



# Response surface designs for crop protection products mixture justification and optimization

JJ Schott  
29/06/2017

Sommertagung AG Landwirtschaftliches Versuchswesen  
Neustadt/Weinstraße

# Objective of the project

- Region : EAME
- Crop : potato
- Target : leaf blight (*Phytophthora infestans*)



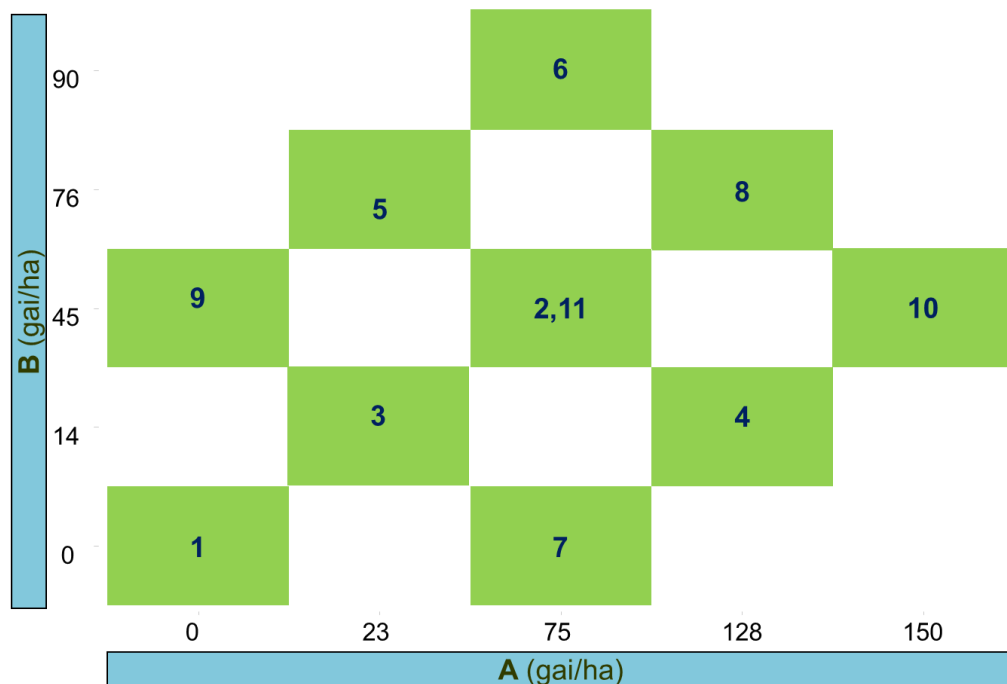
- Justify the ratio and rate of two active ingredients (ai) A and B in mixture
- Define the contribution of the single ai to the mixture
- Define the minimum amount of ai needed to achieve a certain level of efficacy (Eff)

# Protocol

- Experimental design :
  - randomised complete block design (RCBD)
  - 4 replicates
  - small plots (20 to 40 m<sup>2</sup>)
  - 10 trials in 2015 in EAME (3 NL, 3 BE, 1 FR, 2 DE, 1 PL)
- Treatment list structure :
  - response surface design
- Assessments : disease severity (% area) visual estimation at regular intervals after application and Area Under Disease Progression Curve (AUDPC) is calculated with trapezoidal method within each trial, treatment and replicate.
- Efficacy (Abbott's formula) :  $(AUDPC_{CHECK} - AUDPC_{TREAT})/AUDPC_{CHECK} * 100$

# Treatment list structure for response surface design

TRT	A (gai/ha)	B (gai/ha)
1	0	0
2	75	45
3	23	14
4	128	14
5	23	76
6	75	90
7	75	0
8	128	76
9	0	45
10	150	45
11	75	45



Central Composite Design  
 Display and Modify Design

Axial Value:

Rotatable 1.414

Orthogonal 1.078

On Face 1.000

User Specified 1.414

Inscribe

Source : DOE Response surface design JMP® 12.0.0

# Response surface design evaluation for A and B

- Design evaluation

## Power Analysis

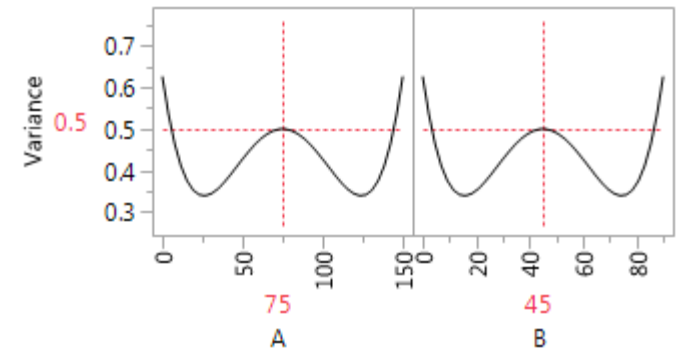
Significance Level 0.05  
 Anticipated RMSE 3.5 -> From Historic trials

Term	Anticipated	
	Coefficient	Power
Intercept	6	0.455
A	-9.21231	0.969
B	-2.94454	0.255
A*A	8.125	0.472
A*B	4.5	0.17
B*B	1.125	0.058

