

Environmental Risks of DMH-11, *Varuna barnase* & EH- 2 *barstar*, AND Unscientific Biosafety Trials

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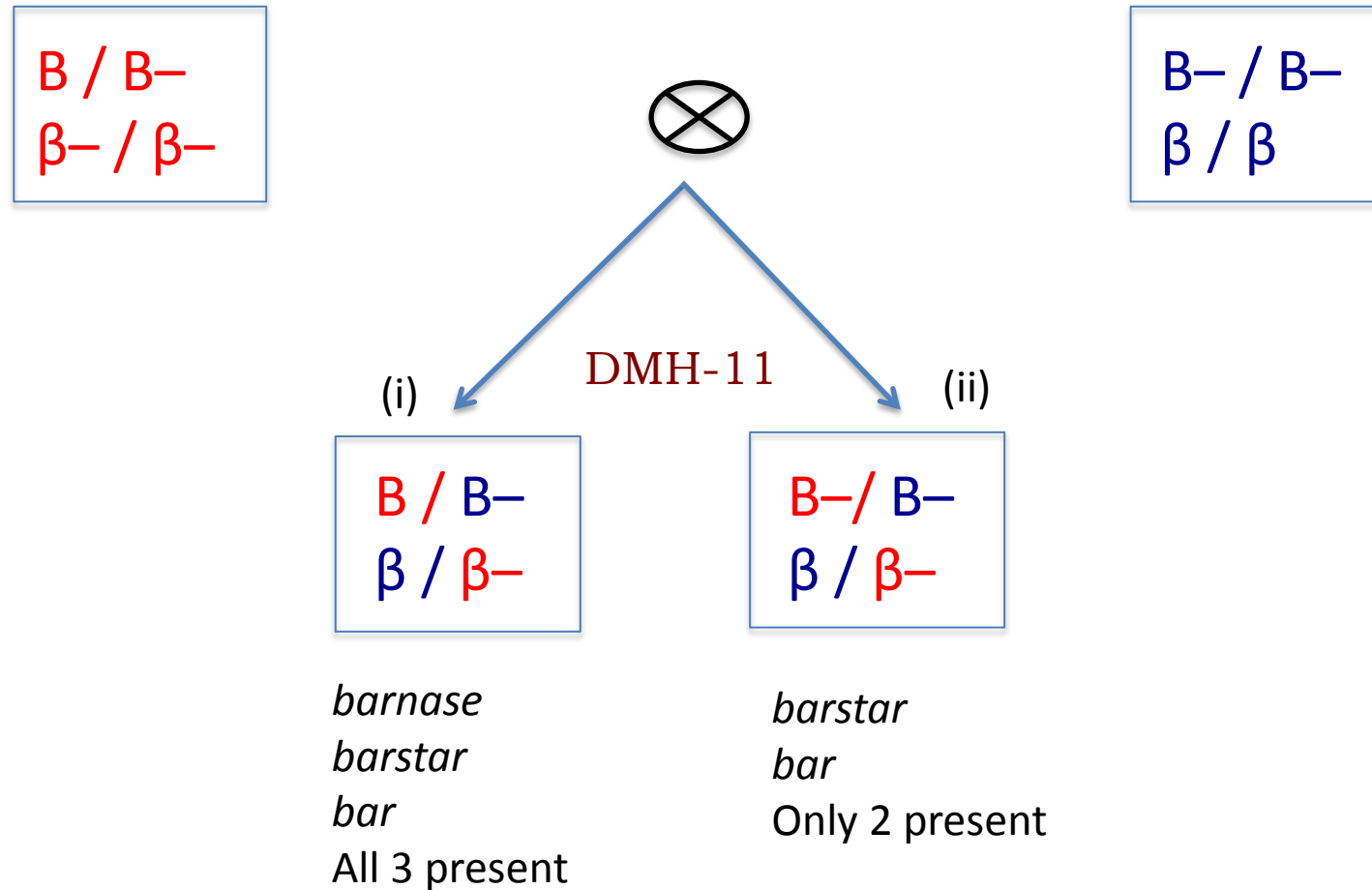
Presentation will cover...

