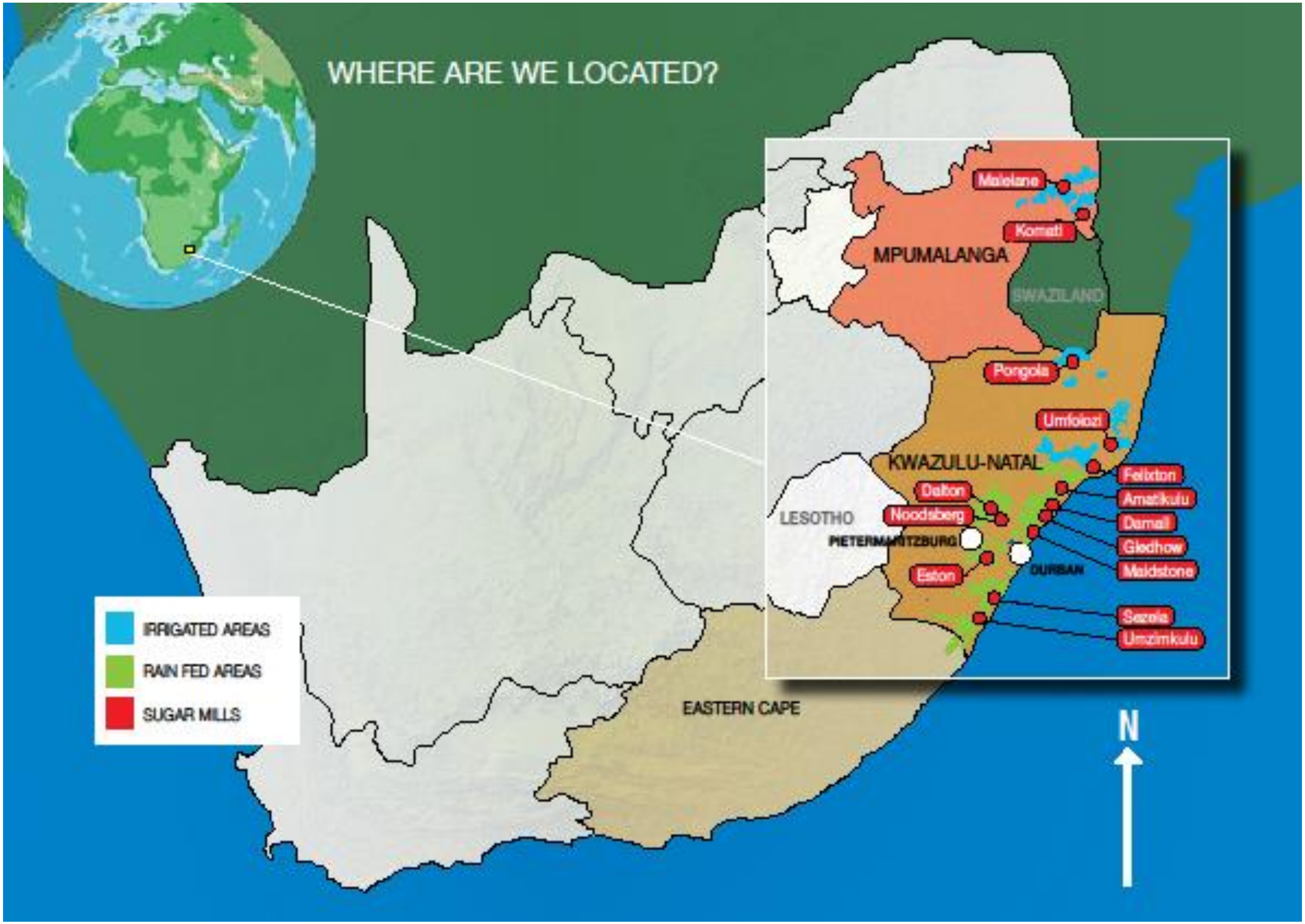
The potential addition of SIR and Bt-sugarcane to the toolbox of an AW-IPM programme against *Eldana saccharina* in South African sugarcane

DE Conlong and RS Rutherford

The South African Sugar Industry



Eldana saccharina

INTERVENTIONS

2015: Ampligo, Coragen and Steward (emergency) registrations
2015: N58 and N59 released for their eldana resistance
2014: Workshop held to develop 5 yr research plan (2015-2020)
2014: IPM manual for eldana control produced
2013: Industry support for GM inclusion in IPM solution to eldana
2012: Sterile Insect Technique 'in cage' proof of concept achieved
2011: Implementation of "push-pull" promoted in the field

2005: Eldana-Fusarium interaction described
2005: Eldana control manual produced
2004: Bt GM cane with Monsanto gene produced and tested at SASRI
2004: First recommendation on use of silicon to increase resistance to eldana
2004: N42 released for its eldana resistance
2004: Aerial use of Fastac approved
2003: Fastac registered
2002: N39 released for its eldana resistance

1999: In-house Bt GM cane produced and tested at SASRI
1991: Potted screening trials conducted routinely in the plant breeding selection programme
1989: N21 released for its eldana resistance
1988: Insect Rearing Unit opened at SASRI
1982: Pest And Disease Committees formed

1975: First exotic parasitoids introduced for biological control of *E. saccharina*

1925: EXPERIMENT STATION ESTABLISHED

2010s
2000s
1990s
1980s
1970s
1960s
1950s
1940s
1930s
1920s

HISTORY

2000s: *E. saccharina* evolves cold tolerance *and* increasingly moves into Midlands regions

1990s: Another borer threatens from Mozambique – *Chilo sacchariphagus*

1980: *E. saccharina* found as far south as Port Shepstone

1975: First report of *E. saccharina* infestation south of Tugela River

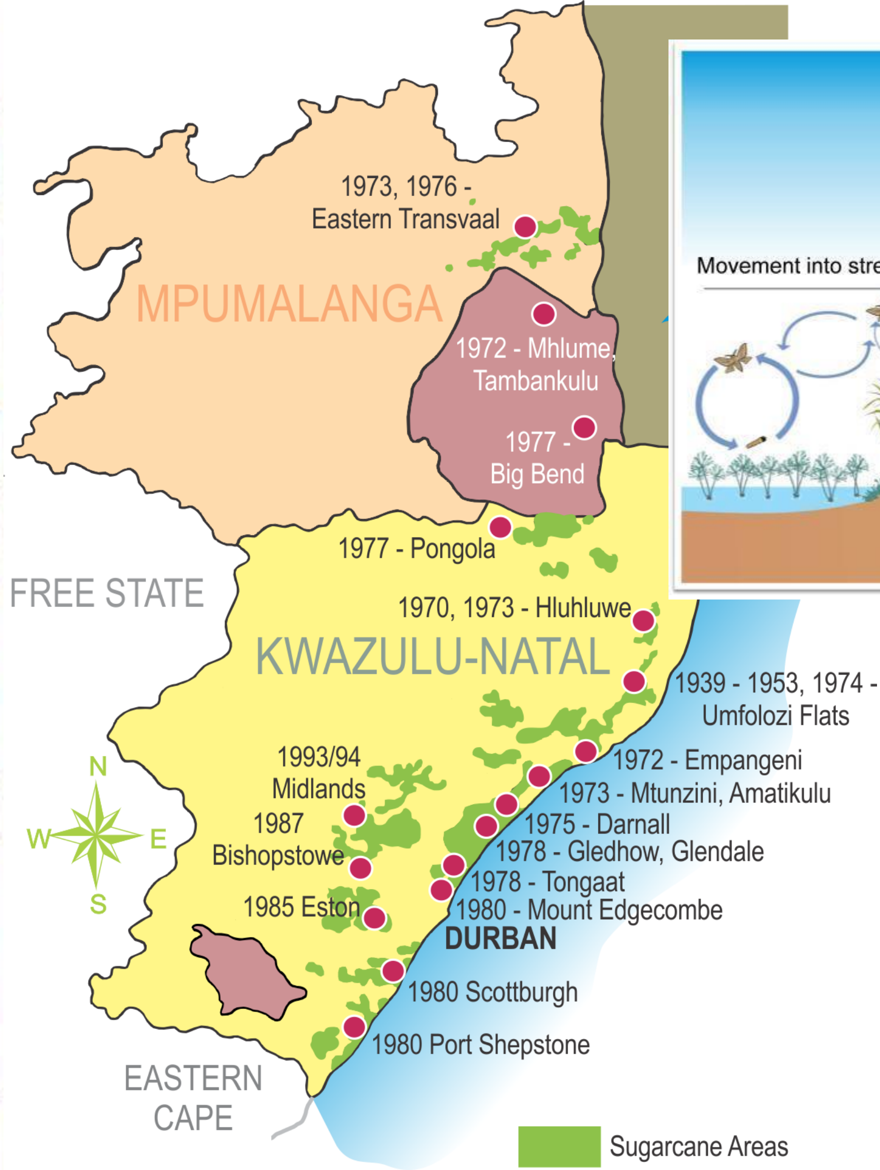
1970: *E. saccharina* observed in Hluhluwe in susceptible NCo376, soon to become widespread and a major pest

