

# **Douglas-fir vegetative propagation – Results to 2006**

**C.B. Low, M.J. Dibley**

**NZ Douglas-fir Cooperative**

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# **DOUGLAS-FIR VEGETATIVE PROPAGATION – RESULTS TO 2006**

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## **ABSTRACT**

Research on setting Douglas-fir cuttings was successfully trialled at the NZFRI in the 1970s and further variations in stool-plant treatment were also researched in the late 1990s. This recent research was designed to look at the effects of different times of stool-plant treatment and the setting of cuttings.

The three treatment times spanned the period of summer flush, with the latest treatment (31<sup>st</sup> October) occurring after the flush. Most stool-plants treated after the flush responded with slower growth and some died, thus providing a clear case in favour of treatment before flushing.

The greenhouse could not maintain summer temperatures below 20° Celsius, and this delayed rooting of the cuttings immediately after setting. Cuttings set in January and February rooted at the same time as those set in March. However, very few rooted cuttings flushed in the spring following setting, possibly due to their bud chilling requirements not being met.

The method of stool-plant treatment delivered a high proportion of rooted cuttings over a range of Douglas-fir provenances. However, the stool-plant management treatments proved labour intensive and time-consuming and should be researched further to increase efficiency. Root systems appeared to be generally good, with only a small percentage rejected for planting out in field trials.

Considering that the origin of the stool-plants spanned 5° of latitude (or 550 km), there was little effect of provenance on height growth, although there was a significant family effect. However, the main source of variation in height growth and survival was observed between clones within families rather than between families, although this effect was confounded with treatment time.

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## **INTRODUCTION AND BACKGROUND**

Forest Research conducted pilot trials in the 1990s to test a nursery stool-bed management technique, which promotes axillary shoots to develop on Douglas-fir stool-plants (Project Record by Faulds *et al.* 2002). The technique was developed and these shoots were set as cuttings. Resultant plants had good root systems and none showed signs of plagiotropism, which is often a major problem in other Douglas-fir propagation systems. Other benefits included the cost-effectiveness of the system and a shortening of the propagation cycle compared with current North American propagation systems. Research was not continued beyond the stage of producing rooted cuttings due to lack of resources.

It is envisaged that stool plants could be maintained for a number of years, and that cutting production would increase over time, but this needs to be tested. Cuttings production needs to be scaled up to test the validity of the method as an operational propagation system. Also, the rooted cuttings must be field tested alongside standard seedling planting stock, to validate the worth of the system. The rooted cuttings must perform at least as well as genetically similar seedling stock.

Controlled-pollinated seed from selected parents in the New Zealand Douglas-fir breeding program has been available in very small quantities since 1994. The first of these seedlots were trialled in a progeny test planted in 1996 and demonstrated excellent gains in growth rate over the best available seedlots from New Zealand seed stands.

It is unlikely that significant quantities of controlled-pollinated seed will be available for at least 10 years, so a system to multiply this seed will have great value. The New Zealand Douglas-fir Cooperative has funded a controlled-crossing program between 185 select parents in PROSEED's Waikuku archive. This seed is destined for progeny trials to rank these parents, but some crosses have produced surplus seed, which was available for research purposes.

The breeding program and propagation systems have finally reached a stage where it is possible to propagate from extremely high quality seed and produce clonal lines of exceptional merit. Such clones would also raise the profile of New Zealand Douglas-fir.

## **MATERIALS AND METHODS**

Controlled-pollinated seed was sown in 2000 for a progeny trial planted at West Tapanui in 2002 and plants from some families were lined out as stool-plants (Table 1).

