

Use of Simple Sequence Repeats (SSR) to Determine Incidence and Effectiveness of Self- and Cross-pollinated Avocado Fruit in Southern California

Year 1 of 3 - End of Year Report

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Benefits to the Industry

SSR technology is a powerful tool to determine the pollen parents of avocado progeny of known maternal genetic background. The various SSR markers we have selected for use to determine pollen parents are powerfully informative for the range of cross pollinizing cultivars available in the selected orchards and, therefore, highly capable of discerning the specific pollen parent of each sampled fruit. The cultivars included in the study are Bacon, Ettinger, 'Fuerte, Harvest, Hass, Lamb Hass, Marvel, Nobel, SirPrize and Zutano. This, coupled with the opportunity to sample fruits in replicated experimental plots comparing cross and self pollinations in trees located various distances from pollinizing cultivars, and comparing retention of cross- vs. self-pollinated fruit over the development season makes this endeavor one of the most comprehensive ever preformed on avocado. As a result of this three-year suite of studies, avocado growers and advisors will, for the first time, unequivocally know the impact of interplanting complimentary cultivars, and how these ultimately influence the crop.

Objectives

The specific primary objective of this research is to determine the pollen parent of each fruit sampled early in fruit development and in those sampled late in fruit development at maturity. Secondly with this knowledge applied to the population of fruits sampled from trees in experimental plots described below, the objectives include:

1. Estimate the proportions of successful self-pollinations with 'Hass' and cross pollinations with specific cultivars that occurred in the individual rows of various proximity to cross-pollinizing cultivars.

2. Determine if the proportion of outcrossed fruit increases during maturity due to preferential abscission of self-pollinated fruit as has been found for certain pollen parents of 'Hass'.
3. Determine if there is preferential retention of cross-pollinated fruit pollinated by a specific cultivar during maturation.

Summary

Fruit Collections

The experiments were conducted at the Debusschere orchard located on the coastal plain near Camarillo in Ventura County on land managed by Somis Pacific Ag. Company near Somis in Ventura County.

'Hass' fruits were first harvested prior to CAC funding on May 27, 2003 when they were about marble size. At the Debusschere orchard, approximately 20 fruits were sampled from each of ten tree rows across a block of trees interplanted every 6 rows with the cultivars listed above and nearby Lamb Hass. All of these fruit were Fed Exed to Homestead for SSR analysis. Unfortunately, the shipment arrived three days late with many of the fruit being ruined, perhaps due to in-flight chilling damage. We were able to get some usable embryo samples from these fruit. The early-harvest Debusschere fruit are still being analyzed in hopes of getting as much data as possible. Sam McIntyre, Somis Pacific, later successfully sent samples of marble-sized fruit taken from trees located in the row next to the 'Bacon' row of trees, 100, 200, and 300 feet away from the Bacon's in the solid block of 'Hass'.

Approximately 20 fruits per row were later harvested near maturity from the Debusschere orchard on October 1, 2003. All fruit arrived safely and were analyzed to determine the pollen parent of each fruit according to protocols. Results are presented in Table 1.

We have again sampled 20 fruits per row of marble sized fruit from the Debusschere orchard following this flowering season. They are currently being analyzed, and preliminary information obtained on some rows is included in Table 2. Sam McIntyre has recently sent a sample of marble sized fruit from this season's flowering. They have been analyzed and results from both years, 2003 and 2004, are presented in Table 3.

DNA Extraction and SSR Analysis:

We used 39 SSR primer pairs and genotyped ten of the most common pollinizing cultivars interplanted in the two orchards. Thirteen primer pairs (15 SSR markers) performed well and produced a total of 90 informative alleles with an average of 6.0 alleles per marker. In most cases, three selected primer pairs were sufficient to determine the paternity of 'Hass' fruits.