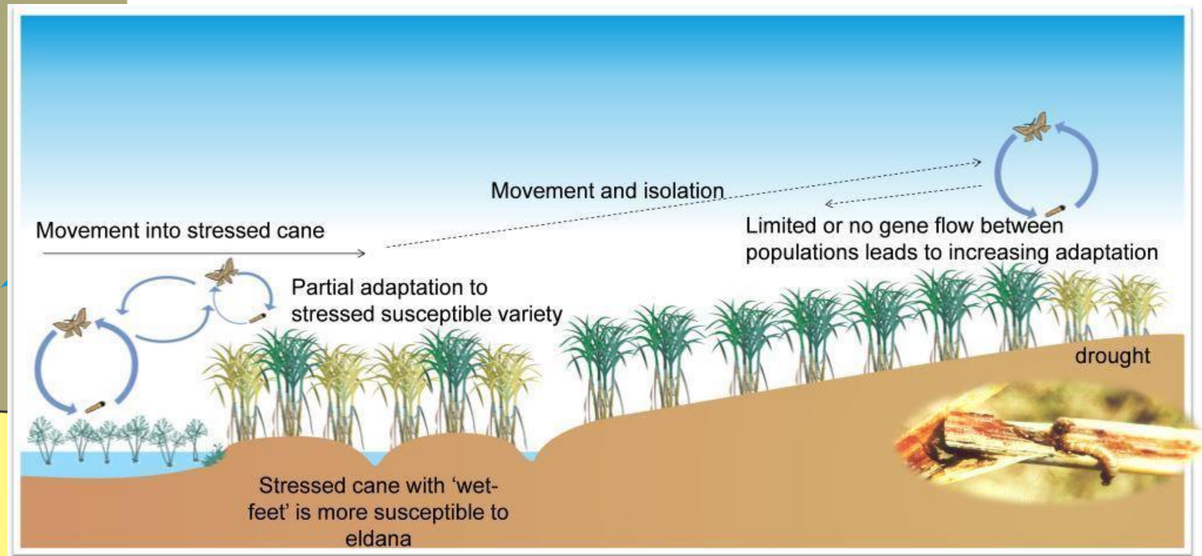
1953: *E. saccharina* disappears

1940s: *E. saccharina* causing extensive damage on Umfolozi Flats, gradual change to resistant Co281

1939: Severe infestation of *E. saccharina* on Umfolozi Flats in POJ2725 (highly susceptible), POJ2878, POJ2714, Co301 also damaged, Co281 more resistant

1929: First report of *Eldana saccharina* in Mtubatuba, South Africa


1973, 1976 -
Eastern Transvaal
MPUMALANGA
1972 - Mhlume,
Tambankulu
1977 -
Big Bend
1977 - Pongola
FREE STATE
1970, 1973 - Hluhluwe
KWAZULU-NATAL
1939 - 1953, 1974 -
Umfolozi Flats
1972 - Empangeni
1973 - Mtunzini, Amatikulu
1975 - Darnall
1978 - Gledhow, Glendale
1978 - Tongaat
1980 - Mount Edgecombe
DURBAN
1993/94
Midlands
1987
Bishopstowe
1985 Eston
1980 Scottburgh
1980 Port Shepstone
EASTERN
CAPE
N
W
E
S
Sugarcane Areas


Movement into stressed cane
Movement and isolation
Limited or no gene flow between
populations leads to increasing adaptation
Partial adaptation to
stressed susceptible variety
drought
Stressed cane with 'wet-
feet' is more susceptible to
eldana

IPM:
Understanding the ecological role of pests

- CULTURAL CONTROL
- BIOLOGICAL CONTROL
- CHEMICAL CONTROL

Area-Wide Integrated Pest Management

Area-Wide:

- Sugarcane: a grass species in a diverse ecosystem
- Habitats: Wetlands Alien weeds
- No farm boundaries!
- Farmer co-operation!

Control of Eldana:

IPM:
Understanding the ecological role of pests

CULTURAL CONTROL

- Crop health & nutrition
- Harvest age
- Variety choice
- Healthy seed cane

BIOLOGICAL CONTROL

- Natural enemies: parasitoids
- Habitat management/push-pull

CHEMICAL CONTROL

- Fastac (alpha-cypermethrin)

Monitoring!

What is habitat management or push-pull?

- "**Habitat management** is an ecologically based approach aimed at favoring natural enemies and enhancing biological control in agricultural systems."
(Landis et al 2000)

- **Push-pull** is a specific example of habitat management where the behaviour of the pest and its natural enemies is manipulated using semio-chemicals or plant volatiles.

Does push-pull work?

Cyperus papyrus

Melinis minutiflora

Lab trials:

PULL:

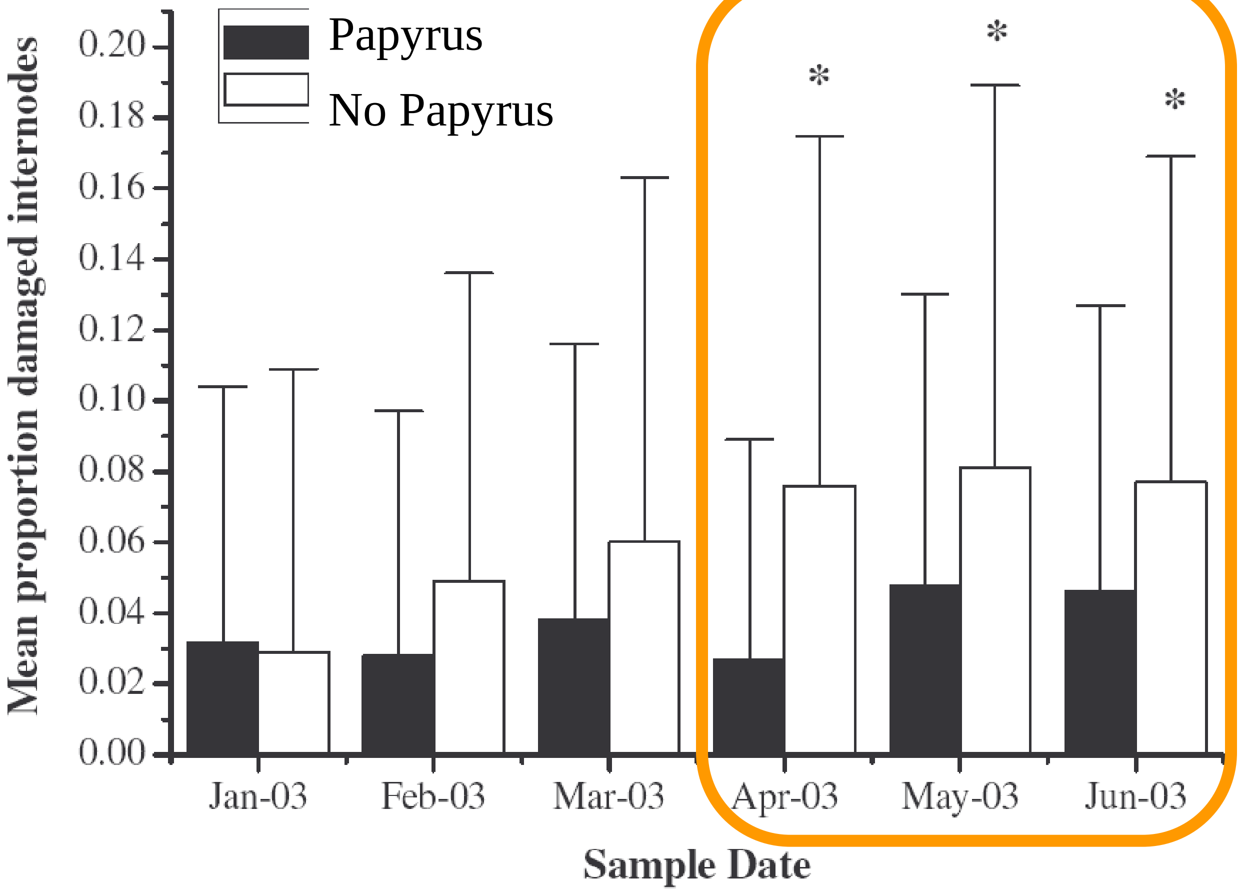
- Eldana larvae prefer sedges (e.g. papyrus) to cane
- Eldana moths prefer laying eggs on mature maize than on sugarcane

