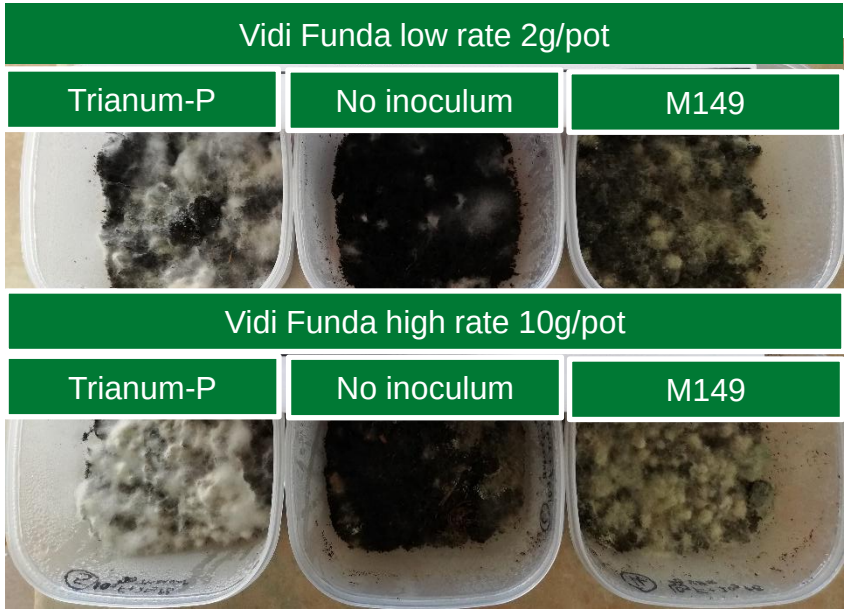
<u>Experiment to determine if Vidi Funda could act as a food base for T22</u>

6 treatments, 3 reps/treatment; 28-day experiment; 16-20^{o}C

All containers (11x11x5.5cm) + 20g Klasmann compost +15ml water

Trianum-P inoculum : 2g/container

M149 = coded trial product containing Trichoderma sp.



- High rate (10g) Vidi Funda yielded more T22 biomass than low rate (2g) Vidi Funda
- Vidi Funda could support the colonization of T22 in commercial growing media
- Rapid establishment could lead to stronger biocontrol activity and better root disease suppression

TRIANUM-P & FORTAFOL EXPERIMENT

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Experiment to test the safety of Fortafol & Trianum-P as a tank mix

<u>Fortafol is a biostimulant which increases the health & resilience of plants</u>

Formulated as a water-soluble emulsion

Contains humic acids, fulvic acids and plant extracts from *Thymus* & *Mentha*

Early studies have shown that Fortafol can reduce fungal spore viability

Clarity regarding the effect of Fortafol on T22 spores is required

TRIANUM-P & FORTAFOL EXPERIMENT

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Experiment to test the safety of Fortafol & Trianum-P as a tank mix

Two commercial fungicides were included for comparative purposes

Commercial rates: Fortafol-D 1-5ml/L; Cercobin 3g/L; Switch 0.8kg/ha

Highest concentration used = 1 ml or 1g per 100 ml (1% v/v or 1% w/v)

0.05g Trianum-P was added to 100ml of test solutions in 150ml bottles

Bottles were kept at 16-20^{o}C and checked daily for growth over 14 days

Product	1%	0.01%	$1x10^{-4}$	$1x10^{-6}$	Water
Fortafol-D	O △	O	+	+	+
Cercobin	O	O	+	+	+
Switch	O	O	+	+	+

- It can be assumed that Fortafol-D would inhibit spore germination at the commercial rate (0.1%v/v) △
- Fortafol-D exhibited '*fungicidal*' activity on a par with Cercobin and Switch
- Current advice : Do not tank mix Fortafol with Trianum-P. Apply Trianum-P first then Fortafol 3 days later.

COMPARISON OF T22 & M149 GROWTH STIMULATION IN TAGETES

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Experiment to compare the stimulation effects of T22 & M149 on the growth of *Tagetes patula* (French marigold)

Experimental details

Two trays per treatment; 10 seeds per tray

Treatments: T22, M149, untreated control (water only)

Applications: T1 – 4 days after sowing; T2 – 14 days after sowing

T1 - 1.5g/l at 5ml solution per cell; T2 - 0.5g/cell followed by irrigation

Assessments per treatment per date

Plant height : Mean of 20 plants

Cotyledons : Mean of 40 cots (2/plant)

First true leaves : Mean of 40 leaves (2/plant)

Second true leaves : Mean of 40 leaves (2/plant)