## Design Diagnostics

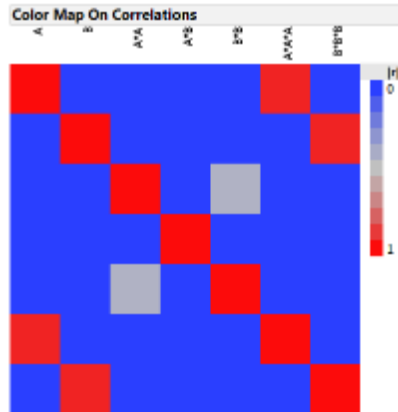
I Optimal Design  
 D Efficiency 25.19842  
 G Efficiency 50.09189  
 A Efficiency 16  
 Average Variance of Prediction 0.544444  
 Design Creation Time (seconds) 0

## Prediction Variance Profile



## Design and Anticipated Responses

Anticipated Response	A	B
6	75	45
20	22	13
5	128	13
10	22	77
6	75	90
10	75	0
4	128	77
26	0	45
4	150	45
6	75	45

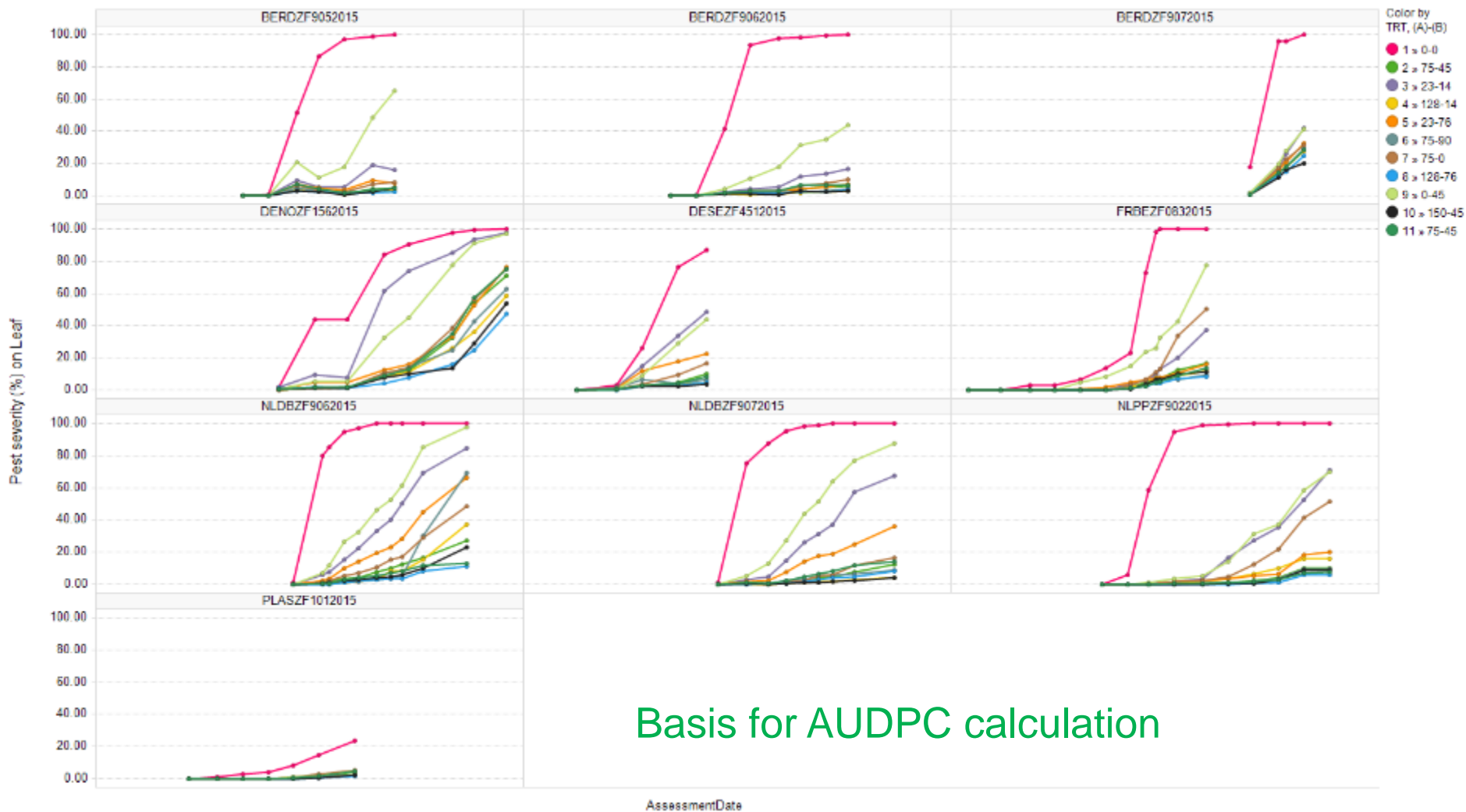


## Alias Matrix

Effect	A*A*A	B*B*B
Intercept	0	0
A	0.75	0
B	0	0.75
A*A	0	0
A*B	0	0
B*B	0	0

I Optimal Design : minimizes the average variance of prediction over the region of the data (appropriate for response surface model)

# Results of the 10 successful trials



# Single trials response surface model on efficacy

- Response variable : Efficacy (%)

- Explanatory variables :

- Replicate
- A (0 to 150 gai/ha)
- B (0 to 90 gai/ha)

- Second degree polynomial model :

$$\text{Efficacy} = \mu + \text{Replicate} + A + B + A^2 + B^2 + A*B + \epsilon$$