PUSH:

- Molasses grass repels moths

Field trials: PULL

Cyperus papyrus

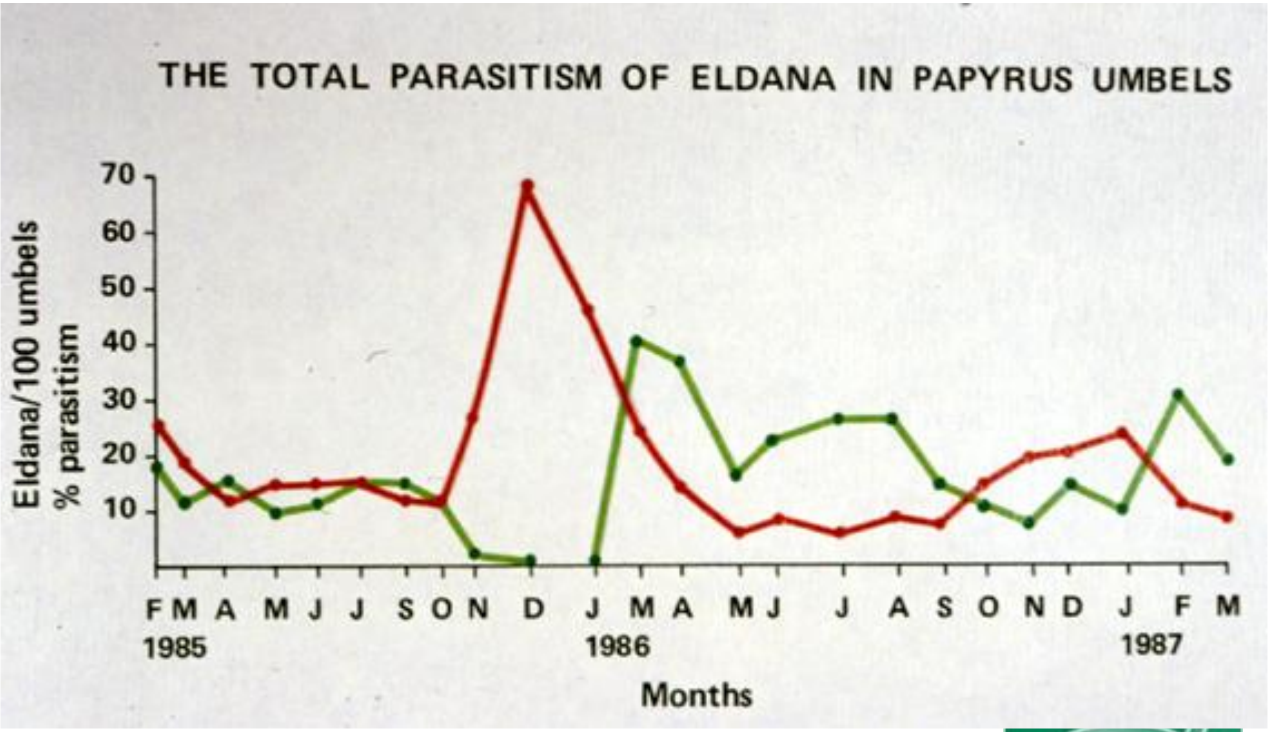


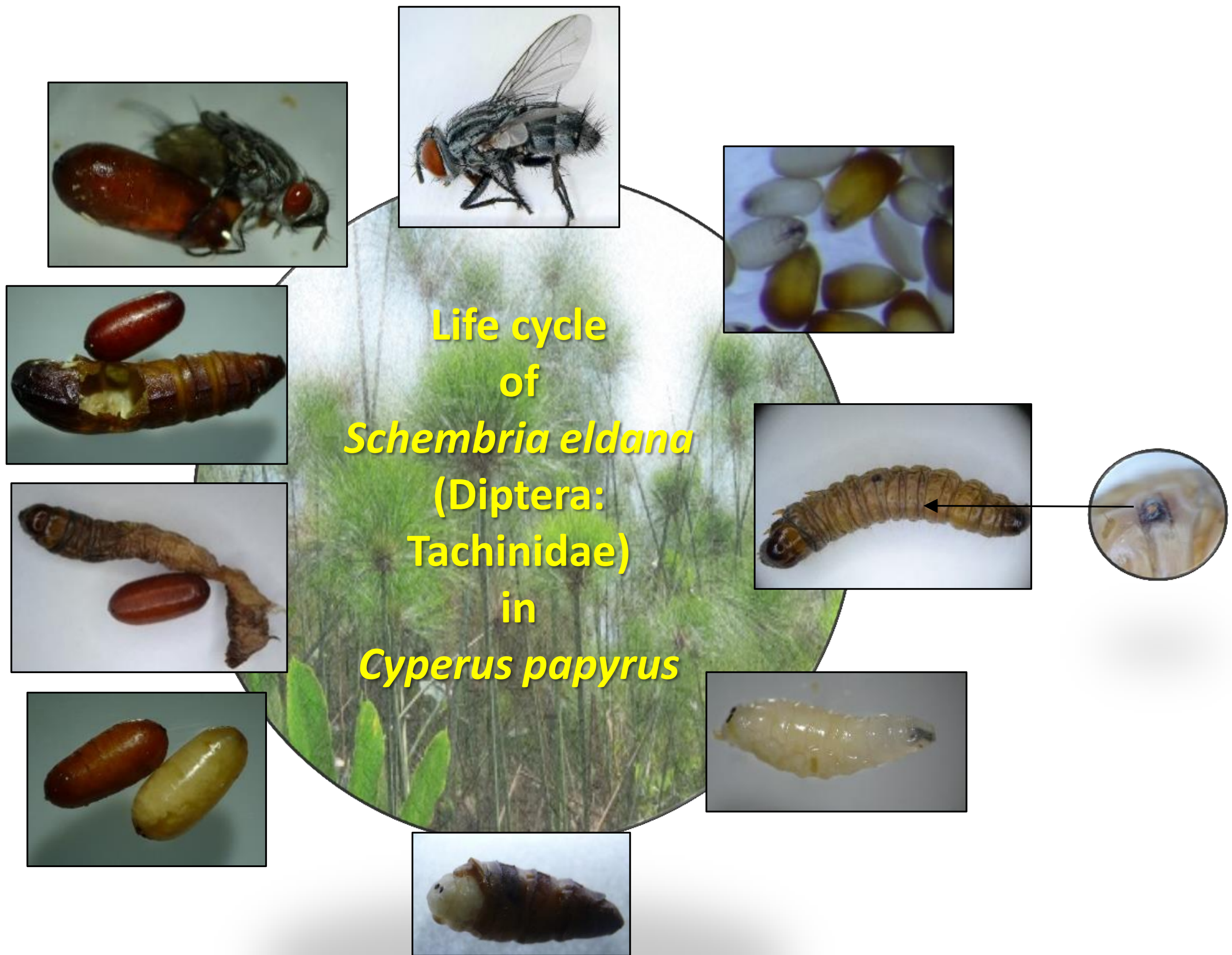
Presence of *Cyperus papyrus* decreases *Eldana* damage in adjacent sugarcane

AW-IPM: *E. saccharina* push-pull. Re-establishment of wetland habitats

- **Papyrus and dives are preferred oviposition sites, rather than sugarcane, for *E. saccharina* females (Atkinson, 1980 and Kasl, 2004)**