Prior to analyzing any fruit, DNA was isolated from the leaves of 'Bacon', 'Ettinger', 'Fuerte', 'Harvest', 'Hass', 'Lamb Hass', 'Marvel', 'Nobel', 'SirPrize', and 'Zutano' using CTAB method

(Murray and Thompson, 1980). These were used to genotype the ten cultivars with 39 SSR primer pairs (Mhameed et al., 1997; Schnell et al., 2003). DNA was isolated from embryos using REDExtract-N-Amp Seed PCR Kit (Sigma, St. Louis, MO) and used for paternity analysis with 3 to 5 SSR primer pairs. PCR was performed in multiplex reactions on DNA from each sample in 5 μ l solution volumes containing each component at concentrations based on manufacturer's instructions (REDExtract-N-Amp Seed PCR Kit manual, Sigma, St. Louis, MO) excepting each forward primer labeled with different fluorescent dyes and adding a PCR additive, betaine, at 1 μ mol/ μ l. Conditions for thermocycling were 94°C for 4 min, 35 cycles of 94°C for 30 sec, 43-45°C for 1 min (depended on primers) and 72°C for 1 min, and a final extension at 72°C for 7 min. PCR products were separated by capillary electrophoresis on an ABI 3100 genetic analyzer (Applied Biosystems Inc., Foster City, CA) following the procedure described by Meerow et al. (2002). Raw microsatellite data were analyzed using Genotyper 2.1 software (Applied Biosystems Inc., Foster City, CA). Maternal alleles of each embryo were confirmed by comparison with the 'Hass' genotype. Paternity was determined by matching the paternal alleles with the genotype of pollinizing cultivar.

Results and Discussion

Pollen distribution, as determined in nearly mature fruit harvested on October 1, 2003 in the Debusschere orchard, was widespread throughout the orchard as evidenced by individual row data in Table 1. Most complimentary B-type cultivars were effective in cross pollination of 'Hass' when such trees were planted in the same row as the 'Hass'. Cross pollination, however, dropped off dramatically in rows further displaced from the interplanted rows. 'Zutano' was the most effective in cross pollination followed by 'Ettinger' and 'Bacon'. Overall average pollination rates throughout the orchard was topped by self pollination within 'Hass' at 30% followed by 'Zutano' at 22%, 'Ettinger' at 18%, 'Bacon' at 10% and the others at lower levels.

The pollen parents of fruit harvested on June 13 of this year are displayed in Table 2. We report information on fruit collected from one of two rows of trees in and next to those interplanted with 'Lamb Has's because we noticed 'Lamb Has's offspring in portions of the interplanted orchard block (Table 1). Little can be said about this year's sampling of marble sized fruit until all of the data analyses are in. It is also hoped that sufficient numbers of the marble sized fruit sampled last year can be successfully analyzed for comparison with the mature fruit data reported in Table 1. This comparison will hopefully address the question of preferred retention of cross pollinated fruit over self-pollinated fruit.

Results obtained from fruit sampled from a solid block of 'Hass' bordered on one side with one row of 'Bacon' trees near Somis is presented in Table 3a for 2003 and Table 3b for 2004. Occurrence of cross pollinizers other than 'Bacon' is apparent in both years. These may be complimentary cultivars that were mistaken for 'Hass' and interplanted with them when the orchard was planted in 1979. The alleles suggest 'Zutano' as a likely candidate and a less likely probability of 'Ettinger' since I do not think it was available then, but cultivars other than these may have alleles of the same size as well. Clearly self pollination dominates, and 'Bacon' is not far reaching in its distribution in the solid block.

Conclusion

These data are only the first of three years we have proposed are needed to study some of the mechanics and events in avocado pollination. This year's data, however, suggest that self pollination is a major component of avocado pollination although pollinizer varieties significantly contribute to fruit pollination and fruit set through harvest. 'Zutano' appears at present to be the most effective in pollen distribution across the orchard block studied..