**Table 1.** Family details for Douglas-fir 1/1 (plug + 1) seedlings lined out as stool plants, August 2002 (Group 1 stool plants)

♀ parent	♂ pollen	No. stools	No. in bed	Provenance	Latitude
888.442	PM	10	66	Santa Cruz	37° 05
888.482	PM	10	42	Mt Tamalpais	37° 53
888.406	PM	10	83	Stewart Point	38° 39
888.432	PM	10	40	Fort Bragg	39° 21
889.617	PM	10	42	Fort Bragg	39° 21
889.528	PM	10	77	Fort Bragg	39° 21
889.574	PM	10	61	Dehaven	39° 36
889.557	PM	10	70	Ashley	44° 00
889.633	PM	10	95	Ashley	44° 00
889.591	PM	10	47	Deadwood	44° 06
889.611	888.499	10	58	Fourmile x Berteleda	43° 02 x 41° 48
889.580	888.461	10	48	Coos Bay x Florence	43° 42 x 43° 58

The parents used for the polymix pollen (PM) were chosen from selected trees from the most vigorous provenances in the 1959 provenance trial (Table 2). The pollen used in 1995 and 1996 was collected from selected edge trees in the Fort Bragg seed stand at Rotoehu, as the Waikuku seed orchard was not producing much pollen. Two pair crosses were also available, both of mainly Oregon origin.

**Table 2.** Pollens used for Douglas-fir GCA (polycross) crossing program 1995 - 1998

1995-96 (Fort Bragg)	1997	1998
895.301	888.406 (Stewart Pt)	888.406 (Stewart Pt)
895.302	888.496 (Berteleda)	888.430 (Fort Bragg)
895.303	889.526 (Berteleda)	888.432 (Fort Bragg)
895.304	889.538 (Stewart Pt)	889.526 (Berteleda)
895.305	889.574 (Dehaven)	889.538 (Stewart Pt)
895.306	889.581 (Berteleda)	889.539 (Fort Bragg)
895.307	889.612 (Berteleda)	889.581 (Berteleda)
895.308	889.615 (Dehaven)	889.612 (Berteleda)
895.309	889.618 (Stewart Pt)	889.618 (Stewart Pt)
895.310	889.620 (Stewart Pt)	889.620 (Stewart Pt)
895.311		

The initial sequence of operations in the nursery began with the sowing of seed and raising plants for the progeny trial in 2002 (Table 3). Operations that were concerned only with vegetative propagation started in August 2002. Further explanation of the details of propagation systems are available in Faulds *et al.* (2003). Three topping treatments were evaluated (3 September, 30 September, and 31 October), and cuttings from these treatments were set at three times in the following January, February and March.

**Table 3.** Sequence of nursery operations

Date	Operation
June 2000	Seed stratified
July 2000	Seed sown into planter boxes in glasshouse
October 2000	Seedlings pricked out into paper pots
March 2001	Seedlings lined out into nursery bed
August 2002	Ten seedlings per family transferred to stool bed at 60 cm spacing
3 <sup>rd</sup> September 2003	2-4 plants per family cut back and de-budded
30 <sup>th</sup> September 2003	“ “ “ “ “ “ “ “
31 <sup>st</sup> October 2003	“ “ “ “ “ “ “ “
January 2004	Ten cuttings per clone set in propagation facility
February 2004	“ “ “ “ “ “ “ “
March 2004	“ “ “ “ “ “ “ “
August 2004	Rooted cuttings counted and lined out into the nursery bed
August 2006	Rooted cuttings measured, counted
September 2006	Cuttings lifted for planting into two field trials

### Analysis

The analysis on numbers of rooted cuttings was carried out on plot totals. Treatment time was confounded with clones within each family as each treatment involved an entire stool-plant or clone. Tree height was measured on every tree in each plot, so there is an extra level of setting\*clone(family) in the analysis of height over the plot survival values. Treatment and setting were considered to be fixed effects and all other effects were treated as random effects.

The equation for the model of analysis of variance was as follows:

$$Y_{ijk} = \mu + T_i + S_j + F_k + S_j * T_i + F_k * T_i + F_k * S_j + E_{ijk}$$

Where :

$Y_{ijk}$  = the observation on the plot of the  $k^{\text{th}}$  family in the  $j^{\text{th}}$  treatment of the  $i^{\text{th}}$  setting

$\mu$  = the overall mean

$T_i$  = the effect of the  $i^{\text{th}}$  treatment

$S_j$  = the effect of the  $j^{\text{th}}$  Setting

$F_k$  = the effect of the  $k^{\text{th}}$  family

$S_j * T_i$  = the interaction effect of the  $j^{\text{th}}$  setting with the  $i^{\text{th}}$  treatment