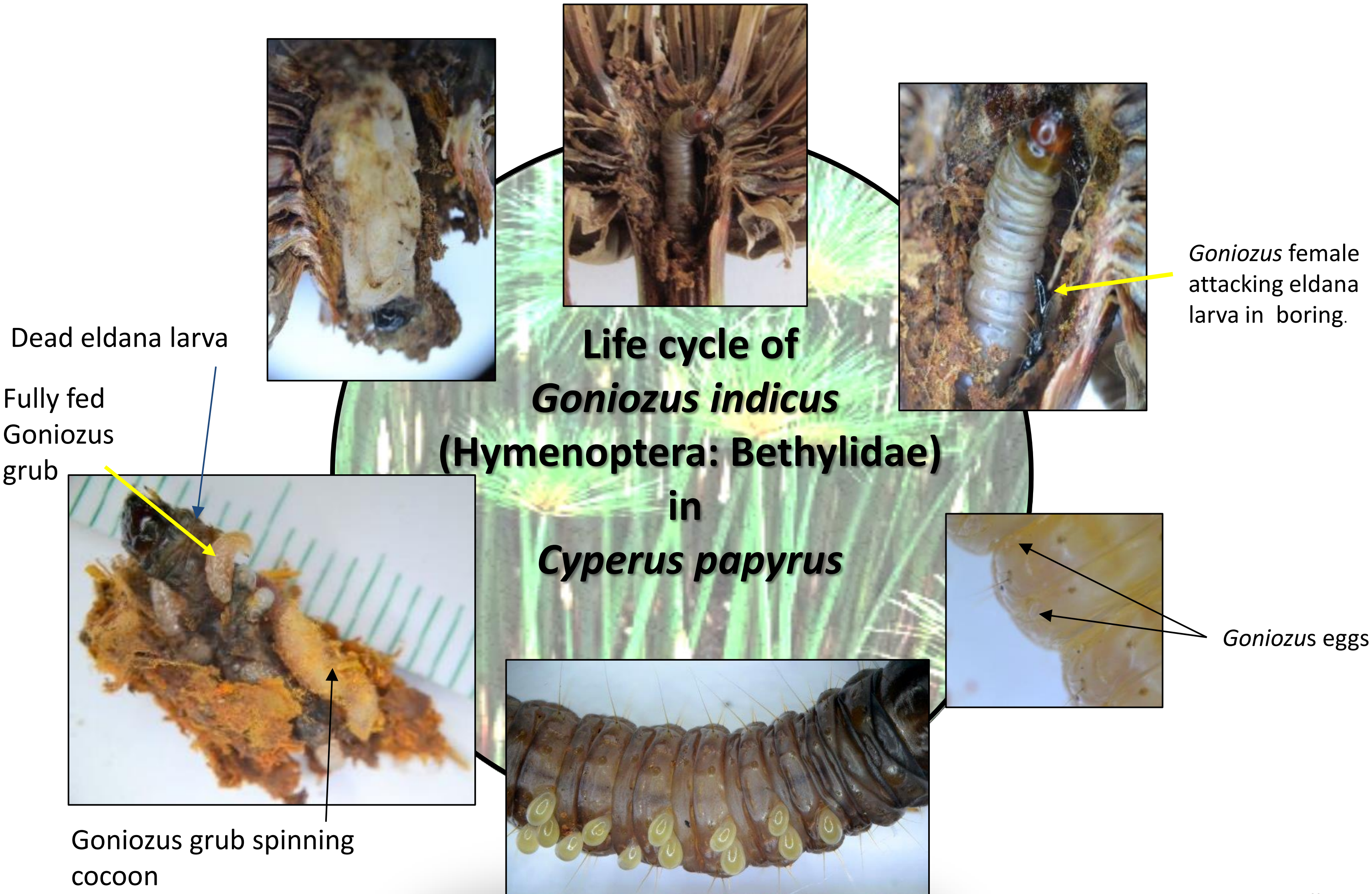
- **In these two indigenous host plants there are good biocontrol agents, giving "text book" control (Conlong, 1990)**





D.Y. Gillespie 2016

Reference: D.A. Barraclough. A new species of Tachinidae (Diptera) parasitic on the sugarcane borer *Eldana saccharina* (Lepidoptera: Pyralidae), in Natal, South Africa. Bulletin of Entomological Research 81(02):133 - 136 · June 1991



D.Y. Gillespie 2016

Reference: DE Conlong, DY Graham and H. Hastings 1988. Notes on natural host surveys and laboratory rearing of *Goniozus natalensis* Gordh (Hymenoptera: Bethylidae), a parasitoid of *Eldana saccharina* Walker (Lepidoptera: Pyralidae) larvae from *Cyperus papyrus* L. in Southern Africa. J. Ent. Soc. S. Afr. 51(1):115-127.

AW-IPM and weed biocontrol?

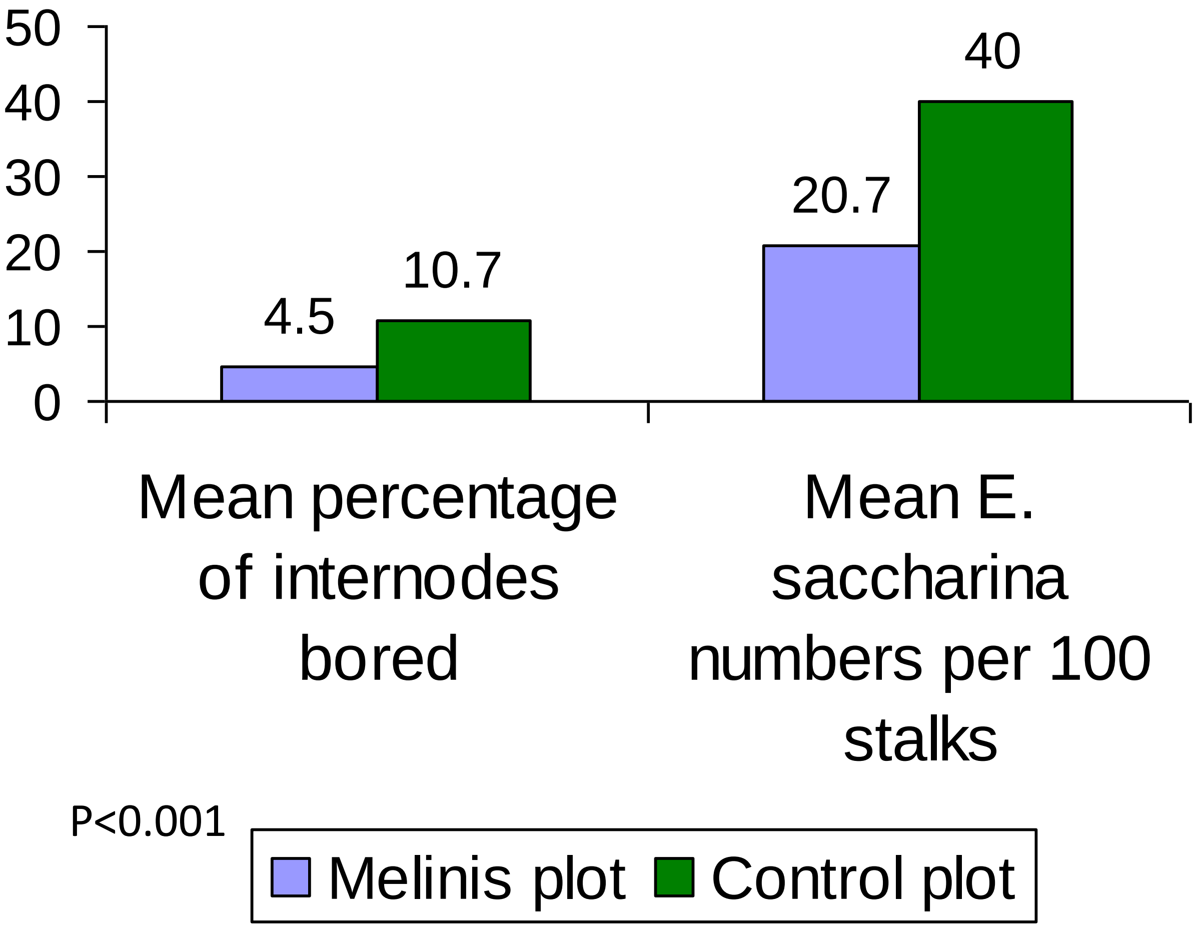
- **Clearing IAP's with biocontrol agents- especially those mass reared?**
- **Increased habitat for indigenous host plants?**
- **Increased habitat for crop pests and their natural enemies?**
- **Habitat management can be accomplished more readily and populations of natural enemies augmented?**