References

Ashworth, V.E.T.M, M. de La Cruz, and M.T. Clegg. 2000. Development of molecular markers, Microsatellite. California Avocado Research Symposium. pp.37-40

Meerow, A.R., R.J. Wisser, J.S. Brown, D. Kuhn, R.J. Schnell, and T.K. Broschat. 2002. Analysis of genetic diversity and population structure within Florida coconut (*Cocos nucifera* L.) germplasm using microsatellite DNA, with special emphasis on the Fiji Darf cultivar. Theoretical and Applied Genetics 106:715-726.

Mhameed, S., D. Sharon, D. Kaufman, E. Lahavi, J. Hillel, C. Degani, and U. Lavi. 1997. Genetic relationships within avocado (*Persea Americana* Mill.) cultivars and between *Persea* species. Theoretical and Applied Genetics 94:279-286.

Murray, M.G. and W.F. Thompson. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research 8:4321-4325.

Schnell, R.J., J.S. Brown, C.T. Olano, E.J. Power, and C.A. Krol. 2003. Evaluation of avocado germplasm using microsatellite markers. J. Amer. Soc. Hort. Sci. 128:881-889.

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Table 1a. Numbers and proportions of nearly mature 'Hass' fruit harvested on October 1, 2003 that were pollinated by all potential pollen donors in the western half of the Debusschere orchard plot. Table representing the eastern half of the plot is shown in table 1b.

Pollinizer					Ettinger												Nobel							
Mary Lou #	12	24	1	3	5	7	9	2	4	6	8	23												
Orig. Row	27	28	29	30	31	32	33	34	35	36	37	38												
N. Row #	1	2	3	4	5	6	7	8	9	10	11	12												
Fruits	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
Total	20	100	20	100	20	100	19	100	19	100	18	100	15	100	2	100	18	100	20	100	19	100	19	100
Zutano	4	20.0	2	10.0	1	5.0	1	5.3	1	5.3	7	38.9	7	46.7	0	0.0	1	5.6	4	20.0	6	31.6	0	0.0
Hass	3	15.0	2	10.0	0	0.0	2	10.5	10	52.6	5	27.8	4	26.7	1	50.0	5	27.8	10	50.0	9	47.4	7	36.8
Fuerte	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	5.6	0	0.0	1	5.3	1	5.3
Ettinger	12	60.0	16	80.0	19	95.0	15	78.9	4	21.1	5	27.8	3	20.0	0	0.0	6	33.3	2	10.0	2	11.8	4	21.1
Bacon	1	5.0	0	0.0	0	0.0	0	0.0	2	10.5	0	0.0	1	6.7	1	50.0	4	22.2	0	0.0	0	0.0	4	21.1
SirPrize	0	0.0	0	0.0	0	0.0	0	0.0	1	5.3	0	0.0	0	0.0	0	0.0	0	0.0	1	5.0	1	6.0	2	10.5
Marvel	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	5.6	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	5.3
Harvest	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Nobel	0	0.0	0	0.0	0	0.0	0	0.0	1	5.3	0	0.0	0	0.0	0	0.0	1	5.6	1	5.0	0	0.0	0	0.0
LambHass	0	0.0	0	0.0	0	0.0	1	5.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	10.0	0	0.0	0	0.0