COMPARISON OF T22 & M149 GROWTH STIMULATION IN TAGETES

KOPPERT BIOLOGICAL SYSTEMS

Experiment to compare the stimulation effects of T22 & M149 on the growth of *Tagetes patula* (French marigold)

Growth assessments are presented as overall means and given in mm

Assessment		Trianum-P (T22)				M149				Control (water only)			
Date	Timing	Stem height	Coty's length	1stTL length	2ndTL length	Stem height	Coty's length	1stTL length	2ndTL length	Stem height	Coty's length	1stTL length	2ndTL length
3rd April	15DAT1	26.0	27.5	20.1	-	20.3	21.8	13.9	-	23.8	25.2	14.4	-
12th April	24DAT1	34.7	-	47.1	23.2	30.3	-	36.2	20.3	33.5	-	40.4	20.7

Growth stimulation

Growth reduction

LAVANDULA HIDCOTE PROPAGATION TRIAL

Nursery propagation trial to evaluate the combination of Trianum-P with the rooting stimulant Vidi Parva

Trial details

1. Trianum-P 2.5g/m^2 + Vidi Parva 5ml/m^2

2. M149 2.5g/m^2 + Vidi Parva 5ml/m^2

3. Untreated control

Number of trays per treatment = 4 (60 cells per tray)

Lavandula hidcote cuttings were planted on 26.4.19

Treatments were applied on 3.5.19

Assessment of disease incidence was carried out after 6 week

% number of diseased cuttings (N=60)			
Tray (rep)	Untreated	Trianum-P + Vidi Parva (TP)	M149 + Vidi Parva
A	11.7	5.0	13.3
B	16.7	1.7	11.7
C	16.7	11.7	10.0
D	13.3	5.0	11.7
Mean	14.6	5.8	11.7
Stats	ac	b	bc

- Significantly fewer diseased plugs occurred in the TP treatment compared to the untreated

LAVANDULA HIDCOTE PROPAGATION TRIAL

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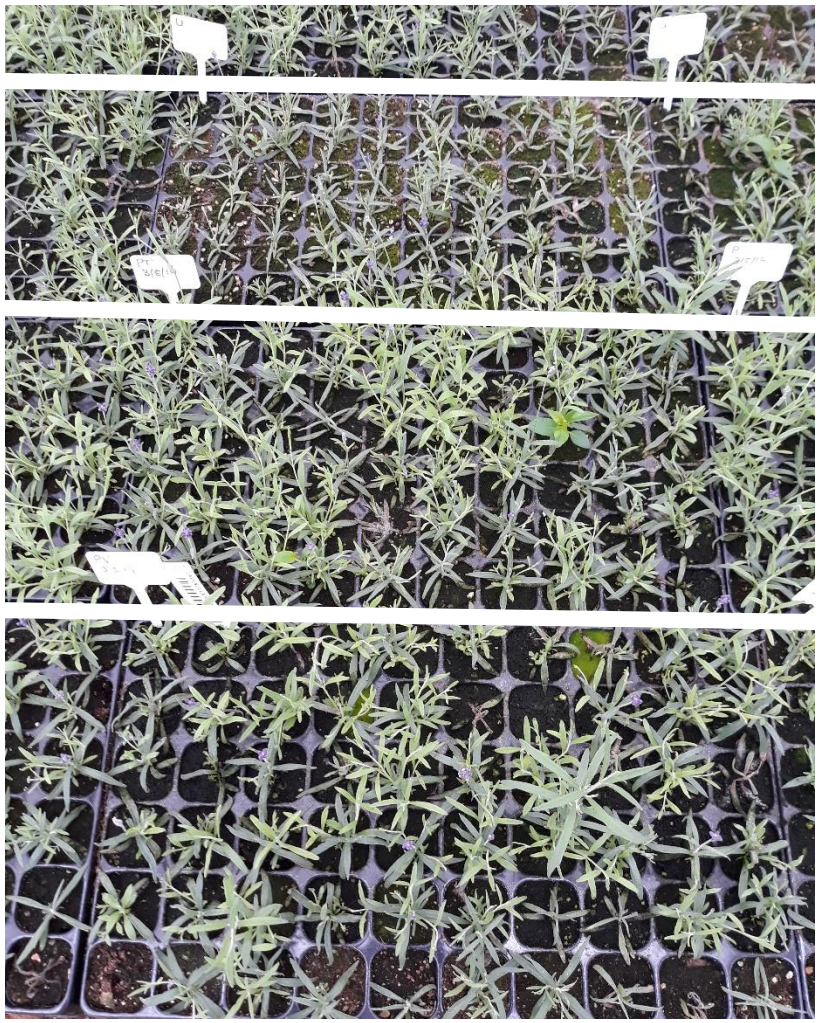
Untreated