12800 *Neochetina* and 14400 *Eccritotarsus* released

Increased habitat for indigenous host plants

AW-IPM: Habitat Management – "Push" plants

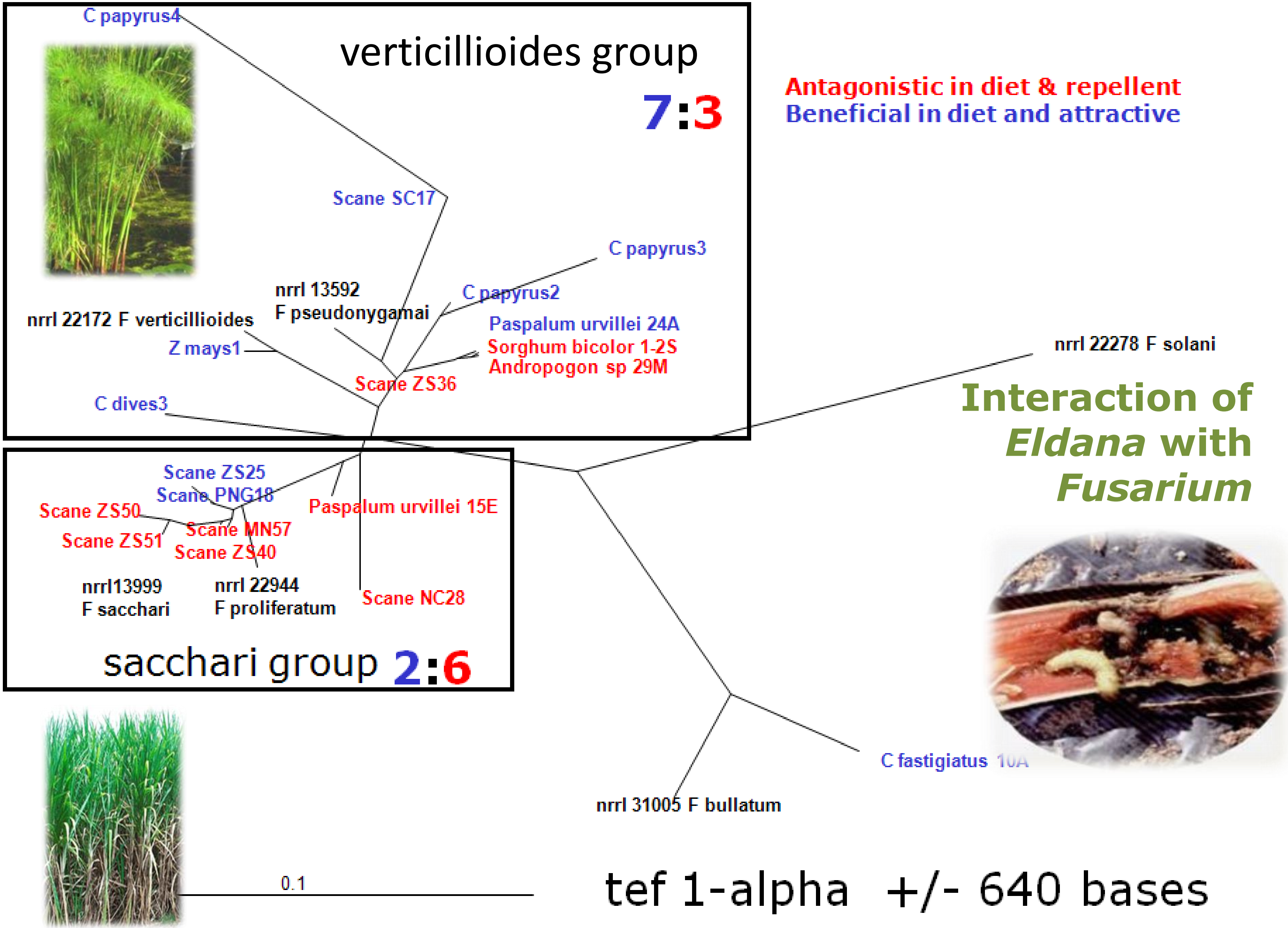
Field trials: PUSH



P<0.001

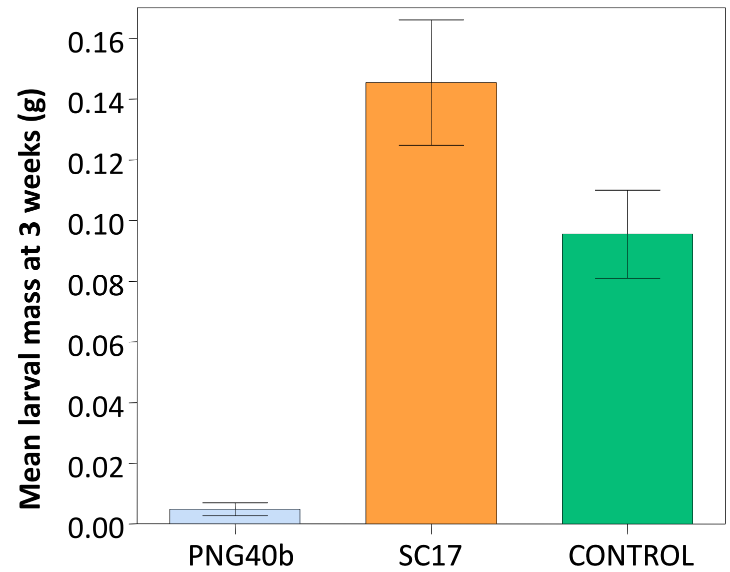
Melinis minutiflora (SA)

MOLASSES GRASS decreases *Eldana* damage and numbers in adjacent sugarcane

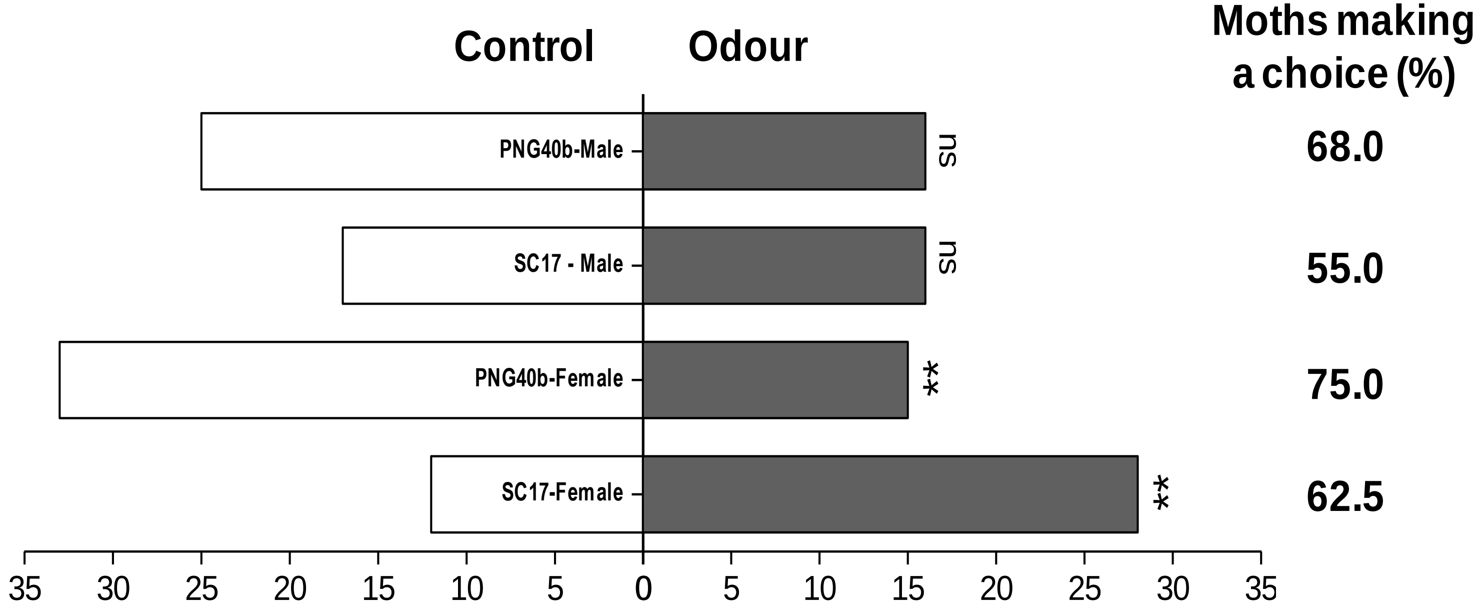


Interaction of *Eldana* with *Fusarium*
Antagonistic in diet & repellent
Beneficial in diet and attractive
verticillioides group
7:3
C papyrus4
Scane SC17
C papyrus3
C papyrus2
nrrl 13592
F pseudonygamai
nrrl 22172 F verticillioides
Paspalum urvillei 24A
Sorghum bicolor 1-2S
Andropogon sp 29M
Z mays1
Scane ZS36
C dives3
nrrl 22278 F solani
Scane ZS25
Scane PNG18
Scane ZS50
Scane ZS51
Scane MN57
Scane ZS40
Paspalum urvillei 15E
nrrl13999
F sacchari
nrrl 22944
F proliferatum
Scane NC28
sacchari group 2:6
C fastigiatus 10A
nrrl 31005 F bullatum
0.1
tef 1-alpha +/- 640 bases

Interaction of *Eldana* with *Fusarium*



Dietary inclusion of fermented maize; effect on larval mass



Number of moths responding to Fusarium volatiles in Y-tube olfactometer bioassays