- 1. Risk of MS (Male Sterility) Trait*
- 2. Spread of HT (Herbicide Tolerance) Trait*
- 3. Breakdown of MS*
- 4. Flaws in Protocols & Methods adopted*
- 5. Crossability between Indian Mustard and Relatives*

The Risk of *barnase* Male Sterility Transmission

1. Gametes from GM MS plants contain *barnase* gene.
2. The *barnase* transgene-induced MS is capable of transmission to non-GM plants.
3. After outcrossing, the recipient non-GM plant will bear seeds with hemizygous *Barnase/B-* combinations.
4. Farm saved seed will contain male sterility

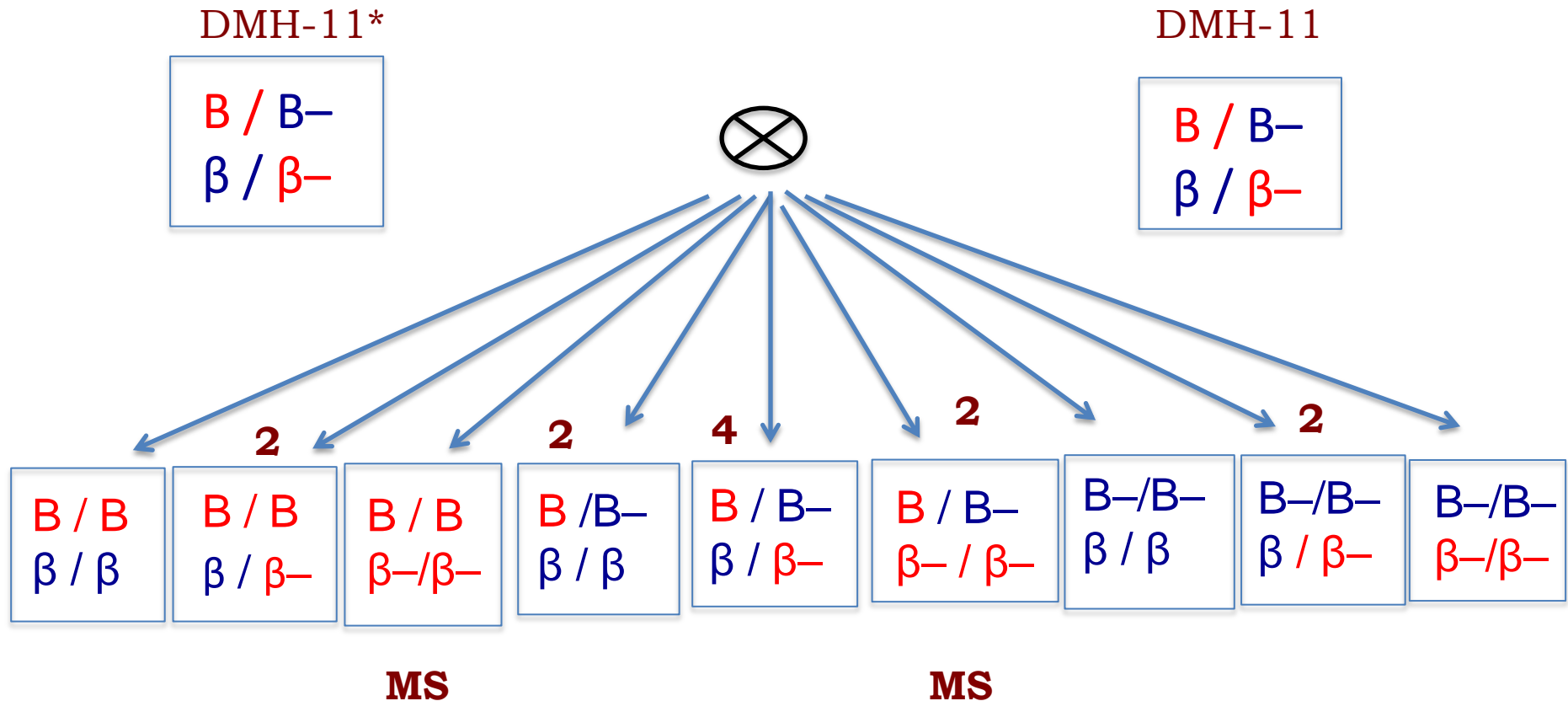
(THIS BUILDS IN INDIAN REALITY OF JUST 25% TO 50% SRRs IN SEVERAL STATES – Not national SRR figures which are misleading in terms of state level variation).