$F_k * T_i$  = the interaction effect of the  $k^{\text{th}}$  family with the  $i^{\text{th}}$  treatment

$F_k * S_j$  = the interaction effect of the  $k^{\text{th}}$  family with the  $j^{\text{th}}$  setting

$E_{ijk}$  = the random error associated with each plot of the  $k^{\text{th}}$  family in the  $j^{\text{th}}$  setting of the  $i^{\text{th}}$  treatment

Mean heights were estimated using PROC MEANS of the SAS<sup>®</sup> software package (SAS Institute Inc. 1990). The first analysis of this trial examined the data as means or totals per plot. Analysis of variance was carried out by PROC GLM of the SAS<sup>®</sup> statistical package (SAS Institute Inc. 1989) and treatment setting and family means were compared using the least significant difference as determined by the Tukey multiple range test. The terms of the analysis

model were treatments, settings, families, a setting by treatment interaction, a family by treatment interaction, a family by setting interaction and error. Families and interaction terms were treated as random effects and treatment and setting were treated as fixed effects.

Individual analyses were carried out for each setting to quantify problems arising from the short period between the last treatment and first setting.

## RESULTS AND DISCUSSION

The stool-plant management treatments proved labour intensive and time-consuming. Timing of spring flush was observed weekly from the 3<sup>rd</sup> of September 2003 and there was surprisingly little difference between families. The buds on most plants started to swell by the 17<sup>th</sup> of September and very few held off past the 30<sup>th</sup> of September. This was not surprising for the polycrossed families, which all shared the same pollen parents, but the two pair-crossed families of Oregon origin were expected to flush later.

The differences between treatments for either the numbers of surviving trees per clone or 2006 height were not significantly different. Treatments were confounded with clones, as each clone could only have a single treatment, so the analysis model could only have clone or treatment as a classification, but not both (Table 4). Most of the families had fully flushed by the time of the 31<sup>st</sup> October treatment and were hit very hard by the pruning and stripping of buds. The 31<sup>st</sup> October treatment resulted in some stool-plant deaths and slowed growth relative to the earlier treatments, which meant fewer plants were produced by this treatment overall (Table 5).

**Table 4.** F tests from analysis of variance

Source	Df	2004 rooting %	2006 survival %	2006 Height
Treatment	2	0.44	0.14	0.23
Setting	2	11.36**	12.81**	1.84
Family	11	0.79	1.30	4.07*
Treatment*Family	21	1.81*	1.82*	2.15**
Treatment*Setting	4	2.07	1.73	0.80
Family*Setting	22	1.96*	1.37	0.71
Error	246			

**Table 5.** Treatment means for stool-plants

Treatment time	N <sup>o</sup> . Stool-plants	2004 rooting %	2006 survival %	Height (cm) 2006
3 <sup>rd</sup> September	105	73.9	64.6	51.7
30 <sup>th</sup> September	113	72.6	62.3	50.4
31 <sup>st</sup> October	91	70.4	63.1	50.7
Least Significant Difference		5.3	5.7	3.57

A further analysis on cutting survival with setting times by treatment was carried out to look more closely at how each treatment interacted with different setting times. F-tests from the analysis are shown in Table 6 and means of setting times are shown in Table 7. The earliest setting time was least successful with the latest treatment (31<sup>st</sup> October), and there was a gradation of survival to the optimum of earliest treatment and latest setting.

**Table 6.** F tests on numbers of plants surviving per year per treatment / setting time

Source	Df	January setting		February setting		March setting	
		2004	2006	2004	2006	2004	2006
Treatment	2	2.77	1.37	0.42	2.41	0.01	0.38
Family	11	1.87	1.33	1.42	3.13**	0.94	1.15
Treatment*Family	21	1.71	1.89*	0.58	0.46	1.17	0.64
Error	68						

**Table 7.** Mean numbers of plants surviving per year per treatment / setting time

Treatment	January setting		February setting		March setting	
	2004	2006	2004	2006	2004	2006
3 <sup>rd</sup> September	67.4 a	57.7	72.8	63.4	81.4	72.6
30 <sup>th</sup> September	64.1 ab	51.6	71.3	64.2	82.1	70.8
31 <sup>st</sup> October	54.8 b	49.0	74.8	69.0	82.4	71.7
Least Sig Diff	10.0	10.3	10.9	11.5	6.8	8.8

Means not sharing a letter are considered to be significantly different at  $P \leq 0.05$

Analysis of variance of families, clones and setting times showed significant differences in setting times for rooting percentage and surprisingly little difference between families (Tables 8 & 9). There were significant differences between clones within families, however (Table 9). There were significant differences between families for height growth, however.