Interaction of *Eldana* with *Fusarium*

0.27g cholesterol

ergosterol

Ingredients	Amount/ volume per kg	Instructions
1. DRY MIX		
Crushed sugarcane stalk (g)	58	Dry mix Mix thoroughly
Wheat flour (brown) (g)	58	
Egg powder (g)	18	
Chickpea (g)	58	
Yeast (g)	2.5	
Milk powder (g)	14	
Ascorbic acid (g)	2.5	
Sugar (g)	0	
2. ANTI-MICROBIALS		
Nipagin (g)	1.4	Dissolve nipagin and dithane in ethanol Add formalin Add to the dry mix Mix well
Ethanol (ml)	25	
Formalin 40% (ml)	3.2	
3. GELLING AGENT		
Agar (g)	3	Dissolve agar in water Autoclave Add to dry mix
Water (ml)	600	
4. ANTI-MICROBIALS		
Propionic acid (ml)	1.5	Add phosphoric acid carefully to the water Add whole volume to the wet mix
Phosphoric acid	0.15	
Water (ml)	100	
5. ACTIVE DRY INGREDIENT		
Fusarium (SC17) fermented maize (g)	56	Add last Mix well

Interaction of *Eldana* with *Fusarium*

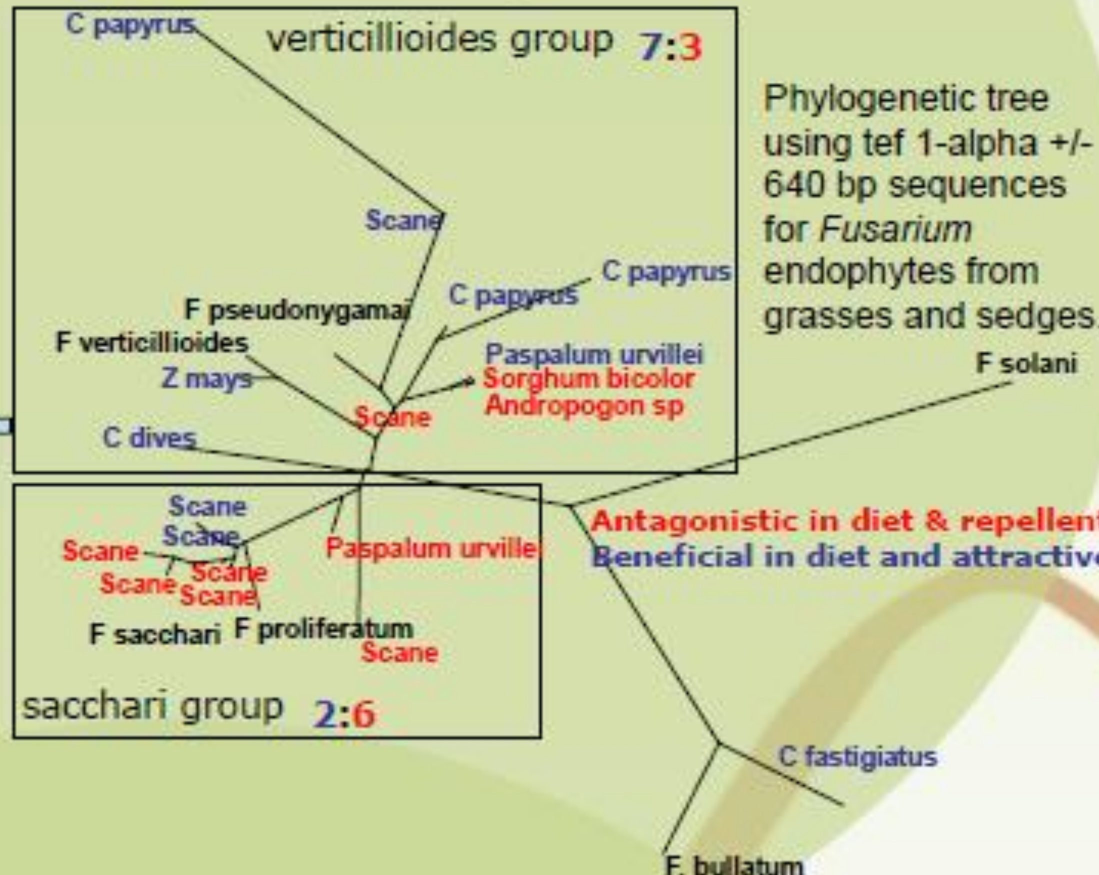
New Diet						
	Cross No.	**Total Num Eggs**	**Fertile**	**%**	**Infertile**	**%**
	1	365	358	98.08	7	1.92
	2	371	361	97.30	10	2.70
	3	342	330	96.49	12	3.51
	4	224	216	96.43	8	3.57
	5	436	420	96.33	16	3.67
	Average	**348**	**337**	**97**	**11**	**3**
Old Diet						
	Cross No.	**Total Num Eggs**	**Fertile**	**%**	**Infertile**	**%**
	1	164	149	90.85	15	9.15
	2	277	266	96.03	11	3.97
	3	132	103	78.03	29	21.97
	4	325	295	90.77	30	9.23
	5	287	270	94.08	17	5.92
	Average	**237**	**217**	**90**	**20**	**10**

4 Endophytes

Endophytic *Beauveria bassiana* in maize can protect against stem-borers. In sugarcane *Fusarium* endophytes are frequently found. Isolates can be beneficial or antagonistic to *Eldana saccharina*.

Isolates beneficial to *E. saccharina*:
- do not produce beauvericin
- digest tissues, stalk - rot
- produce volatile *E. s* attractants
- produce antibiotics/antimicrobial proteins
- ergosterol supply
- detoxify DIMBOA
- induce SA dependent defences

SA/JA ⇐ [Auxin, Fumonisin B1, GalOX (H_2O_2)] ⇐



Phylogenetic tree using tef 1-alpha +/- 640 bp sequences for *Fusarium* endophytes from grasses and sedges.

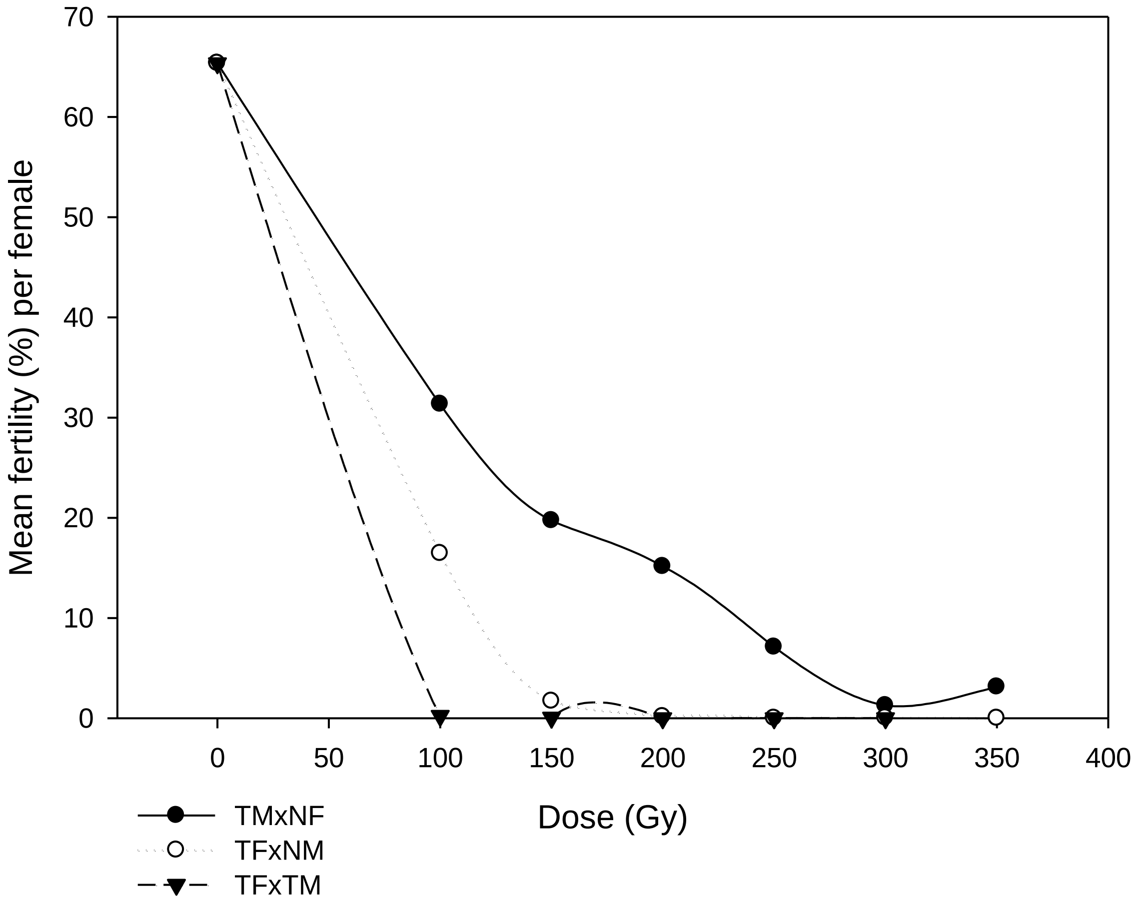
Isolates antagonistic to *E. saccharina*:
- produce beauvericin
- produce volatile *E. s* repellents