# Single trials response surface analysis results on efficacy

Summary of fit											
	BERDZF905	BERDZF906	BERDZF907	DENOZF156	DESEZF451	FRBEZF083	NLDBZF906	NLDBZF907	NLPF902	PLASZF101	Grand Total
Mean	87.14	83.26	58.11	66.93	82.66	85.44	79.35	87.20	90.81	89.87	81.08
RMSE	5.73	5.66	5.60	7.79	7.06	8.44	7.74	6.74	7.11	2.24	6.41
R <sup>2</sup>	0.77	0.63	0.66	0.87	0.85	0.66	0.86	0.87	0.67	0.76	0.76
CV (%)	6.58	6.80	9.63	11.64	8.54	9.88	9.76	7.73	7.83	2.50	8.09

Effects tests (p-values)											
Replicate	0.86	0.33	0.02	0.02	0.05	0.67	0.01	0.00	0.47	0.67	
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A*A	0.00	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09
B	0.16	0.20	0.11	0.00	0.02	0.01	0.06	0.08	0.00	0.00	0.00
B*B	0.46	0.58	0.16	0.96	0.05	0.66	0.04	0.68	0.12	0.88	0.88
A*B	0.47	0.22	0.05	0.00	0.01	0.64	0.25	0.01	0.09	0.41	0.41

Significant alpha 5%

Solution (gai/ha)											Average**
A (0-150)	110	116	140	115	124	101	114	108	100	513*	114
B (0-90)	33	47	29	71	39	117*	50	61	66	-1156*	50

\* Solution outside data range

\*\* Average without outside range solutions



# Multi trials ANOVA on efficacy

## Summary of Fit

RSquare	0.929963
RSquare Adj	0.896502
Root Mean Square Error	5.367631
Mean of Response	81.0764
Observations (or Sum Wgts)	400

## Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	129	103292.89	800.720	27.7917
Error	270	7779.10	28.811	<b>Prob &gt; F</b>
C. Total	399	111071.99		<.0001*

## Effect Tests

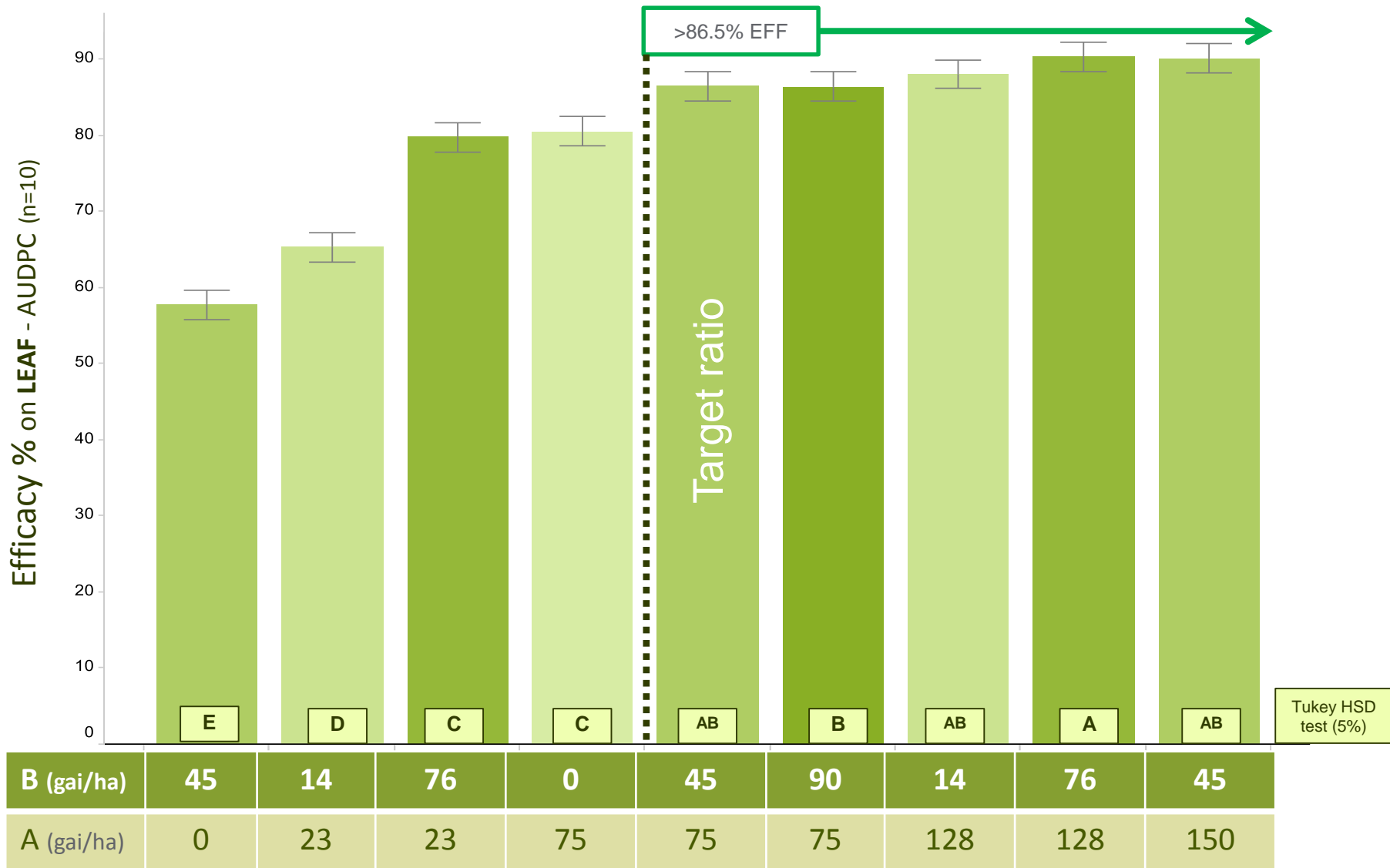
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
TIAL Trial Number	9	9	40121.249	154.7272	<.0001*
ASSE Repetition no[TIAL Trial Number]	30	30	3674.046	4.2507	<.0001*
TRT Treatment Num	9	9	44285.672	170.7872	<.0001*
TRT Treatment Num*TIAL Trial Number	81	81	15211.925	6.5183	<.0001*