**Table 8.** F tests from analysis of variance

Source	Df	2004 rooting %	2006 survival %	2006 height
Setting	2	17.25***	22.49***	1.84
Family	11	0.88	1.49	3.49***
Clone(Family)	93	1.86***	1.90***	6.27***
Setting*Family	22	2.29**	1.44	1.86*
Setting*Clone(Family)	180			2.00***
Error	1647			

Unfortunately, the greenhouse was not able to maintain ambient temperature below 20°C in January and February, so all setting treatments rooted at the same time. This evidently stressed the cuttings set earlier, as survival was lowest for the earliest setting time and improved for later settings (Table 9). However, the improved height growth of the earliest setting suggests that we should try earlier setting if we can reduce summer temperature sufficiently.

**Table 9.** Means by time of setting

Setting time	N. stool-plants	2004 rooting %	2006 survival %	Height (cm) 2006
January	103	62.4 c	52.9 b	52.3 a
February	104	72.9 b	65.4 a	50.8 b
March	102	82.0 a	71.7 a	50.3 b
Least Significant Difference		7.7	6.6	1.47

Means not sharing a letter are considered to be significantly different at  $P \leq 0.05$

Families did not differ significantly in numbers of rooted cuttings for either the 2004 or 2006 counts (Table 10). However, there were substantial differences in height growth, with all clones of family 528 from Fort Bragg producing plants between 50 cm and one metre in height. No particular trend of variation with latitude of the mother plant was observed, but this may have been due to the pollen parents being the same latitude for most families.

**Table 10.** Family means

Family	Latitude	Clones	N. trees	2004 %	2006 %	Height
888.442	37° 05	10	200	71	67	48.8 cd
888.482	37° 53	10	209	75	70	41.8 e
888.406	38° 39	7	142	73	68	52.4 bc
888.432	39° 21	10	183	67	63	55.7 b
889.617	39° 21	10	166	68	57	49.2 cd
889.528	39° 21	10	205	79	68	62.3 a
889.574	39° 36	10	149	68	53	53.5 b
889.557	44° 00	3	62	79	69	55.0 b
889.633	44° 00	6	115	75	64	55.3 b
889.591	44° 06	10	150	69	54	47.3 d
889.611	43° 02 x 41° 48	10	194	71	65	52.4 bc
889.580	43° 42 x 43° 58	9	181	80	67	42.6 e
Least Significant Difference				24	20	4.29

The majority of the cuttings had produced very good planting stock by the winter of 2006. A threshold height of 30 cm was set for plants going to field trials and a few plants fell below this limit (Appendix 1); occasional clones had all plants below 30 cm and were left out of the field trials. The differing effects of treatment on family and setting time can be seen in Appendix 2 (Means by family clone treatment and setting time).

The performance of the greenhouse was a major disappointment as it was unable to maintain the ambient summer temperature below 20° C. This delayed the formation of roots until autumn. The cuttings were kept in the greenhouse over winter and after lining out in a bare-root nursery bed in August, the cuttings sat in the nursery bed for one year without flushing or putting on height growth. This was possibly caused by the cuttings not having their bud chilling requirements for flushing.

Most plants had good root systems but a small proportion of plants that appeared sufficiently tall were rejected as planting stock. Up to eight ramets per clone were planted on each of two sites, one near Taihape in the Central North Island and the other in the Rangitata valley in the South Island.

## CONCLUSIONS

The three treatment times spanned the period of summer flush, with the latest treatment (31<sup>st</sup> October) occurring after the flush. Most stool-plants treated after the flush responded with slower growth and some died, thus providing a clear case in favour of treating before flushing.