Pollinizer					Marvel												Harvest							
Mary Lou #	41	39	37	35	40	38	34	42	44	46	43	45												
Orig. Row	27	28	29	30	31	32	33	34	35	36	37	38												
S. Row #	1	2	3	4	5	6	7	8	9	10	11	12												
Fruits	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
Total	18	100	18	100	18	100	17	100	20	100	11	100	19	100	19	100	19	100	15	100	14	100	10	100
Zutano	5	27.8	1	5.6	1	5.6	1	5.9	2	10.0	1	9.1	1	5.3	1	5.3	0	0.0	1	6.7	1	7.1	0	0.0
Hass	4	22.2	4	22.2	7	38.9	4	23.5	9	45.0	7	63.6	15	78.9	14	73.7	9	47.4	8	53.3	10	71.4	3	30.0
Fuerte	0	0.0	0	0.0	0	0.0	0	0.0	2	10.0	0	0.0	0	0.0	0	0.0	0	0.0	1	6.7	0	0.0	0	0.0
Ettinger	2	11.1	5	27.8	7	38.9	8	47.1	2	10.0	1	9.1	0	0.0	2	10.5	1	5.3	0	0.0	0	0.0	0	0.0
Bacon	4	22.2	2	11.1	0	0.0	1	5.9	1	5.0	0	0.0	0	0.0	0	0.0	3	15.8	2	13.3	0	0.0	3	30.0
SirPrize	0	0.0	1	5.6	0	0.0	1	5.9	0	0.0	1	9.1	1	5.3	1	5.3	5	26.3	0	0.0	0	0.0	3	30.0
Marvel	0	0.0	0	0.0	3	16.7	0	0.0	1	5.0	1	9.1	1	5.3	1	5.3	0	0.0	0	0.0	0	0.0	0	0.0
Harvest	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1.0	6.7	0	0.0	1	10.0
Nobel	0	0.0	3	16.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
LambHass	3	16.7	2	11.1	0	0.0	2	11.8	3	15.0	0	0.0	1	5.3	0	0.0	1	5.3	2	13.3	3	21.4	0	0.0

Table 1b. Numbers and proportions of nearly mature ‘Hass’ fruit harvested on October 1, 2003 that were pollinated by all potential pollen donors in the eastern half of the Debusschere orchard plot. Table representing the western half of the plot is shown in table 1a.

		Fuerte												Zutano													
21		19		22		20		18		10		14		16		11		13		15		17					
39		40		41		42		43		44		45		46		47		48		49		50					
13		14		15		16		17		18		19		20		21		22		23		24		Sum			
#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	Fruits	
18	100	19	100	20	100	19	100	17	100	18	100	20	100	19	100	17	100	19	100	14	100	12	100	421	100	Total	
3	16.7	3	15.8	6	30.0	0	0.0	5	29.4	10	55.6	16	80.0	10	52.6	15	88.2	16	84.2	8	57.1	7	58.3	133	34.0	Zutano	
3	16.7	4	21.1	3	15.0	4	21.1	2	11.8	6	33.3	1	5.0	1	5.3	1	5.9	1	5.3	3	21.4	1	8.3	88	22.5	Hass	
5	27.8	5	26.3	8	40.0	1	5.3	1	5.9	2	11.1	0	0.0	2	10.5	0	0.0	1	5.3	0	0.0	0	0.0	28	7.2	Fuerte	
1	5.6	2	10.5	1	5.0	0	0.0	1	5.9	0	0.0	2	10.0	1	5.3	0	0.0	1	5.3	2	14.3	1	8.3	100	25.6	Ettinger	
1	5.6	0	0.0	0	0.0	3	15.8	1	5.9	0	0.0	0	0.0	2	10.5	1	5.9	0	0.0	0	0.0	2	16.7	23	5.9	Bacon	
2	11.1	0	0.0	0	0.0	2	10.5	4	23.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	7.1	0	0.0	14	3.6	SirPrize	
0	0.0	0	0.0	0	0.0	2	10.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	4	1.0	Marvel	
0	0.0	0	0.0	0	0.0	1	5.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.3	Harvest	
0	0.0	0	0.0	0	0.0	0	0.0	1	5.9	0	0.0	0	0.0	1	1.3	0	0.0	0	0.0	0	0.0	0	0.0	5	1.3	Nobel	
3	16.7	5	26.3	2	10.0	6	31.6	2	11.8	0	0.0	1	5.0	2	10.5	0	0.0	0	0.0	0	0.0	0	0.0	24	6.1	L.Hass	