Tukey HSD test (5%)

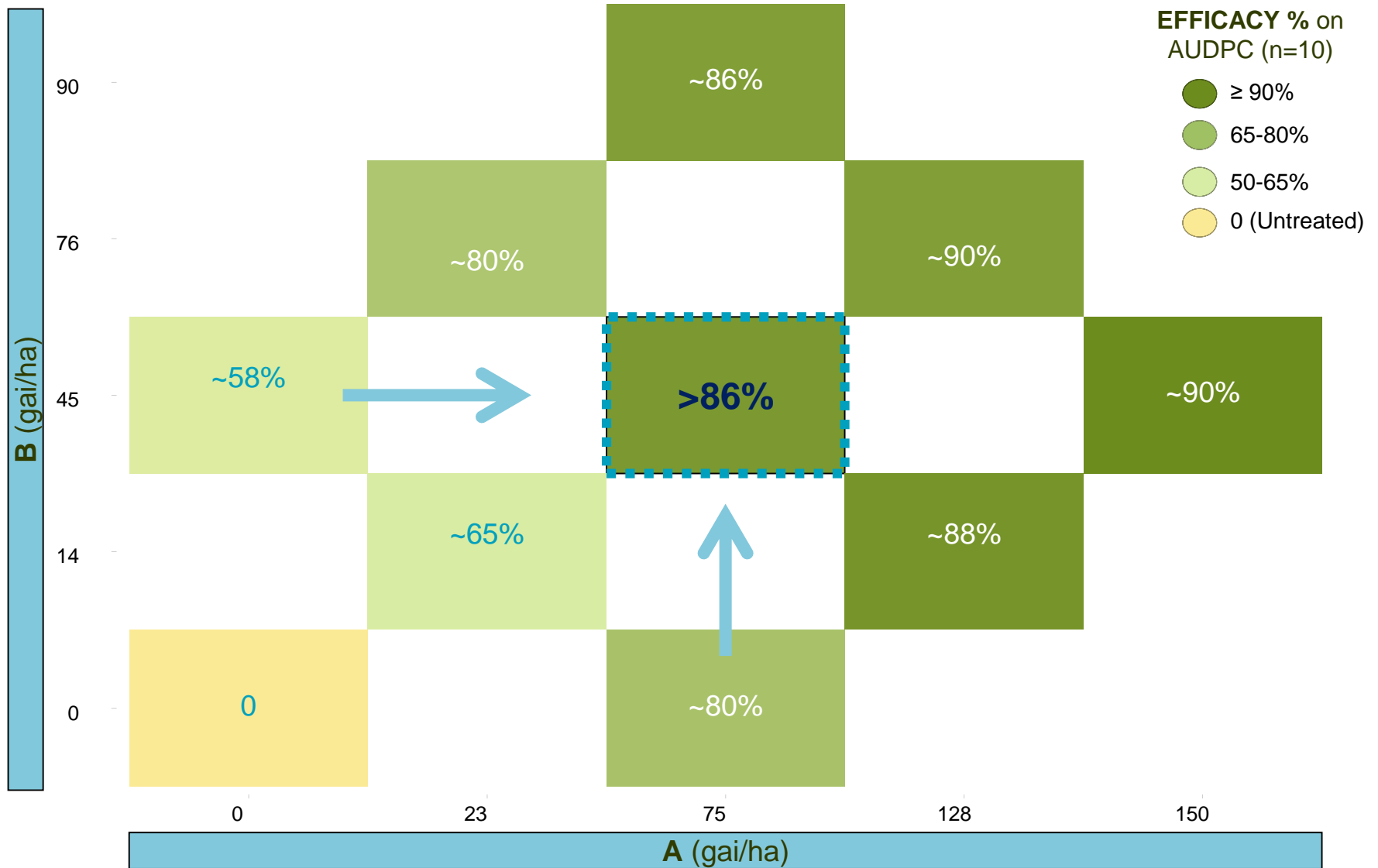
Level	Least Sq Mean
8 A	90.334250
10 A B	90.092000
4 A B	88.037500
2 A B	86.546250
11 B	86.466500
6 B	86.365250
7 C	80.504750
5 C	79.579250
3 D	65.251000
9 E	57.587250

Levels not connected by same letter are significantly different.