The greenhouse could not maintain summer temperatures below 20° Celsius, prevented the cuttings from rooting immediately after setting. The cuttings set in January and February rooted at the same time as those set in March and very few rooted cuttings flushed in the spring following setting. This was possibly caused by the cuttings not having their bud chilling requirements for flushing.

Thus, it required two years in the nursery to produce plantable cuttings, so further attempts should be made to set cuttings earlier in a greenhouse that could maintain lower temperatures in summer.

The method of stool-plant treatment delivered a high proportion of rooted cuttings to cuttings set over a range of Douglas-fir provenances. However, the stool-plant management treatments proved labour intensive and time-consuming. Further research is needed to increase efficiency. Root systems appeared to be generally good, with a small percentage rejected for planting out in field trials.

Considering that the origin of the stool-plants spanned 5° of latitude (or 550 km), there was little effect of provenance on height growth, although there was a significant family effect. The main source of variation in height growth and survival was observed between clones within families rather than between families.

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## APPENDIX 1. CLONE PLANT NUMBERS AND MEAN HEIGHT

Code	FAMILY	CLONE	Total	over 30cm	Height(cm)
101	406	2	17	15	40
102	406	3	20	20	52
103	406	5	23	23	52
104	406	6	20	18	44
105	406	8	24	24	59
106	406	9	20	20	64
107	406	10	18	18	52
108	432	1	22	22	57
109	432	2	17	16	51
110	432	3	9	8	39
111	432	4	24	23	44
112	432	5	22	22	62
113	432	6	13	13	56
114	432	7	16	16	71
115	432	8	23	23	53
116	432	9	18	18	61
117	432	10	19	19	58
118	442	1	14	14	54
119	442	2	18	17	39
120	442	3	23	21	47
121	442	4	14	9	38
122	442	5	25	25	64
123	442	6	19	19	51
124	442	7	23	21	46
125	442	8	24	24	64
126	442	9	20	16	39
127	442	10	20	17	38
128	482	1	14	13	40
129	482	2	25	25	49
130	482	3	25	21	39
131	482	4	21	11	32
132	482	5	23	19	41
133	482	6	24	23	47
134	482	7	18	17	45
135	482	8	19	19	49
136	482	9	21	13	35
137	482	10	19	15	39
138	528	1	14	14	62
139	528	2	19	19	58
140	528	3	18	15	49
141	528	4	25	25	57
142	528	5	23	22	65
143	528	6	24	24	55
144	528	7	20	20	64
145	528	8	20	19	66
146	528	9	23	23	67
147	528	10	19	19	83
148	557	1	18	18	55
149	557	2	24	24	61
150	557	6	20	19	48

## APPENDIX 1. CLONE MEANS (CONTINUED)

Code	FAMILY	CLONE	Total	over 30cm	Height(cm)
151	574	1	11	10	51
152	574	2	5	0	19
153	574	3	14	14	55
154	574	4	15	15	56
155	574	5	19	19	68
156	574	6	24	19	41
157	574	7	16	16	74
158	574	8	19	19	56
159	574	9	7	4	34
160	574	10	19	17	50
161	580	1	19	16	37
162	580	2	17	4	36
163	580	4	21	12	33
164	580	5	24	21	37
165	580	6	16	14	40
166	580	7	17	13	37
167	580	8	24	24	57
168	580	9	24	24	51
169	580	10	19	16	50
170	591	1	17	16	49
171	591	2	13	13	51
172	591	3	14	13	52
173	591	4	16	15	46
174	591	5	14	14	60
175	591	6	25	20	38
176	591	7	15	10	38
177	591	8	15	12	42
178	591	9	8	8	48
179	591	10	13	13	60
180	611	1	22	19	48
181	611	2	13	9	32
182	611	3	10	10	59
183	611	4	21	21	55
184	611	5	21	21	51
185	611	6	19	12	33
186	611	7	23	23	60
187	611	8	22	22	63
188	611	9	21	20	60
189	611	10	22	22	55
190	617	1	14	14	71
191	617	2	15	12	44
192	617	3	20	20	59
193	617	4	18	17	62
194	617	5	16	0	21
195	617	6	17	15	52
196	617	7	18	18	57
197	617	8	16	12	46
198	617	9	19	10	32
199	617	10	13	12	48
200	633	1	21	21	58
201	633	3	22	22	53
202	633	6	21	19	54
203	633	7	21	21	52
204	633	8	21	20	65
205	633	9	9	7	45