		Bacon												SirPrize													
47		32		30		28		26		33		31		29		27		25		48		36					
39		40		41		42		43		44		45		46		47		48		49		50					
13		14		15		16		17		18		19		20		21		22		23		24		Sum			
#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	Fruits	
17	100	20	100	15	100	18	100	18	100	17	100	19	100	20	100	20	100	20	100	19	100	19	100	420	100	Total	
4	23.5	1	5.0	2	13.3	3	16.7	4	22.2	2	11.8	1	5.3	0	0.0	3	15.0	5	25.0	2	10.5	7	36.8	49	11.6	Zutano	
3	17.6	1	5.0	1	6.7	9	50.0	9	50.0	7	41.2	10	52.6	7	35.0	12	60.0	5	25.0	6	31.6	4	21.1	168	39.6	Hass	
0	0.0	1	5.0	1	6.7	0	0.0	0	0.0	0	0.0	0	0.0	1	5.0	0	0.0	0	0.0	0	0.0	0	0.0	6	1.4	Fuerte	
1	5.9	0	0.0	2	13.3	1	5.6	0	0.0	4	23.5	4	21.1	4	20.0	1	5.0	5	25.0	3	15.8	1	5.3	54	12.7	Ettinger	
8	47.1	13	65.0	7	46.7	4	22.2	1	5.6	1	5.9	2	10.5	1	5.0	0	0.0	1	5.0	1	5.3	4	21.1	59	13.9	Bacon	
0	0.0	1	5.0	0	0.0	1	5.6	4	22.2	2	11.8	1	5.3	5	25.0	4	20.0	4	20.0	3	15.8	0	0.0	38	9.0	SirPrize	
1	5.9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	5.3	0	0.0	9	2.1	Marvel	
0	0.0	2	10.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	4	0.9	Harvest	
0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	5.3	0	0.0	0	0.0	0	0.0	1	5.3	1	5.3	6	1.4	Nobel	
0	0.0	1	5.0	2	13.3	0	0.0	0	0.0	1	5.9	0	0.0	2	10.0	0	0.0	0	0.0	2	10.5	2	10.5	27	6.4	L.Hass	

Figure 2a. Numbers and proportions of marble sized ‘Hass’ fruit harvested on June 13, 2004 that were pollinated by all potential pollen donors in the western half of the Debusschere orchard plot. Table representing the eastern half of the plot is shown in table 2b.

Pollinizer					Ettinger												Nobel							
Orig. Row	27		28		29		30		31		32		33		34		35		36		37		38	
N. Row #	1		2		3		4		5		6		7		8		9		10		11		12	
Fruits	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
Total			21	100	23	100																		
Zutano			0	0	0	0																		
Hass			10	48	1	4																		
Fuerte			0	0	0	0																		
Ettinger			10	48	20	87																		
Bacon			0	0	0	0																		
SirPrize			0	0	0	0																		
Marvel			1	5	0	0																		
Harvest			0	0	1	4																		
Nobel			0	0	1	4																		
LambHass			0	0	0	0																		

Pollinizer					Marvel												Harvest							
Orig. Row	27		28		29		30		31		32		33		34		35		36		37		38	
S. Row #	1		2		3		4		5		6		7		8		9		10		11		12	
Fruits	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
Total	18	100	23	100	19	100					22	100	24	100	23	100								
Zutano	0	0	0	0	0	0					1	5	1	4	1	4								
Hass	9	50	11	48	8	42					20	91	20	83	17	74								
Fuerte	0	0	0	0	0	0					0	0	0	0	0	0								
Ettinger	1	6	3	13	0	0					0	0	1	4	2	9								
Bacon	0	0	0	0	0	0					0	0	0	0	0	0								
SirPrize	0	0	0	0	0	0					0	0	0	0	0	0								
Marvel	6	33	5	22	7	37					1	5	0	0	2	9								
Harvest	0	0	0	0	0	0					0	0	0	0	0	0								
Nobel	2	11	2	9	4	21					0	0	2	8	1	4								
LambHass	0	0	2	9		0					0	0		0		0								