In All Reports, DMH-11 is Referred to as (i) Above.
How was (ii) Eliminated?

B is Barnase; B- is absence of Barnase; β is Barstar; β- is absence of Barstar

SCENARIO WITH FARM SAVED SEED OF DMH11



**FARMER WILL END UP WITH 18.76 % MS SEED;
6.25% herbicide susceptible**

* Based on DMH-11's protein expression studies & with a Q as in earlier slide

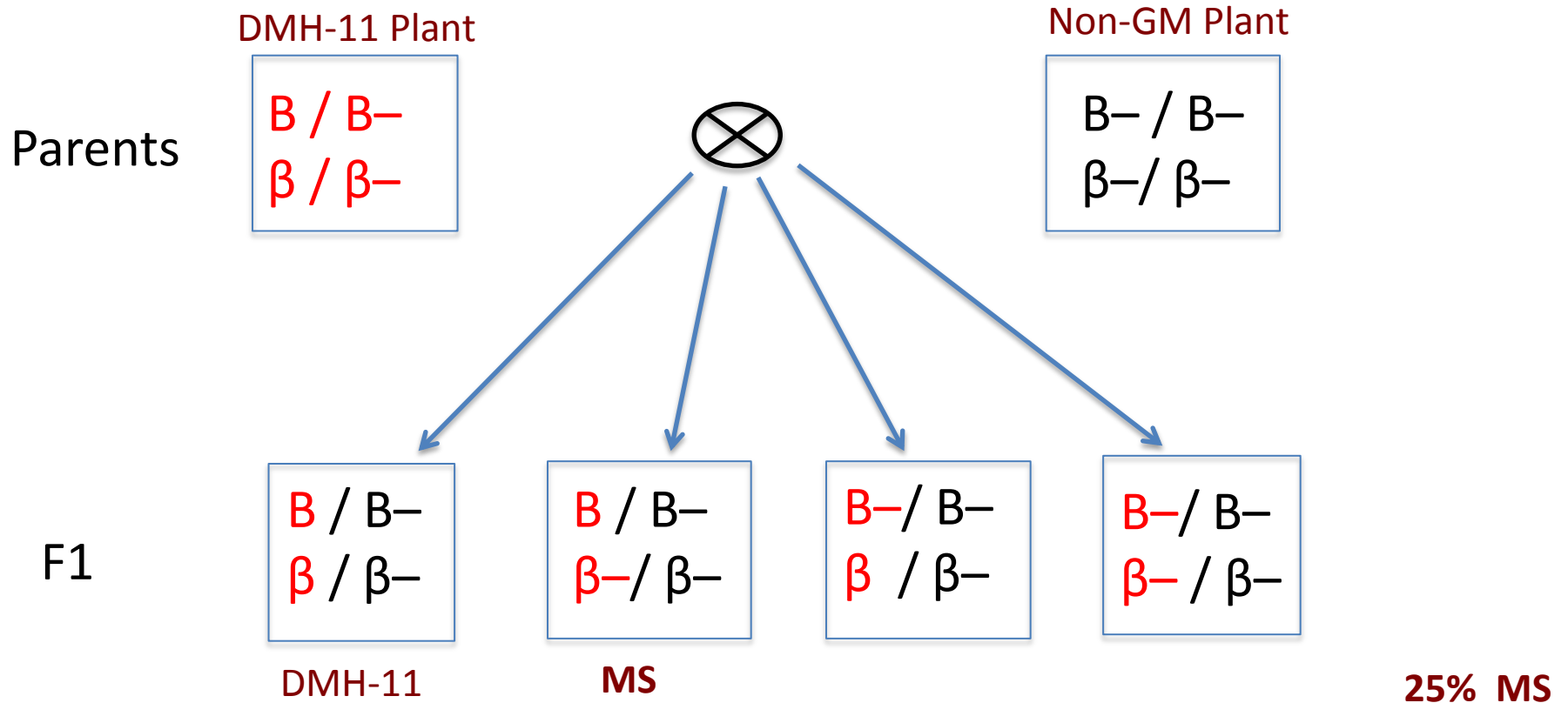
Natural Outcrossing in *B. juncea*

- Pollen flow studies did not measure rate and level of outcrossing.
- However, the Outcrossing Range (from literature) is **20%** to **30%**.
- The Range of Outcrossing on Individual Basis is from **7.1%** to **47.1%** (based on glossiness marker).
 - Singh et al. 2011. *Cruciferae Newsletter* 30: 55-7.

Therefore, DMH-11's outcrossing with neighboring non-GM crop can be upto 30% (and upto 47.1% for Individual Plants).

After the First Year of Out-Crossing with Non-GM Mustard, the F1 Progeny of the contaminated crop will contain 25% MS.