Trianum-P 2.5g + Vidi Parva 5ml

M149 2.5g+ Vidi Parva 5ml



- There was significantly more shoot extension growth in the Trianum-P + Vidi Parva treatment compared to the untreated

HONS NURSERY TRIAL

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Nursery trial to evaluate microbial inoculants and biostimulants in the production of three nursery stock plant species

Trial plants - 126 plugs were potted into 9cm pots on 26.3.19

Deutzia nikko, Hebe x andesonii ‘Variegata’, Ceanothus repens

Reps/treatment: 6 plug plants / treatment x 3 plant species

Klasmann Container substrate; 1g Vidi Funda/pot

7 treatments x 2 applications (1st April & 2nd May)

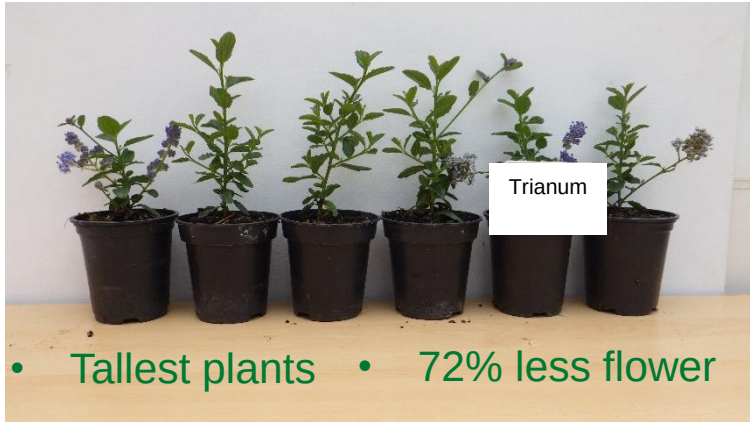
1. Untreated (plain water only)
2. M149 (2g/m^2)
3. Trianum-P (2g/m^2)
4. Vidi Parva (2.8ml/m^2)
5. M149 (2g/m^2) + Vidi Parva (2.8ml/m^2)
6. Trianum-P (2g/m^2) + Vidi Parva (2.8ml/m^2)
7. Fortafol (2.8ml/m^2)

Solution volume : 100ml per pot

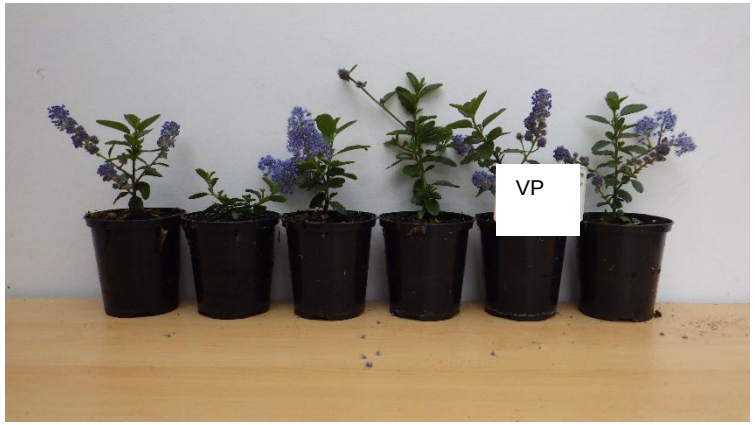
Commercial rates achieved: Trianum-P 15g/1000 pots; Vidi Parva 3l/ha; Fortafol-D 3l/ha; M149 15g/1000 pots