# Multi trials mean separation test on efficacy



# Multi trials heat map on efficacy



## Multi trials response surface **complete** model

- Response variable : Efficacy (%)
- Explanatory variables :
  - Trial
  - Replicate within trial
  - A (0 to 150 gai/ha)
  - B (0 to 90 gai/ha)
- Second degree polynomial complete model :
$$\text{Efficacy} = \mu + \text{Trial} + \text{Replicate}(\text{Trial}) + A + B + A^2 + B^2 + A*B + \text{Trial}*A + \text{Trial}*B + \text{Trial}*A*B + \epsilon$$

# Multi trials surface response **complete** model

## Summary of Fit

RSquare	0.854711
RSquare Adj	0.823262
Root Mean Square Error	7.014248
Mean of Response	81.0764
Observations (or Sum Wgts)	400

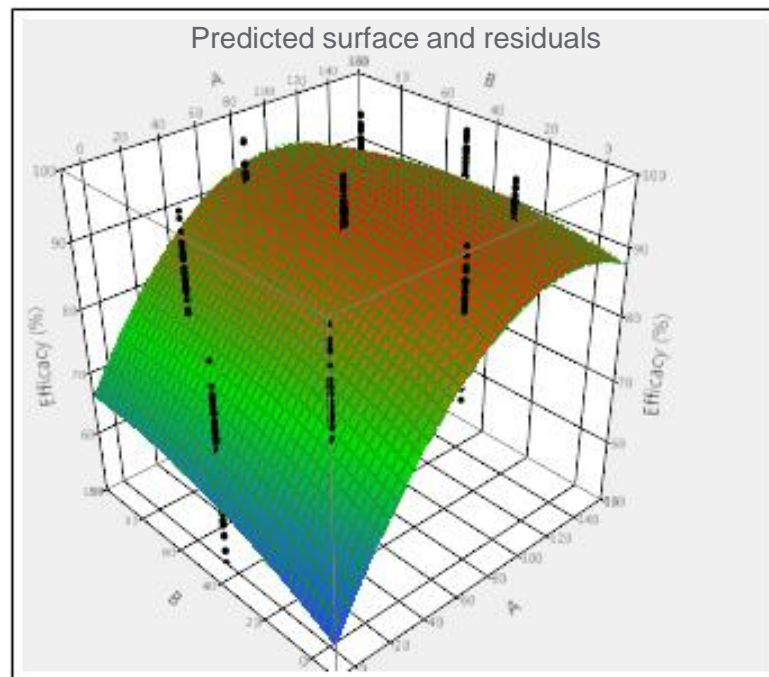
**AICc**  
2793.266

Source	LogWorth	PValue
TIAL Trial Number	82.749	0.00000
A	78.697	0.00000
A*A	24.088	0.00000
TIAL Trial Number*A	18.794	0.00000
B	13.112	0.00000
A*B	6.748	0.00000
ASSE Repetition no[TIAL Trial Number]	4.324	0.00005
TIAL Trial Number*B	1.793	0.01610
TIAL Trial Number*A*B	1.710	0.01948
B*B	1.292	0.05107

## Solution

Variable	Critical Value
A	110.55648
B	55.876824

Solution is a Maximum  
Predicted Value at Solution 95.006445



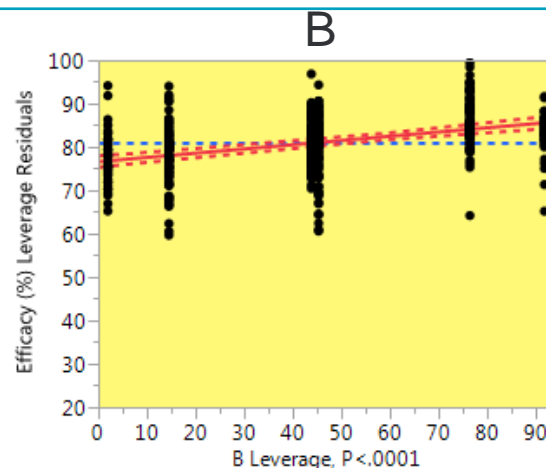
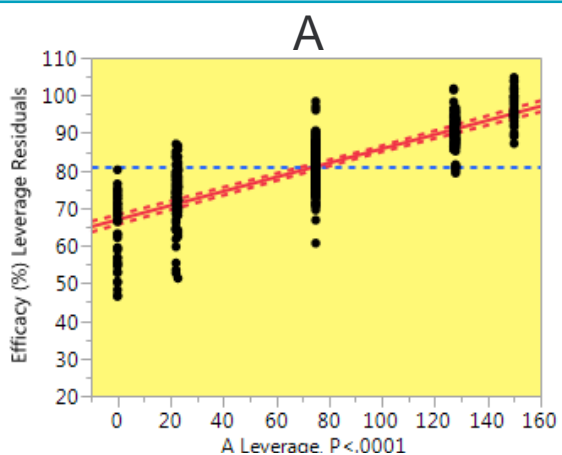
## Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
TIAL Trial Number	9	9	40121.249	90.6087	<.0001*
ASSE Repetition no[TIAL Trial Number]	30	30	3674.046	2.4892	<.0001*
A	1	1	31721.865	644.7576	<.0001*
B	1	1	3002.005	61.0168	<.0001*
TIAL Trial Number*A	9	9	6339.877	14.3178	<.0001*
A*A	1	1	6153.384	125.0696	<.0001*
TIAL Trial Number*B	9	9	1019.635	2.3027	0.0161*
A*B	1	1	1400.307	28.4617	<.0001*
B*B	1	1	188.638	3.8341	0.0511
TIAL Trial Number*A*B	9	9	991.044	2.2381	0.0195*