Insecticides

-reduced dose immunosuppression

Stalk/foliar application					
Product	**Actives g/L**	**Formulation**	**MOA (IRAC group)**	**Rate/ha**	**Comments**
Genesis	Pyriproxyfen 100	EC	Juvenile hormone analogue (7C) Egg hatch and pupation failure. Sub-lethal: Pupal weight, adult longevity, fecundity and fertility reduced. Various defective adults. Decreased cellular immunity.	50 mls	May disrupt mating and increase susceptibility to pathogens Partner with: Coragen (mating disruption thru semi-paralysis), Broadband (*Beauveria*), Dipel (*Bt*), chitin biosynthesis inhibitor
Denim Fit	Lufenuron 400 Emamectin 100	WG water dispersible granule	Chitin biosynthesis inhibitors (15) Egg mortality & molting failure. Sub-lethal: Increased ease of pathogen penetration; reduced adult fecundity Decreased cellular immunity.	100g	
Broadband	*Beauveria bassiana*	EC	Fungal entomopathogen	500 mls	Combine with suppressors of immunity from classes 7C and 15 above
Dipel	*Bacillus thuringiensis*	DF water dispersible granule	Bacterial entomopathogen (11)	500 g	

SIT: Parental sterilisation

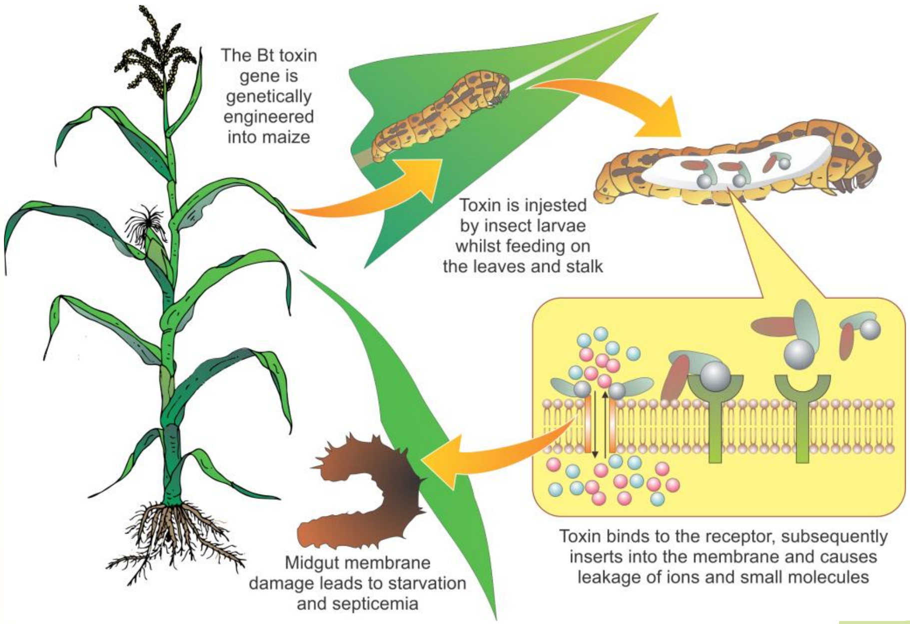


Marking of lab reared radiated adults

SIT for eldana

- Radiation biology study of eldana showed 200Gy to be a good radiation dose, with no effects on eldana reproduction biology, and F1 sterility could be induced (A Walton- MSc Thesis March 2011)
- Moths could be marked with calco red with no effects on reproduction biology (A Walton- MSc Thesis March 2011
- Mating behaviour and competiveness of 200Gy radiated moths as good as wilds, and cage study showed population suppression and cane damage reduction in SIT treatment compared to control (P. Mudavanhu- PhD thesis December 2012)

Bt



Bt crop			non-Bt refugia
rr	rS	SS	SS SS SS SS SS SS SS
rare but survival rate high	may survive low doses	no survivors	

- Single or major resistance gene appears in the pest by mutation (r)
  - rr is rare.
- Susceptible is dominant (S). Resistance is a recessive trait (r).
  - Refuge supplies SS
- Mating -
  rr x rS gives progeny rr, rr, rS, rS (very rare cross)
  rr x SS gives progeny rS, rS, rS, rS (rare cross)
  rS x SS gives progeny rS, rS, SS, SS (prevents emergence of rr)

High Bt dose > 95% rS will be killed and > 99.9% of the SS will be killed

Bt advantage - Approximately 18% of borer damage is due to *Sesamia*.

Surveys:	1st June 2014 to 31st May 2015.			
Mill	*Eldana*	*Sesamia*	Total	%*Sesamia*
ML	604	1	605	0.2
PG	520	36	286	12.6
UF	479	95	574	16.6
FX	3647	377	4024	9.4
AK	3104	128	3232	4
EN	5625	404	6029	6.7
MS	7037	2774	9811	28.3
GH	8040	4061	12101	33.6
DL	4443	1682	6125	27.5
ES	2899	599	3498	17.1
NB	843	553	1396	39.6
SZ	11523	693	12216	5.7
UK	4150	261	4411	5.9
IND	**52914**	**11664**	**64308**	**18.1**

BIOSECURITY

Thank you for helping us keep track of this pest

Biosecurity Hotline:
+27 (0)60 544 5393

South African Sugarcane Research Institute

www.sugar.org.za

July 2013

BIOSECURITY ALERT

The *Chilo* stalk borer poses a real threat to sugarcane in South Africa. Your assistance is requested to look out for damage and larvae as illustrated inside.

If you find damage or larvae, please inform your local Extension Specialist or contact us via the Biosecurity Hotline.

Estimated indirect losses due to a shortened crop duration

Age of crop (months)	**Tonnes tRV/ha**
12	5.12
18	10.54
Annualised yield for 18 month	6.97 tRV/ha/annum
Model increase/annum	1.85 (36%)
Commercial RV increase/annum	[#]0.7 x 1.85 = 1.295
Grower + miller value of RV gain (no eldana)	**R8 569 /ha/annum**

1 Euro = approx. R15

One of the consequences of a shortened crop duration

Estimated direct losses due to borer damage

PD&VCC area	Average yield tRV/ha	Average age at harvest (mo)	Average age at survey (mo)	Average % IB in surveys	Estimated Average % IB at harvest	corrected tRV/ha gain per 1% reduction in Internodes Bored	max gain Rands/ha harvested	total hectares	ha harvested /annum	max gain Rands/mill area/annum
Malelane/ Komati	11.4	13.5	11.0	1.5	2.0	0.143	1907	39816	35392	R 67 498 932
Pongola	11.2	14.1	11.7	0.6	1.0	0.150	1001	16869	14357	R 14 374 304
Irrigated SSG	8.4	13.8	-	-	1.5	0.146	1462	8252	7176	R 10 489 541
Umfolosi	10.4	12.6	10.5	0.3	0.4	0.134	347	17163	16346	R 5 664 661
Umfolosi SSG	2.4	12.6	-		0.4	0.134	339	4759	4532	R 1 534 589
Felixton	7.9	13.0	11.1	1.0	1.5	0.156	1556	20226	18670	R 29 056 510
Amatikulu	5.0	14.0	11.4	2.3	3.5	0.168	3896	20000	17143	R 66 785 074
Entumeni	5.2	19.0	13.4	1.7	3.0	0.228	4531	8870	5602	R 25 384 416
Zululand SSG	3.5	15.3	-	-	1.9	0.184	2317	18483	14465	R 33 512 458
Maidstone	6.7	15.0	13.5	3.7	4.5	0.180	5366	22572	18058	R 96 905 749
Gledhow	6.7	14.8	12.4	3.3	4.0	0.178	4707	27553	22340	R 105 165 187
Darnall	6.7	14.3	11.6	2.8	3.5	0.172	3981	19595	16443	R 65 459 033
NCoast SSG	3.5	14.7	-	-	3.5	0.176	4089	9389	7664	R 31 338 840
Eston	10.4	25.0	16.2	1.6	2.0	0.300	3981	33768	16209	R 64 519 626
Noodsberg/ UCL	10.4	24.0	19.0	0.6	1.0	0.288	1916	48088	24044	R 46 069 891
Midlands SSG	8.4	24.5	-	-	1.0	0.294	1954	5532	2710	R 5 293 915
Sezela	8.3	17.7	14.8	2.2	3.0	0.212	4225	34375	23305	R 98 455 882
Umzimkulu	8.3	19.1	14.1	1.8	3.0	0.229	4558	22205	13951	R 63 589 761
SCoast SSG	5.6	18.4	-	-	3.0	0.221	4389	5249	3423	R 15 023 668
RV price R/ton	**R 6 617**			**Average**	**2.3**		**Totals**	**382764**	**281829**	**R 846 122 038**

1 Euro = approx. R15

Guideline of licensing costs (US$) obtained from biotechnology developer companies and their licensing consultants.

