

Qualitative and Quantitative Impact of Honeybee Pollination on Apple Production in Shimla Hills of Himachal Pradesh, India

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Abstract: The experiment on quantitative and qualitative effects of honeybee pollination on apple crop were carried out on European bee *Apis mellifera* L. and Indian hive bee *Apis cerana* F., by placing two colonies of each species in experimental apple orchards located at Padola (2200 m), Kalzar (2514 m) and Jarol (2648 m) areas in Shimla hills of Himachal Pradesh. There was no significant difference ($P>0.01$) in the fruit set in different experimental designs (honeybees pollinated flowers, open pollinated flowers and in control) in self-compatible varieties of apple (Golden Delicious and Red Gold), but the differences in these experimental designs were significant ($P<0.01$) in self-incompatible varieties like Royal Delicious and Red Delicious. There was some difference in the fruit drops of self-incompatible varieties between open and honeybees pollinated flowers but it was not significant ($P>0.01$). In Golden Delicious and Red Gold, the qualitative pollination data showed that weight, length, breadth, volume and number of seeds per fruit developed were significantly maximum in honeybee pollinated flowers ($P<0.01$) and minimum in fruits developed under control experimental conditions in all the three orchards. Whereas, in Royal Delicious and Red Delicious varieties, the weight, length, breadth, volume and number of seeds per fruit were significantly more ($P<0.01$) in fruits developed from honeybees pollinated flowers than in fruits from open pollinated flowers.

Keywords: Honeybee pollination, quantitative and qualitative effects, apple crop, Shimla hill

1. Introduction

Honeybees and flowering plants are dependent upon each other for their existence. Most of the plants are dependent on insects for their pollination requirements, whereas, insects in turn depend upon plants for energy to maintain their activities (Seeley, 1985). This energy relationship between plants and nectar gathering insects is the necessary basis for studying the crop pollination, honey production and their foraging strategies (Morse, 1976; Akaratanakul, 1987). Bees and certain flowering plants have, therefore, evolved a well adjusted system of interdependence, which is very important in the process of their organic evolution (Deodikar, 1962; Martin, 1992).

Apple (*Malus domestica*) is one of the most important fruit crops globally with 2010 production across 93 countries worth US\$ 64bn (FAOStat, 2013). Apple cultivars are self-incompatible to varying extents and require pollen transfer from polliniser cultivar to set fruit in marketable quantities (Delaplane and Mayer, 2000). Insects, such as bees and hoverflies, are the predominant pollination vectors for apple and thus their activity in orchards is essential for apple production (Klein *et al.*, 2007). Increasing insect pollinator numbers in apple orchards has shown improved fruit set and yield (Kumar, 1997) and there is some evidence that levels of pollination affect seed number with associated impacts on size and calcium concentration (Matsumoto *et al.*, 2012).

The vital role which honeybees play in the pollination of large number of cultivated crops is often underestimated in developing countries. As a matter of fact, the main significance of honeybees and beekeeping is pollination. Therefore, income from agriculture by the use of honeybees in crop pollination is many times higher than their value as

honey and beeswax producers (Verma, 2003; Mattu, 2013). Honeybees are considered as the most efficient pollinators of cultivated crops because of their potential for long working hours, presence of pollen baskets, floral fidelity, micromanipulation of flowers, maintainability of high population and adaptability to different climatic conditions (Verma, 1990; Mattu and Mattu, 2013). Further, honeybees can be domesticated, marketed and transported from place to place (Crane, 1990; Mattu *et al.*, 1996, 2006). In the absence of honeybee pollination, the important factors that lacked in the complex of agronomical practices in apple cultivation were the total cross-pollination and fertilization of flowers and this led to the flower as well as fruit drop and a higher fruit drop in Golden Delicious and Red Gold cultivars (Kolesnikov, 1972; Dulta and Verma, 1987; Mattu *et al.*, 2012; Mattu, 2014) but pollination by *Apis cerana* enhanced increase in fruit setting and quality of fruit set in apple crop (Mattu and Mattu, 2007).

2. Methodology

Quantitative and qualitative effects of honeybee pollination on apple crop were investigated on European bee, *Apis mellifera* L. and Indian hive bee, *Apis cerana* F., by placing two colonies of each species in experimental apple orchards located at Padola (2200 m), Kalzar (2514 m) and Jarol (2648 m) areas in Shimla hills of Himachal Pradesh.

2.1 Effect of Honeybee Pollination on Fruit Set

The following experimental designs were set to study the effect of honeybee pollination on fruit set at the time of bloom in the apple orchards located at different altitudes.

2.1 (a) Experiment A (Honeybee pollinated flowers)

In honeybee pollinated flowers, four trees, one each of Golden Delicious, Red Gold, Royal Delicious and Red Delicious were enclosed in an insect proof net in which two colonies of honeybees (one of *A. mellifera* and one of *A. cerana*) were placed as pollinators of apple bloom. In this experiment, four branches of each variety, containing approximately eight hundred pink buds were chosen at random. These branches of experimental trees were chosen in such a way that they were of same dimensions with respect to their spread, phase of flowering and height above the ground. The bees were kept inside the net till the last flower on experimental trees. Fruit set was observed after ten days of petal fall and the percentage was measured as below:

$$\text{Percentage of Fruit Set} = \frac{\text{Number of Fruits}}{\text{Number of Pink Buds}} \times 100$$

2.1(b), Experiment B (Open, besides honeybees other natural insect pollinators were present)

In open pollinated flowers, honeybees and other natural insect pollinators could visit the apple flowers freely. In this experiment also, equal number of branches of ten experimental trees, two trees each of Golden Delicious and Red Gold and three trees each of Royal Delicious and Red Delicious were chosen in the same way as in case of honeybees pollinated experiment and each branch contained approximately eight hundred pink buds. The results were expressed in terms of percentages of fruit set and calculated as honeybee pollinated flowers.