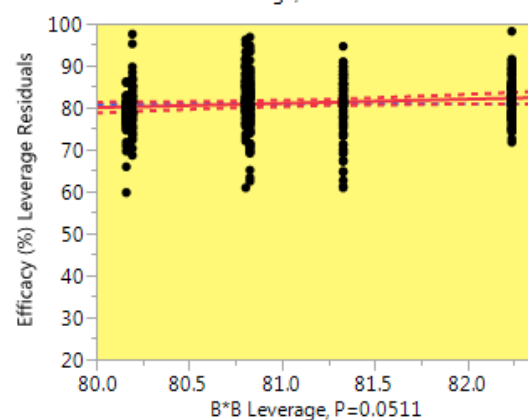
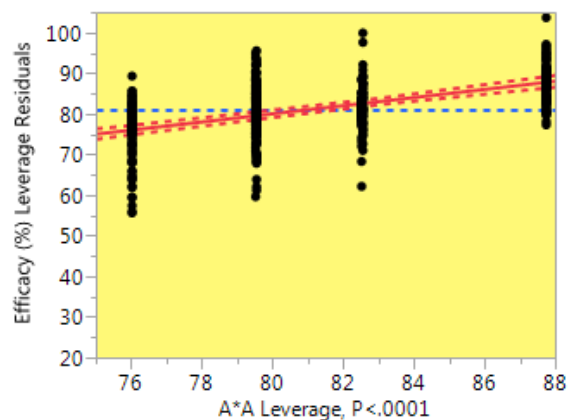
# Estimates of multi trials surface response **complete** model

Term	Estimate	Std Error	t Ratio	Prob> t	Lower 95%	Upper 95%
A	0.1887	0.0074	25.39	<.0001*	0.174	0.203
B	0.0976	0.0125	7.81	<.0001*	0.073	0.122
A*A	-0.0021	0.0002	-11.18	<.0001*	-0.002	-0.002
B*B	-0.0010	0.0005	-1.96	0.0511	-0.002	0.000
A*B	-0.0018	0.0003	-5.33	<.0001*	-0.002	-0.001

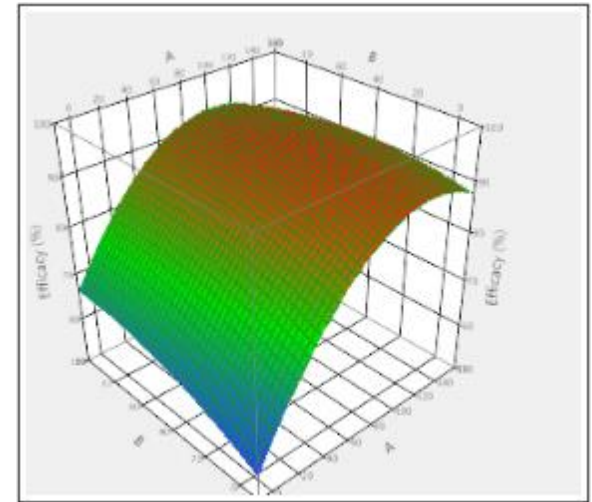
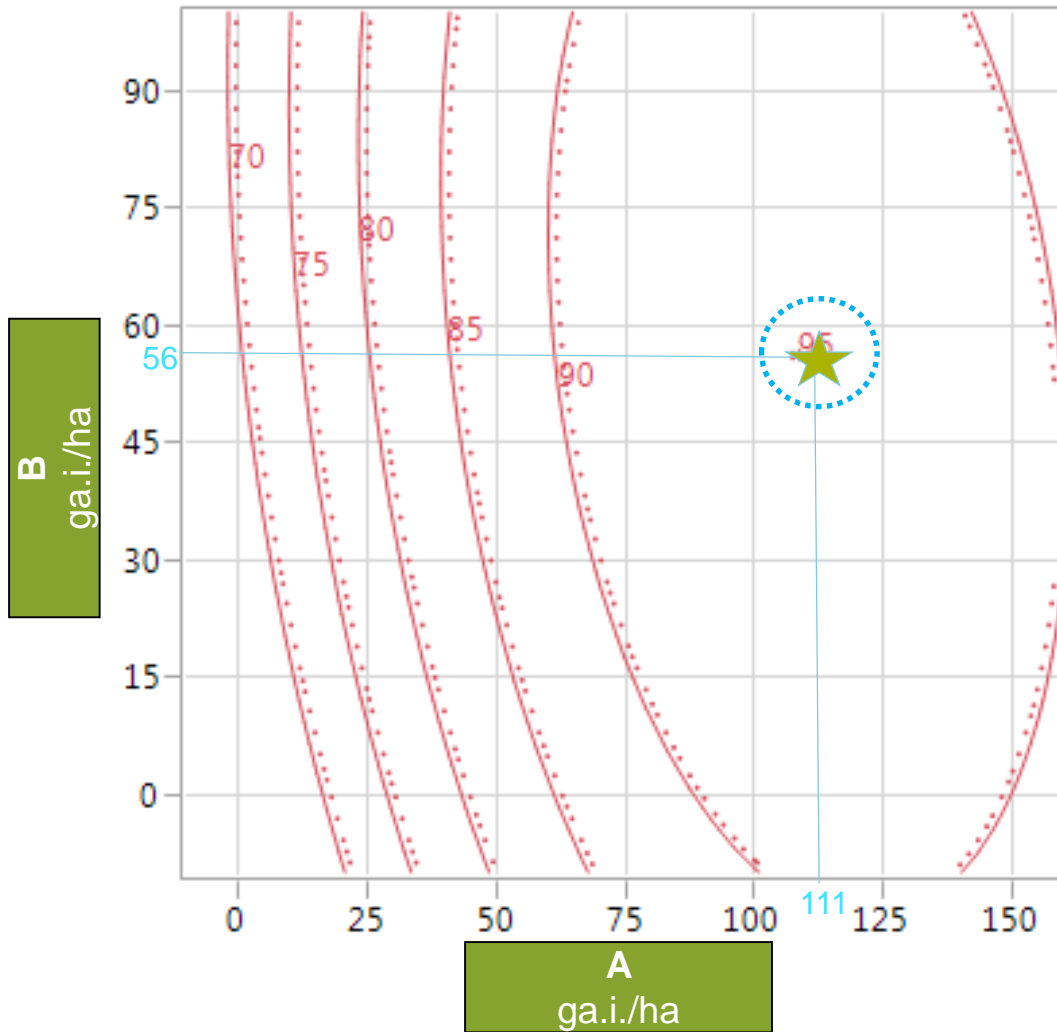
Linear