## APPENDIX 2. CLONE AND TREATMENT MEANS

FAMILY	TREAT	CLONE	SETTING	N. 2004	N. 2006	HTC00
406	1	2	1	3	3	47
406	1	2	2	8	6	46
406	1	2	3	8	8	34
406	1	5	1	9	9	56
406	1	5	2	8	6	49
406	1	5	3	9	8	50
406	1	8	1	8	8	56
406	1	8	2	9	9	58
406	1	8	3	8	7	64
406	2	3	1	7	5	46
406	2	3	2	6	7	56
406	2	3	3	9	8	52
406	2	6	1	5	5	47
406	2	6	2	8	8	42
406	2	6	3	7	7	44
406	2	9	1	4	4	66
406	2	9	2	8	8	59
406	2	9	3	9	8	68
406	3	10	1	5	4	50
406	3	10	2	7	7	54
406	3	10	3	9	7	52
432	1	1	1	7	7	61
432	1	1	2	9	8	53
432	1	1	3	9	7	58
432	1	4	1	8	8	41
432	1	4	2	8	8	43
432	1	4	3	8	8	48
432	1	7	1	4	4	80
432	1	7	2	6	6	63
432	1	7	3	6	6	74
432	1	10	1	7	6	59
432	1	10	2	8	7	55
432	1	10	3	6	6	62
432	2	2	1	3	3	55
432	2	2	2	8	7	46
432	2	2	3	8	7	54
432	2	5	1	7	7	65
432	2	5	2	6	6	61
432	2	5	3	9	9	60
432	2	8	1	8	6	41
432	2	8	2	9	9	55
432	2	8	3	9	8	61
432	3	3	1	4	4	45
432	3	3	2	6	5	34
432	3	6	1	1	1	60
432	3	6	2	6	6	65
432	3	6	3	6	6	48
432	3	9	1	5	5	60
432	3	9	2	7	7	65
432	3	9	3	6	6	58

**APPENDIX 2. CLONE AND TREATMENT MEANS (CONTINUED)**

FAMILY	TREAT	CLONE	SETTING	N. 2004	N. 2006	HTC00
442	1	3	1	7	6	49
442	1	3	2	9	9	47
442	1	3	3	8	8	46
442	1	6	1	8	8	48
442	1	6	2	4	3	51
442	1	6	3	9	8	55
442	1	9	1	7	7	37
442	1	9	2	7	7	38
442	1	9	3	6	6	41
442	2	1	1	4	4	51
442	2	1	2	4	4	49
442	2	1	3	8	6	59
442	2	4	1	6	5	46
442	2	4	2	5	5	42
442	2	4	3	4	4	22
442	2	7	1	6	6	48
442	2	7	2	9	8	49
442	2	7	3	7	9	42
442	2	10	1	6	5	41
442	2	10	2	9	9	36
442	2	10	3	9	6	38
442	3	2	1	6	4	51
442	3	2	2	8	7	36
442	3	2	3	8	7	36
442	3	5	1	7	6	56
442	3	5	2	9	10	73
442	3	5	3	9	9	58
442	3	8	1	7	7	63
442	3	8	2	9	9	63
442	3	8	3	9	8	66
482	1	2	1	8	8	44
482	1	2	2	9	8	55
482	1	2	3	9	9	48
482	1	5	1	7	7	47
482	1	5	2	9	9	39
482	1	5	3	9	7	38
482	1	8	1	6	6	53
482	1	8	2	4	4	45
482	1	8	3	9	9	48
482	2	3	1	8	7	45
482	2	3	2	9	9	37
482	2	3	3	9	9	37
482	2	6	1	7	7	46
482	2	6	2	9	9	44
482	2	6	3	8	8	52
482	2	9	1	7	7	34
482	2	9	2	8	7	34
482	2	9	3	9	7	35
482	3	1	1	2	2	30
482	3	1	2	7	6	43
482	3	1	3	6	6	41
482	3	4	1	5	4	34
482	3	4	2	8	8	30
482	3	4	3	9	9	34
482	3	7	1	5	5	40
482	3	7	2	8	6	46
482	3	7	3	9	7	47
482	3	10	1	8	5	37
482	3	10	2	7	6	41
482	3	10	3	8	8	40