SCENARIO WHEN DMH-11 OUTCROSSES WITH NON GM MUSTARD

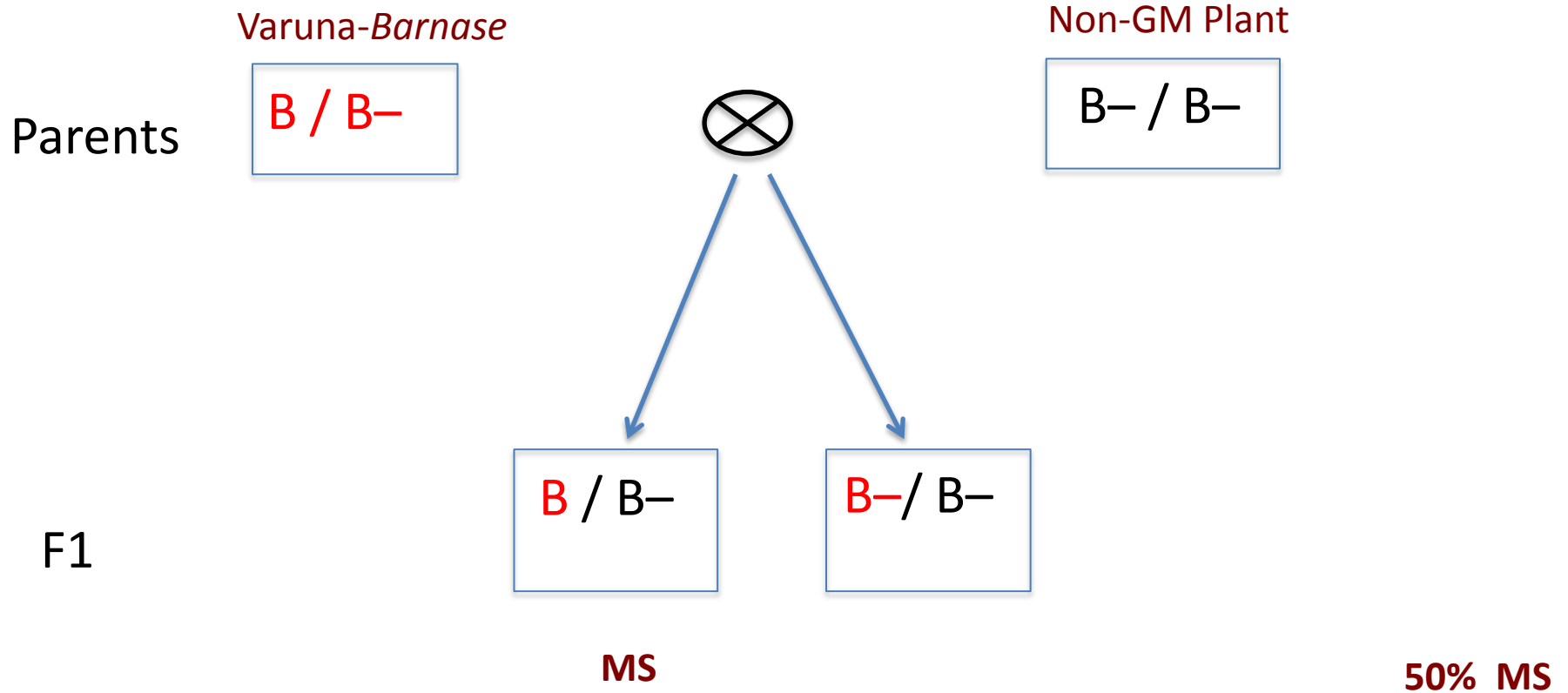


THIS OUTCROSSING WILL PRODUCE 25% DMH-11 AND 25% MALE STERILE OFFSPRING.

Application of Herbicides will Eliminate 25% of the F1 Population.
In the Absence of Herbicide Spray, Panmixis in F1 will Produce Hybrids with 14.29% MS Phenotype in F2.

HOWEVER, ADDITION OF DMH-11 POLLEN IN SUBSEQUENT GENERATIONS WILL INCREASE MALE STERILITY PERCENTAGE.

SCENARIO WHEN Varuna-*Bn* OUTCROSSES WITH Non-GM Mustard



THIS OUTCROSSING WILL PRODUCE 50% MALE STERILE OFFSPRING.

Application of Herbicides will Eliminate 50% of the F1 Population.

In the Absence of Herbicide Spray, Panmixis in F1 will Produce Hybrids with 50% MS Phenotype in F2

2. HERBICIDE RELATED YIELD LOSSES

- In All the Transgenic Varieties (Parental lines & hybrid), Glufosinate Spray will keep all plants intact (100% HT crop).
- However, 6.25% yield loss in NEXT GENERATION for Farm Saved Seeds of DMH-11
- In Case of DMH-11 Outcrossing with A Non-GM Mustard, Glufosinate Application will Cause 25% Loss in First Generation

Implications for Mustard Farmers

- Any farm saved seed (from DMH-11 or from contaminated non-GM crop) will have male sterility ranging from 18% to 25%. This will have yield implications
- Farmers, to avoid such losses, will be forced to turn to external seed sources – THIS IS LOSS OF SOVEREIGNTY & mustard GENETIC DIVERSITY