ACTIVITY	SCENARIO 1:	SCENARIO 2:	SCENARIO 3: “GO-IT-ALONE’
Licence agreement fee	250 000	Approx. 25% of the trait value spread evenly over milestone payments **and/or** royalties. Payment structure designed during negotiations.	0
Annual R&D fee	0		0
Milestone payments:			
Transformation success	300 000		0
Greenhouse efficacy	0		0
Event PoC field testing	500 000		0
CFT: commercial events	300 000		0
Lack of yield impact	0		0
Regulatory submission	0		0
Regulatory approval	500 000		0
Royalties (value of trait)	3 to 9% (e.g. 7%)	25%	0%

Cost of Bt development up to deregulation (year 14).

	SCENARIO 1	SCENARIO 2	SCENARIO 3
Year 1	R8 291 000	R291 000	R291 000
Years 2 - 9	R11 370 000	R1 770 000	R13 770 000
Years 10 - 14	R55 010 000	R39 010 000	R39 010 000
Total Investment to year 14	**R74 671 000**	**R41 071 000**	**R53 071 000**
Royalties /ha/annum	R234	R418	R0

1 Euro = approx. R15

INTEGRATED PEST MANAGEMENT

| Sustainability | Resilience | Innovation |

SASRI Research focuses on the development of the **comprehensive** technology **toolkit** that is required for sustainable, resilient and innovative **Eldana IPM** on an area-wide basis

HABITAT MANAGEMENT

Restoration of wetlands and natural vegetation corridors

Fallow crops

Crop residue management

STERILE INSECT TECHNOLOGY

Potential release of irradiated males for sterile F_1 generation Eldana population control

CROP STRESS MANAGEMENT

Crop nutrition

Soil Health

Surface water management

Irrigation management

ELDANA IPM RESEARCH AT SASRI

VARIETY RESISTANCE

Production of Eldana resistant cultivars through classical, mutation and precision (marker-assisted) breeding

Potential expansion of IPM toolkit to encompass Bt GM technology

CHEMICAL & BIOLOGICAL CONTROL

Evaluation of novel blue and green label alternative chemistries

Evaluation of biological agents and plant resistance inducing chemistries

TRAIT DEVELOPMENT

Classical and emerging technologies to introduce novel sources of resistance from:

ancestral species

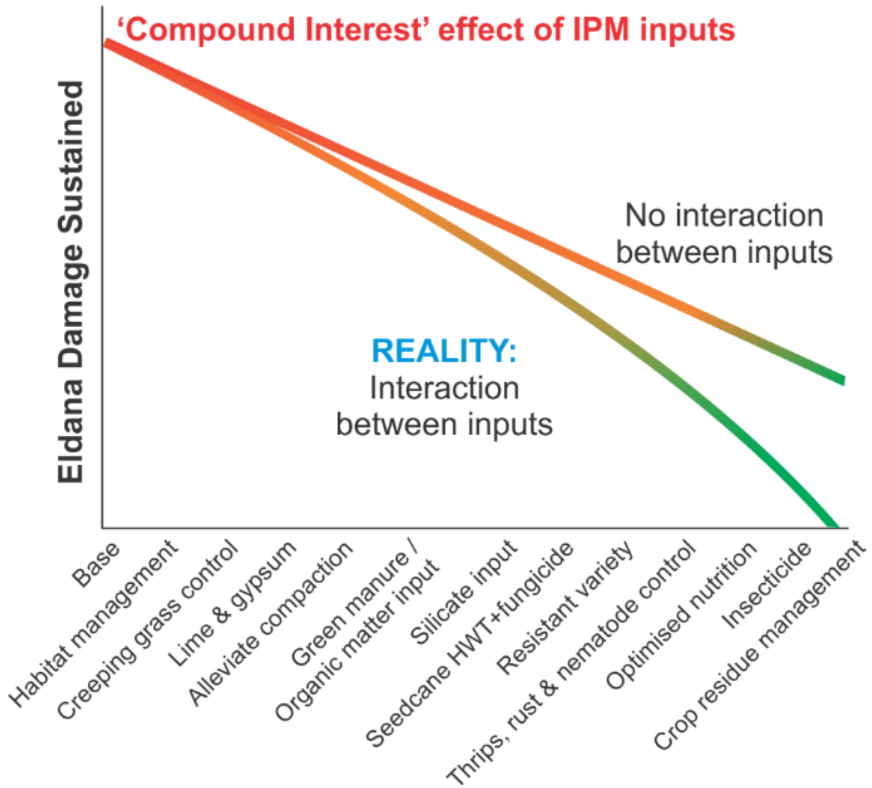
closely related species, unrelated species

INTEGRATED PEST MANAGEMENT

| Sustainability | Resilience | Innovation |

Efficacy of Eldana IPM is dependent on the **sum** of the **several technologies** and **practices** that comprise the toolkit

Whilst **individual** technologies and practices may **decrease population size**, effective and **sustainable** Eldana control on an area-wide basis requires the co-ordinated implementation of as many of the IPM technologies and practices that is practicable

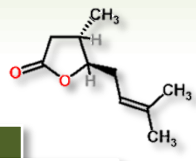


Source: SASRI Eldana IPM Manual

INTEGRATED PEST MANAGEMENT

| Sustainability | Resilience | Innovation |

STERILE INSECT TECHNOLOGY: PROGRESS



ELDANA TRAP

1980s

- Complexities of Eldana calling described (VOC and acoustic cues)
- Eldanolide identified

1990s

- Other terpenoid, aromatic and unbranched chain compounds identified in wing glands

2000s

- Eldanolide synthesised for research purposes
- Pheromone (eldanolide) traps as effective as light traps
- Synthesis of eldanolide for commercial applications prohibitively expensive

2010s

- Progress stalled

DEPARTMENT OF CONSERVATION ECOLOGY AND ENTOMOLOGY

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THRIP TECHNOLOGY AND HUMAN RESOURCES FOR INDUSTRY PROGRAMME

ELDANA SIT

- Eldana irradiation biology and moth marking technique established
- Eldana rearing QC and thermal biology established for SIT
- SIT efficacy demonstrated in confined release studies on potted cane
- Pilot SIR Project: Release of F1 irradiated males at release site placed on-hold
- Transport and handling requirements for irradiated Eldana under investigation

DEPARTMENT OF LOGISTICS

sasri ©2014

ELDANA SIT

- Modeling to aid SIT decision-making

INTEGRATED PEST MANAGEMENT

| Sustainability | Resilience | Innovation |

Sterile Insect Technology as a Component of Eldana IPM

KEY CONSIDERATIONS FOR DISCUSSION

POTENTIAL EFFICACY OF SIT IN ELDANA CONTROL: **DEMONSTRATED**

- Thermal and radiation biology of Eldana established.
- Contained-release (cage) studies on potted sugarcane plants indicate significant reduction in Eldana damage through SIT.

PROOF-OF-CONCEPT OF SIT: **REQUIRED**

- Irradiation facility for KZN.
- Increased Eldana rearing throughput.
- Proof-of-concept pilot release programme.

A BUSINESS CASE: **REQUIRED**

Outcomes of pilot release programme to enable Business Case preparation.

INVESTMENT PARTNERSHIPS: **REQUIRED**

Business Case to secure investment partners for independent commercially-viable enterprises.

Estimated (speculated) that …

- Release of ~300 million irradiated Eldana moths per week required per sugar mill area
- A facility equivalent to XSIT in Citrusdal required per mill area

Summary

- Eldana eradication is probably not possible
    - *however* SIT and Bt are complimentary approaches
    - Proven for pink bollworm control in cotton in USA

- ‘go-ahead’ for the Bt approach has been given
    - *but* impact will not be felt for at least 20 years
    - -candidate genes: Bt Cry1Ac, Bt Cry2Ab, ALS, Fumonisin esterase

- Irradiator acquisition for proof of concept Pilot SIT a problem
    - *but* see our poster –F1: Fast tracking *Eldana saccharina* moths for Sterile Insect Release.