Quadratic



# Efficacy predicted curve of multi trials surface response **complete** model



## Multi trials response surface **reduced** model

- Response variable : Efficacy (%)
- Explanatory variables :
  - Trial
  - Replicate within trial
  - A (0 to 150 gai/ha)
  - B (0 to 90 gai/ha)
- Second degree polynomial reduced model :
$$\text{Efficacy} = \mu + \text{Trial} + \text{Replicate}(\text{Trial}) + A + B + A^2 + B^2 + AB + \epsilon$$



# Multi trials surface response **reduced** model

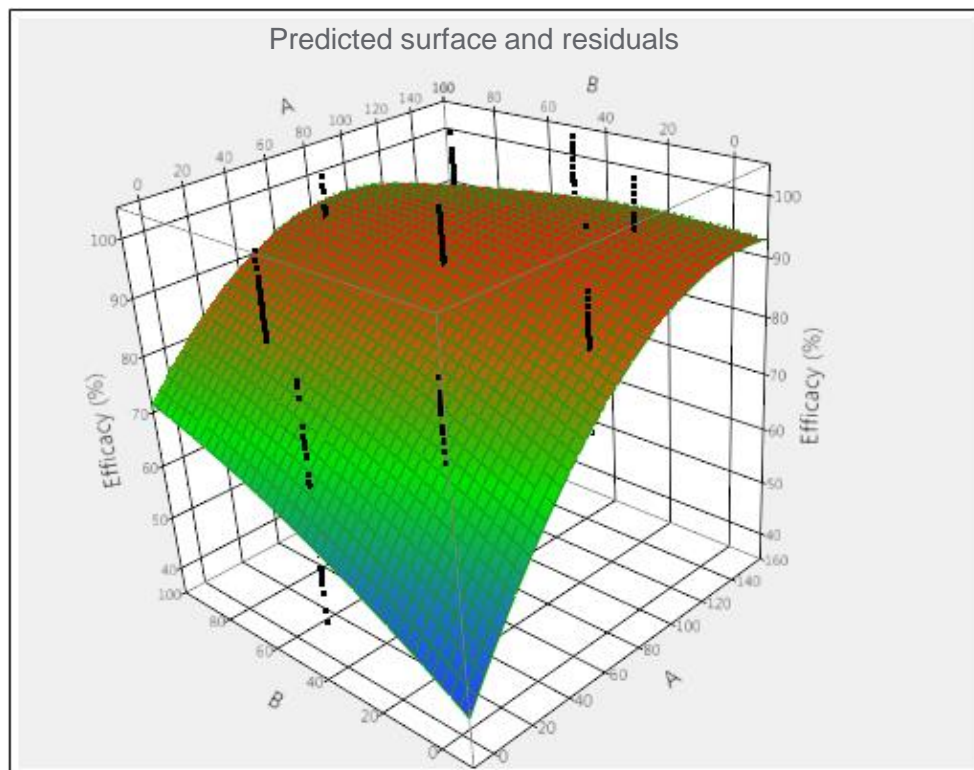
## Summary of Fit

RSquare	0.779685
RSquare Adj	0.752378
Root Mean Square Error	8.302526
Mean of Response	81.0764
Observations (or Sum Wgts)	400

### AICc

2884.909

Source	LogWorth	PValue
TIAL Trial Number	68.715	0.00000
A	65.333	0.00000
A*A	18.321	0.00000
B	9.822	0.00000
A*B	5.049	0.00001
ASSE Repetition no[TIAL Trial Number]	2.073	0.00845
B*B	1.005	0.09896