- This is quite apart from Herbicide Related Impacts

3. Male Sterility Breakdown in Varuna bn3.6

- The Central Compliance Committee field visits note this in ALL trial locations in 2014-15 in BRL-II
- Non-standardisation of test protocol an issue as seen in BRL reports
- Tabulated data on seed set in bagged MS branches from Ludhiana indicate **7.43%** breakdown of MS.
- Lies in summing up : “No sterility breakdown observed in extensive backcrosses and numerous field observations”; “Pollination behaviour of all the lines remained true to their expected characteristics.”
- **Q is how much of the Yield of Varuna-Bn line is due to Male Sterility Breakdown and how much due to Cross Pollination?**

4

Unreliable Methodology and Spurious Data

Crop Developers State with Reference to Pollen Flow That:

1. "The Maximum Distance of Bee Pollination is **20 m.**" !!

Bee Pollination Distance Can Exceed 6 km.

[see Pasquet et al. 2008. *PNAS* 105: 13456-61]

Specifically for Mustard, "gene flow was detected at the **800 m limit** of the experiment." [Beckle et al. 2003. *Ecol. Appl.* 13: 1276-1294]

2. "Farmers who want to keep their own [*sic*] seed are advised to collect seed from the centre of the field."

It's Unreasonable to Assume that Bees will Visit only the Peripheral Plants, and Plants at the Centre of the Field will only Self-Pollinate!