**APPENDIX 2. CLONE AND TREATMENT MEANS (CONTINUED)**

FAMILY	TREAT	CLONE	SETTING	N. 2004	N. 2006	HTC00
528	1	1	1	6	4	68
528	1	1	2	8	6	62
528	1	1	3	9	4	55
528	1	4	1	9	8	56
528	1	4	2	8	8	47
528	1	4	3	9	9	66
528	1	7	1	8	7	59
528	1	7	2	9	6	73
528	1	7	3	8	7	62
528	1	10	1	6	4	78
528	1	10	2	9	8	80
528	1	10	3	8	7	89
528	2	2	1	9	7	64
528	2	2	2	8	6	49
528	2	2	3	6	6	60
528	2	5	1	9	9	67
528	2	5	2	6	6	62
528	2	5	3	8	8	64
528	2	8	1	9	6	59
528	2	8	2	7	7	62
528	2	8	3	8	7	75
528	3	3	1	6	5	42
528	3	3	2	7	7	50
528	3	3	3	7	6	53
528	3	6	1	8	8	50
528	3	6	2	9	9	57
528	3	6	3	8	7	61
528	3	9	1	7	7	58
528	3	9	2	9	8	71
528	3	9	3	8	8	70
557	1	1	1	8	5	61
557	1	1	2	7	7	54
557	1	1	3	9	6	52
557	2	2	1	8	8	69
557	2	2	2	9	8	54
557	2	2	3	8	8	60
557	2	6	1	8	7	53
557	2	6	2	7	7	45
557	2	6	3	7	6	46

**APPENDIX 2. CLONE AND TREATMENT MEANS (CONTINUED)**

FAMILY	TREAT	CLONE	SETTING	N. 2004	N. 2006	HTC00
574	1	3	1	5	4	50
574	1	3	2	2	2	51
574	1	3	3	9	8	58
574	1	6	1	8	8	51
574	1	6	2	7	7	38
574	1	6	3	9	9	36
574	1	9	2	4	2	34
574	1	9	3	5	5	34
574	2	1	1	7	2	54
574	2	1	2	8	6	56
574	2	1	3	9	3	38
574	2	4	1	8	7	57
574	2	4	2	2	1	56
574	2	4	3	9	7	55
574	2	7	1	7	5	69
574	2	7	2	7	3	74
574	2	7	3	7	8	77
574	2	10	1	8	5	46
574	2	10	2	7	7	44
574	2	10	3	7	7	59
574	3	2	1	1	1	20
574	3	2	2	6	4	18
574	3	5	1	6	5	55
574	3	5	2	9	8	77
574	3	5	3	9	6	67
574	3	8	1	7	6	55
574	3	8	2	9	7	55
574	3	8	3	7	6	57
580	1	1	1	8	3	39
580	1	1	2	9	8	38
580	1	1	3	9	8	36
580	1	4	1	8	7	37
580	1	4	2	7	6	48
580	1	4	3	9	8	18
580	1	7	1	7	5	40
580	1	7	2	6	5	39
580	1	7	3	7	7	33
580	1	10	1	9	6	60
580	1	10	2	8	6	52
580	1	10	3	9	7	41
580	2	2	1	9	4	40
580	2	2	2	7	5	35
580	2	2	3	10	8	34
580	2	5	1	7	7	35
580	2	5	2	8	8	38
580	2	5	3	9	9	38
580	2	8	1	9	9	54
580	2	8	2	8	8	66
580	2	8	3	9	7	50
580	3	6	1	6	5	44
580	3	6	2	7	6	46
580	3	6	3	5	5	30
580	3	9	1	8	7	51
580	3	9	2	9	8	52
580	3	9	3	9	9	50