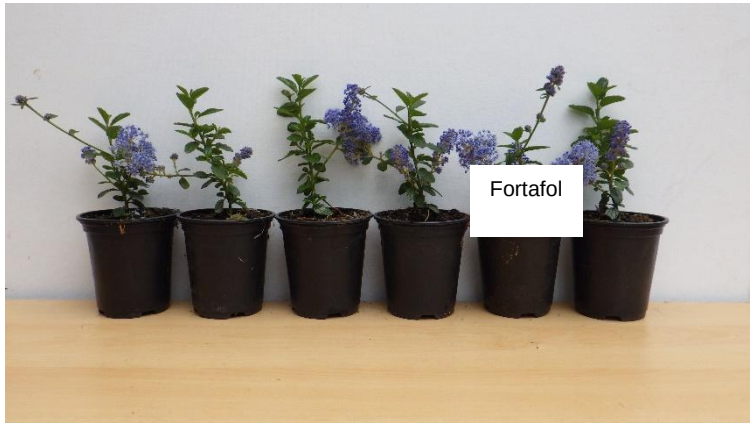
CEANOTHUS REPENS

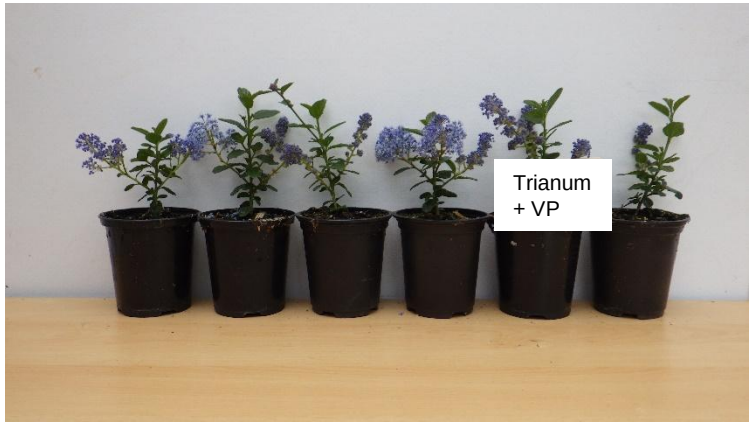
41DAT1 (11TH MAY 2019)



- Tallest plants
- 72% less flower



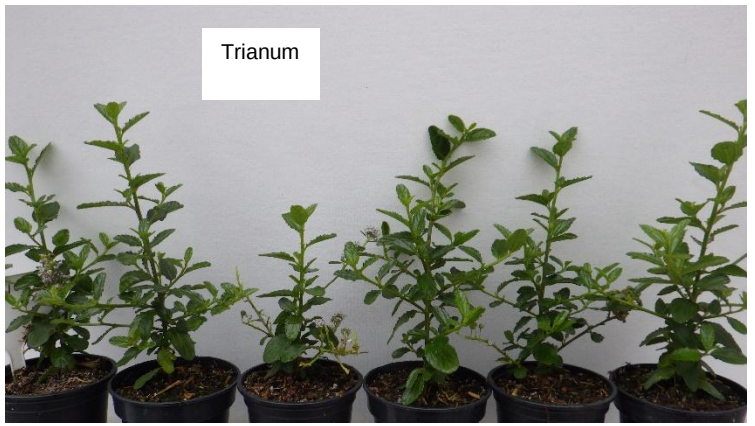




CEANOTHUS REPENS

68DAT1 (7TH JUNE 2019)

- Moderate growth overall
- Reasonable canopy shape
- Canopy appears less dense
- Some lateral shoot extension
- Some pale leaves
- More leaves with small surface area



- Good spring growth overall
- Good canopy shape
- Dense growth in bottom half of plant
- Good lateral shoot & leaf production
- Good colour and plant health
- Some leaves with a large surface area

- Moderate spring growth
- Spreading plant shape
- Open less dense
- Variable lateral shoot & leaf production
- Good colour although some pale leaves
- Some leaves with a large surface area

HEBE X ANDERSONII ‘VARIEGATA’ 41DAT1 (12TH MAY 2019)

Some plugs were diseased when potted-on (26.3.19)

1=low 4=high

	Untreated	Trianum + Vidi Parva	M149 + Vidi Parva	Vidi Parva	Fortafol	M149	Trianum-P
A	1	4	4	3	2	1	3
B	1	3	3	3	2	1	3
C	4	1	3	3	4	4	2

DEUTZIA NIKKO 69DAT1 (9TH JUNE 2019)

Some plugs were diseased when potted-on (26.3.19)

Untreated	Trianum-P	Vidi Parva	M149 + Vidi Parva	M149	Trianum-P + Vidi Parva	Fortafol

- Compared to the untreated plants, more root biomass and best root quality was produced in the Trianum-P and Trianum-P + Vidi Parva treated plants

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Advances in our understanding of the disease biocontrol potential & enhancement of *Trichoderma harzianum* strain T22 (Trianum®)

Summary

The plant biostimulant Vidi Parva promoted the growth of *Trichoderma harzianum* T22 in liquid culture
The pelleted product Vidi Funda increased T22 biomass production in commercial growing media
Growth stimulation in *Tagetes patula* occurred at the earliest stages of plant development
The combination treatment of Trianum-P + Vidi Parva gave rooting development & disease control benefits in HONS
Fortafol exhibited fungicidal like activity against T22 spores, so practical use must be managed accordingly
Plant biostimulants may provide a means to increase disease biocontrol activity under a wide range of conditions

THANK YOU FOR YOUR ATTENTION

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