3. *Even Assuming Short Pollination Distance (we question study protocol),
Rate & Extent of Outcrossing NOT Studied.*

4. No Pollen Flow Studies at all For Parental Lines!

Test for Aggressiveness and Weediness

- BEHAVIOUR OF WILD RELATIVES DUE TO TRANSFER OF THE NEW TRAITS/ TRANSGENES NOT STUDIED.
- FOR NEITHER THE PARENTAL LINES NOR DMH-11, NO STUDY UNDERTAKEN TO EXAMINE POSSIBLE COMPETITIVE ADVANTAGE OF GM MUSTARD LINES WITH HERBICIDE EXPOSURE
- VOLUNTEER BEHAVIOUR IN SUBSEQUENT SEASONS NOT STUDIED.
- Even Data related to Plant Height or Biomass Production has not been statistically analysed across all locations: Weediness Potential of NEITHER DMH-11 NOR ITS PARENTS is NOT clear with the INADEQUATE DATA & ANALYSES.

Pod Shattering

- A very important trait for farmers
- More importantly, this trait is related to biosafety risks (weediness, contamination of non-GM etc.)

Yet, for Neither the Parental Lines (VarunaBn and EH2 modbs) Nor the F1 Hybrid (DMH-11): Pod Shattering trait was studied in any quantitative manner

Protein Expression Levels

- Varuna *barnase* does not have any expression of barnase protein even in whole bud. What explains this?*
- What explains such variability in protein expression levels across different reports? For instance, bar protein expression in whole bud of DMH-11 being 14.84 mg/kg as per April 2014 report and 0.31 only in 2015 report?

(To the first point, the GEAC scientists explained that because the tapetum of the Varuna-Barnase 3.6 mustard is killed, no barnase protein could be detected)*

Precision? Predictability?

Summary Report (p. 9) states **Variable Expression of *bar* gene**

- “from location to location
- from plant to plant in the same line
- from leaf to leaf in the Same Plant”

If this Unpredictability and Uncertainty Prevails Even in the Plants HOMOZYGOUS for *bar* gene,

* What is the Level of Certainty of Expression of the Other Transgenes?

* How Truthful is the Promise to Farmers of the Crop's Advantage?

Counts of Beneficial Insects

- *Predators*: Coccinellid Beetles, Lacewings, Syrphid Larvae
- *Pollinators*: Honeybees (1 sp.)
- No Other Taxa are Considered in the Tests.

Mustard fields are habitats for

Ants *e.g.* 2 spp. (Roy et al. 2014)

Gryllids *e.g.* 2 spp. (Amer 2011)

Hoppers *e.g.* 2 spp. (Banuelos et al. 1999)

Spiders *e.g.* 39 Spp (Rana et al. 2016)

Butterflies *e.g.* 7 spp. (Roy et al. 2014)

WHY NO EFFORT TO TEST EFFECTS OF GMO ON
THESE ARTHROPODS?

Periodic Observation Results:

Unreliable, with No Complementary Method Employed

- For Aphids
 - Suction Sampler; Drop Cloth; Sticky Card
- For Beetle Adults
 - Drop Cloth, Vacuum/ Suction, Sweep net, Pitfall trap, Videography
- For Beetle Larvae
 - Drop Cloth, Sweep Net, Pitfall trap
- For Honeybees (which species? Only one? Really?)
 - Sweep Net; Soap bowl; Videography
- For Lacewings
 - Sweep Net, Videography

Pedigo & Buntin 1993. *Handbook of sampling methods for arthropods in agriculture*;
Woltz & Landis 2014. *J. Appl. Entomol.* 138: 475–484

Counts of Insect Pests: An Ecologically Unfeasible Scenario

Trial	Location	Mustard Aphid	Painted Bug	Leaf Miner	Cabbage Butterfly	Mustard Sawfly	Termites
BRL-I, 1st Year (2010-11)	Kumher	✓	Nil	Nil	Nil	Nil	Nil
	Alwar	✓	✓	Nil	Nil	Nil	Nil
	SG nagar	✓	Nil	Nil	Nil	Nil	Nil
BRL-II, 2nd Year (2011-12)	Kumher	✓	Nil	Nil	Nil	Nil	Nil
	Alwar	✓	Nil	Nil	Nil	Nil	Nil
	SG nagar	Nil	Nil	Nil	Nil	Nil	Nil
BRL-III, 3rd Year (2014-15)	Delhi	✓	Nil	Nil	Nil	Nil	Nil
	Bhatinda	Nil	Nil	Nil	Nil	Nil	Nil
	Ludhiana	Nil	Nil	Nil	Nil	Nil	Nil