**APPENDIX 2. CLONE AND TREATMENT MEANS (CONTINUED)**

FAMILY	TREAT	CLONE	SETTING	N. 2004	N. 2006	HTC00
591	1	3	1	7	5	63
591	1	3	2	6	3	50
591	1	3	3	9	6	43
591	1	6	1	8	8	42
591	1	6	2	9	8	36
591	1	6	3	9	9	37
591	1	9	1	9	8	48
591	2	1	1	8	5	42
591	2	1	2	9	3	49
591	2	1	3	9	9	53
591	2	4	1	3	1	70
591	2	4	2	8	7	41
591	2	4	3	9	8	47
591	2	7	1	5	5	51
591	2	7	2	5	5	38
591	2	7	3	7	5	24
591	2	10	1	3	3	59
591	2	10	2	5	5	61
591	2	10	3	8	5	59
591	3	2	1	8	6	54
591	3	2	2	6	4	42
591	3	2	3	7	3	58
591	3	5	1	2	2	67
591	3	5	2	8	8	55
591	3	5	3	10	4	65
591	3	8	1	1	1	52
591	3	8	2	6	6	50
591	3	8	3	9	8	35
611	1	2	1	6	5	40
611	1	2	2	4	3	28
611	1	2	3	7	5	26
611	1	5	1	7	6	52
611	1	5	2	7	7	53
611	1	5	3	8	8	49
611	1	8	1	7	6	58
611	1	8	2	8	8	62
611	1	8	3	8	8	69
611	2	3	1	5	2	69
611	2	3	2	1	1	77
611	2	3	3	7	7	54
611	2	6	1	3	2	33
611	2	6	2	9	9	40
611	2	6	3	9	8	26
611	2	9	1	6	6	60
611	2	9	2	9	9	59
611	2	9	3	9	6	60
611	3	1	1	9	9	49
611	3	1	2	7	7	55
611	3	1	3	8	6	40
611	3	4	1	6	6	53
611	3	4	2	5	5	54
611	3	4	3	10	10	57
611	3	7	1	6	5	70
611	3	7	2	9	9	58
611	3	7	3	9	9	57
611	3	10	1	9	8	56
611	3	10	2	5	5	54
611	3	10	3	9	9	54

**APPENDIX 2. CLONE AND TREATMENT MEANS (CONTINUED)**

FAMILY	TREAT	CLONE	SETTING	N. 2004	N. 2006	HTC00
617	1	1	1	2	2	53
617	1	1	2	7	7	81
617	1	1	3	7	5	66
617	1	4	1	3	3	62
617	1	4	2	7	7	56
617	1	4	3	8	8	67
617	1	7	1	4	2	53
617	1	7	2	8	8	60
617	1	7	3	8	8	54
617	1	10	1	3	1	55
617	1	10	2	9	6	46
617	1	10	3	9	6	49
617	2	2	1	7	3	40
617	2	2	2	8	5	33
617	2	2	3	7	7	54
617	2	5	2	9	8	19
617	2	5	3	10	8	22
617	2	8	1	5	4	52
617	2	8	2	6	6	30
617	2	8	3	9	6	58
617	3	3	1	5	5	61
617	3	3	2	8	8	60
617	3	3	3	9	7	57
617	3	6	1	2	2	48
617	3	6	2	8	8	54
617	3	6	3	9	7	52
617	3	9	1	4	4	17
617	3	9	2	7	7	38
617	3	9	3	9	8	34
633	1	8	1	9	8	71
633	1	8	2	8	4	60
633	1	8	3	8	9	61
633	2	1	1	7	6	62
633	2	1	2	9	9	64
633	2	1	3	9	6	46
633	2	6	1	6	5	64
633	2	6	2	9	7	46
633	2	6	3	9	9	56
633	2	9	1	3	2	64
633	2	9	2	2	2	56
633	2	9	3	8	5	32
633	3	3	1	7	7	49
633	3	3	2	7	6	61
633	3	3	3	9	9	50
633	3	7	1	7	6	56
633	3	7	2	9	7	46
633	3	7	3	9	8	54