Pollinizer					LambHass												LambHass							
Orig. Row	27		28		29		30		31		32		33		34		35		36		37		38	
S. Row #	1		2		3		4		5		6		7		8		9		10		11		12	
Fruits	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
Total																			17	100				
Zutano																			0	0				
Hass																			14	82.4				
Fuerte																			0	0.0				
Ettinger																			1	5.9				
Bacon																			0	0.0				
SirPrize																			0	0.0				
Marvel																			0	0.0				
Harvest																			0	0.0				
Nobel																			0	0.0				
LambHass																			2	11.8				

Figure 2b. Numbers and proportions of marble sized ‘Hass’ fruit harvested on June 13, 2004 that were pollinated by all potential pollen donors in the eastern half of the Debusschere orchard plot. Table representing the western half of the plot is shown in Table 2a.

39		40		41		42		43		44		45		46		47		48		49		50		Sum		
13		14		15		16		17		18		19		20		21		22		23		24				
#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	Fruits
																15	100	22	100							Total
																5	33	0	0							Zutano
																5	33	12	55							Hass
																0	0	0	0							Fuerte
																1	7	3	14							Ettinger
																0	0	0	0							Bacon
																0	0	0	0							SirPrize
																0	0	2	9							Marvel
																0	0	0	0							Harvest
																0	0	3	14							Nobel
																4	27	2	9							L.Hass

				Bacon										SirPrize												
39		40		41		42		43		44		45		46		47		48		49		50		Sum		
13		14		15		16		17		18		19		20		21		22		23		24				
#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	Fruits
24	100	24	100	23	100																					
1	4	2	8	3	13																					
13	54	16	67	13	57																					
0	0	2	8	1	4																					
0	0	0	0	0	0																					
1	4	0	0	1	4																					
0	0	0	0	0	0																					
3	13	2	8	0	0																					
1	4	0	0	0	0																					
1	4	0	0	1	4																					
4	17	2	8	4	17																					

				LambHass										LambHass													
39		40		41		42		43		44		45		46		47		48		49		50		Sum			
13		14		15		16		17		18		19		20		21		22		23		24					
#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	Fruits	

Table 3a. Marble-sized fruit harvested July 7, 2003 from an orchard managed by Somis Pacific near Somis. Block was solid 'Hass' edged on one side by one row of 'Bacon' trees. Samplings were taken at various distances from the 'Bacon' row.

Distance (Rows)	Hass	Bacon	Zutano	Ettinger	Others else	# Fruits tested	% of Bacon	% of selfing
Next to Bacon	2	11	0	0	1	14	78.6	14.3
100' Away	12	4	0	1	0	17	23.5	70.6
200' Away	8	2	1	0	4	15	13.3	53.3
300' Away	7	2	0	1	6	16	12.5	43.8
Sum	29	19	1	2	11	49	38.8	59.2
Percentage	74.4	48.7	2.6	5.1	28.2			

Table 3b. Marble-sized fruit harvested July 9, 2004 from an orchard managed by Somis Pacific near Somis. Block was solid 'Hass' edged on one side by one row of 'Bacon' trees. Samplings were taken at various distances from the 'Bacon' row.

Distance (Rows)	Hass	Bacon	Zutano	Ettinger	Others else	# Fruits tested	% of Bacon	% of selfing
Next to Bacon	4	6	0	0	0	10	60.0	40.0
100' Away	8	2	0	0	0	10	20.0	80.0
200' Away	8	1	0	0	0	9	11.1	88.9
300' Away	6	1	3	0	0	10	10.0	60.0
Sum	26	10	3	0	0	39	25.6	66.7
Percentage	66.7	25.6	7.7	0.0	0.0			