Ref : 1) BRL Trial Reports

STATISTICS IN THE TRIAL: INACCURATE & UNRELIABLE

Predators	Varuna-Barnase	EH-2	DMH-11	Questions
Coccinelids (Grubs + Beetles) 60/70/80/90/100/110/120 DAS	8.75	6.50	10.25	Mean per Replicates or per Day of test?
<i>Chrysoperla</i> Larvae	Nil	Nil	Nil	
Honey bee visits per 5 minutes	13.1	15.56	19.44	<i>ditto</i>
Honey bee visits per 10 minutes	26.94	31.94	36.63	<i>ditto</i>

Doctored Report:

INSECT DATA (Rabi 2011)

	Mustard Aphid	All Other Prey	COCCINELID ABUNDANCE
Tables 8 A & 10 A	4.9	Nil	Nil
Tables 8 B & 10 B	3.26	Nil	10.25
Tables 8 C & 10 C	Nil	Nil	Nil

A Global Ecological Rule

* Low or No Predator in Presence of Prey is Possible.

* When Prey Abundance is Low, 3 Times Higher Predator Abundance is Ecologically Unfeasible.

- The Trial Report Boldly Defies This Rule !

Honey Bee Visitation Counts : *What Exactly was Counted?*

A) **Foraging Rate** → The No. of Flowers Probed by an Individual Bee in X minutes

e.g. Honeybees: 9.2 Flowers/min; Beetles: 2.2/min

(Couvillon et al. 2015 *Psyche*, Article ID 134630)

B) **Visitation Counts** → No. of Bees Observed per Flower in X minutes

e.g. *A. dorsata*: 7/ Fl/ 5 min; *A. florea*: 1/ Fl/ 5 min.

(Balachandran et al. 2014 *Curr. Sci.*106: 1379-86)

C) **Visitation-Abundance** → No. of Bees in a Patch of Flowers in X minutes

e.g. 7.53 Bees/ 10 Flowers/ 5 min.

(Sivaram et al. 2013 *Braz. Arch. Biol. Tech.* 56: 365-70)

ALL Trial Reports: Incompetent Methodology: Untrustworthy.

Statistical Fallacy, Again!
Bee Visitation in 10 min Cannot Be
Double that in 5 min in the Field!!
(– Unless The Bees are Tutored)

Predators	Varuna-Barnase	EH-2	DMH-11
Honey bee visits per 5 minutes	13.1	15.56	19.44
Honey bee visits per 10 minutes	26.94	31.94	36.63

**At this rate, Honey Bee Visits to DMH-11 would Exceed 2332
if Observed for 10 hr !**

Crossability Studies

- The Report *Falsely* Claims: “No Reports are Available on Naturally Occurring Inter-specific Crosses among Cultivated and Wild Species of *Brassica* Cenospecies in India.”
- MANY Studies DO Report Natural Crossability between *B. juncea* and *B. napus*, (Oilseed Rape) and *B. rapa* (Yellow Sarson).

Bing et al. 1996; Gupta 1997; Choudhary & Joshi 1999; Ghosh Dastidar & Varma 1999; Choudhary et al. 2002; Gupta et al. 2006

The Frequency of Hybridization between *B. napus oleifera* and *B. juncea* in Nature is 5.91%.

Salisbury 2006

**INCIDENTALLY, PARENTAL LINES WERE NOT TESTED FOR
CROSSABILITY**

CURRENT TESTING & DATA RELATED
TO Varuna-barnase, EH2 modbs and
DMH-11 TRANSGENIC MUSTARD
LINES INCOMPLETE, COMPLETELY
UNRELIABLE AND EVEN DOCTORED
FOR ANY DECISION-MAKING

MANY RISKS REMAIN UNASSESSED

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