## Solution

Variable	Critical Value
----------	----------------

A 114.89916

B 57.723324

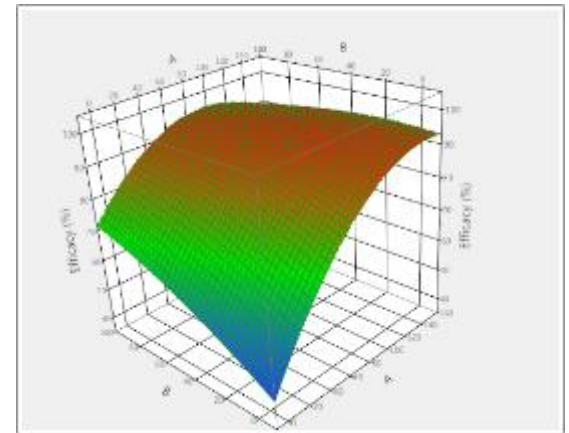
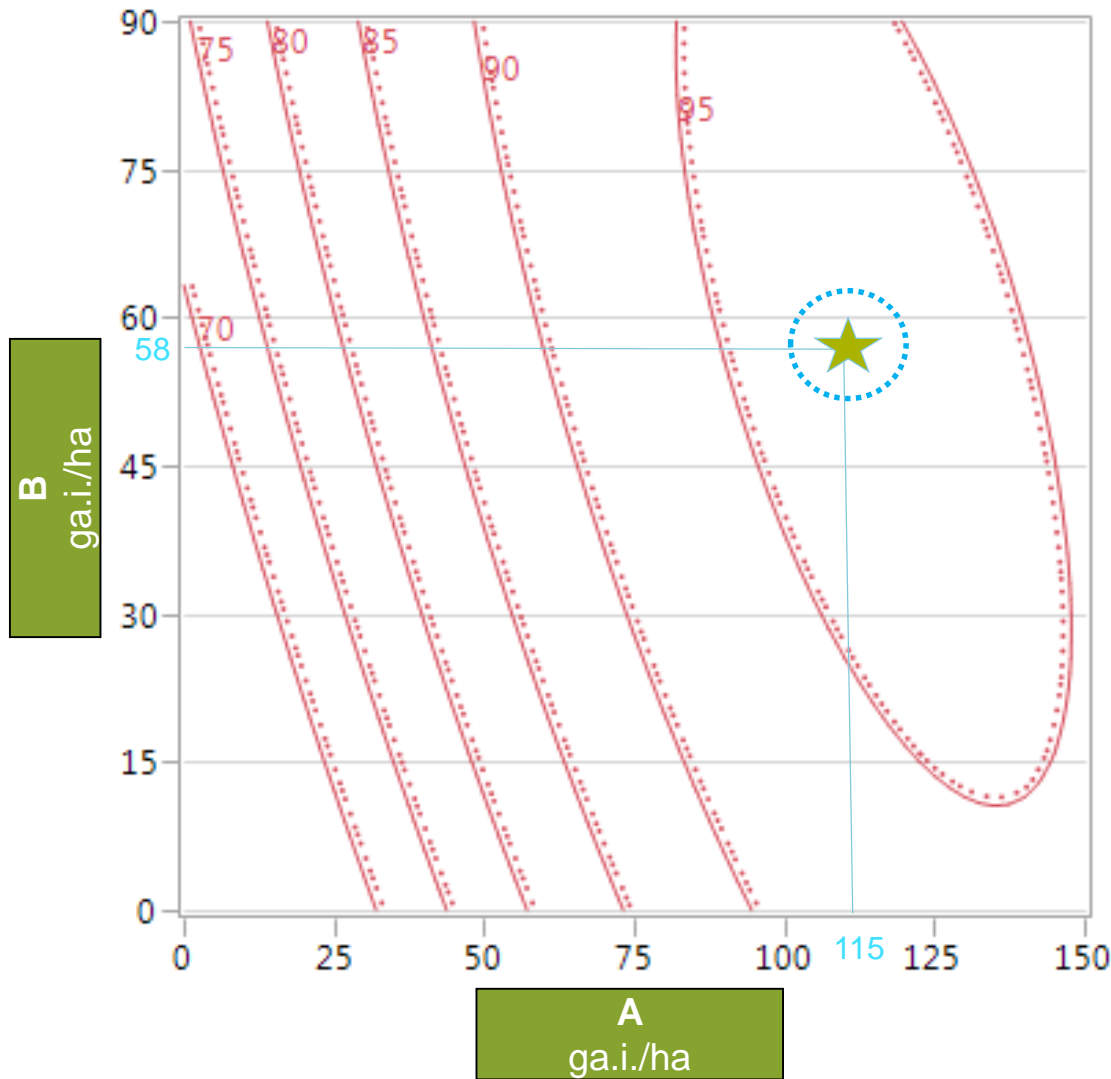
Solution is a Maximum

Predicted Value at Solution 96.383651

## Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
A	1	1	31721.865	460.1911	<.0001*
B	1	1	3002.005	43.5503	<.0001*
A*A	1	1	6153.384	89.2675	<.0001*
A*B	1	1	1400.307	20.3143	<.0001*
B*B	1	1	188.638	2.7366	0.0990
TIAL Trial Number	9	9	40121.249	64.6713	<.0001*
ASSE Repetition no[TIAL Trial Number]	30	30	3674.046	1.7767	0.0085*

# Efficacy predicted curve of multi trials surface response **reduced** model



# Conclusions

- Optimum stationary point at **111** gai/ha of A + **56** gai/ha of B with complete model (115 / 58 with reduced model)
- The predicted efficacy is 95% at the stationary point
- Product A is the strongest driver of efficacy in the mixture
- The optimal model for one response may not be optimal for other responses (eg : tuber blight, yield)

## Points for discussion

- How to select trials which enter the analysis (validity, precision, level of infestation, progression curve, significant surface response...)?
- Which approach to prefer: aggregate estimates (and errors) from multiple single trials, univariate combined complete model, select the best fit (AIC) , adding random trial effects to generalize the conclusions ?
- How to deal with the interactions ?
- How to optimize multi-variate surface response analysis ?
- Alternative approaches to surface response (classical factorial design, non independence of rates) ?
- Easy extension to 3+ way mixtures ?