2.1(c) Experiment C (Control, where no insect pollinator was present)

In control experiment ten trees, two each of self-compatible varieties (Golden Delicious, Red Gold) and three each of self-incompatible varieties (Royal Delicious and Red Delicious) were selected and in each tree four branches, each containing approximately five hundred flowers at pink bud stage were chosen at random and covered with muslin bags so that no insect pollinator could enter the bag for pollination. These branches of experimental trees were chosen in the same way as in case of honeybee pollinated experiment. Before covering with muslin bags, the numbers of pink buds were counted in each experimental branch. The fruit set was observed after ten days of the petal fall, calculated as honeybee pollinated flowers.

Similar experimental designs were set up in all the three orchards and the percentage of fruit set was calculated.

2.2 Effect of honeybee pollination on fruit drop

After calculating the fruit set in different experimental designs, the number of fruits dropped in the month of June, was also noted in different experimental designs i.e. open, honeybee pollinated and control. The fruit drop was observed from the ratio of fruits dropped to the total number of fruits set. This fruit drop was also calculated in terms of percentage (Dulta, 1986).

2.3 Quality improvement in the apple fruit due to honeybee pollination

Quality improvement in the fruit due to pollination was assessed in terms of increase in weight, length, breadth,

volume and number of seeds per fruit. Weight of fruit in grams was measured with the help of a top pan electric balance; length and breadth in centimeter with the help of Vernier callipers; volume in ml on the line of the principle that the volume of an object is equal to the amount of water displaced by it. Finally, the number of seeds in a fruit was counted by cutting it. For each parameter, ten fruits were taken to get the mean values of weight, length, breadth, volume and number of seeds (Dulta, 1986; Kumar, 1997).

3. Results and Discussion

3.1 Effect of insect pollinators on fruit set of apple

In apple orchard at Padola (2200 m), self-compatible varieties like Golden Delicious and Red Gold, the fruit sets in control experiment were 17.53% and 15.74% respectively, whereas, in open pollinated flowers of Golden Delicious and Red Gold the fruit set was 20.77% and 17.91% respectively. In honeybees pollinated flowers, the fruit set in Golden Delicious and Red Gold were 31.33% and 23.69% respectively. However, in case of self-incompatible varieties of apple, such as Royal Delicious and Red Delicious, the role of honeybees in pollination was very significant. For example, the fruit set in Royal Delicious were 0%, 15.71% and 22.52% percent in control, open and honeybee pollinated flowers respectively. Similarly, at the same orchard, the fruit set in Red Delicious were 0%, 13.50% and 21.02% in control, open and honeybee pollinated flowers respectively (Table 1).

At Kalzar, (2514 m) in Golden Delicious, Red Gold, Royal Delicious and Red Delicious the fruit set in control experiment were 16.89%, 15.93%, 0% and 0% respectively. In open pollinated flowers fruit set were 18.51%, 16.33%, 15.57% and 11.95% in Golden Delicious, Red Gold, Royal Delicious, Red Delicious respectively, whereas, in honeybees pollinated flowers the fruit set in Golden Delicious, Red Gold, Royal Delicious and Red Delicious were 29.74%, 22.82%, 21.61% and 20.51% respectively (Table 1).

In Golden Delicious, Red Gold, royal Delicious and Red Delicious the fruit set were 17.22%, 17.37%, 0% and 0% respectively in control experiment, whereas, the fruit set was 19.82%, 20.04%, 8.74% and 8.63% respectively in open pollinated flowers at Jarol (2648 m). Moreover, at the same height, the fruit set in honeybee pollinated flowers of Golden Delicious, Red Gold, Royal Delicious and Red Delicious was 22.58%, 21.71%, 17.39% and 16.62% respectively (Table 1). In self-compatible varieties of apple (Golden Delicious and Red Gold) there was no significant difference ($P > 0.01$) in the fruit set in different experimental designs (honeybees pollinated flowers, open pollinated flowers and in control) but the differences in these experimental designs were significant ($P < 0.01$) in self-incompatible varieties like Royal Delicious and Red Delicious.

3.2 Effect of insect pollinators in apple fruit drop

In the present study it has been found that fruit drop was significantly higher ($P > 0.01$) in self-compatible varieties of apple under controlled experiment as compared to the fruits

from open and honeybees pollinated flowers. At Padola, Kalzar and Jarol in Golden Delicious, the fruit drops were maximum (37.73%, 39.20% and 40.17% respectively) under control experiment and minimum (25.74%, 27.21% and 28.92% respectively) in honeybee pollinated flowers. In open pollinated flowers of Golden Delicious, the fruit drops were 28.31%, 28.73% and 30.99% at Padola, Kalzar and Jarol respectively (Table 2). Similarly at Padola, Kalzar and Jarol in Red Gold, the fruit drops were maximum (37.33%, 38.90% and 37.52% respectively) under control experiment and minimum (24.69%, 26.47% and 28.27% respectively) in honeybee pollinated flowers. In open pollinated flowers of Red Gold, the fruit drops were 30.88%, 32.89% and 31.99% respectively. No significant difference ($P>0.01$) was observed in fruits drops of self-compatible varieties between open and honeybees pollinated flowers. In self-incompatible variety like Royal Delicious, the fruit drops in open pollinated flowers were 30.20%, 30.93% and 31.32% at Padola, Kalzar and Jarol respectively, whereas, in honeybees pollinated flowers the fruit drops were 26.20%, 26.47% and 27.58% respectively. In other self-incompatible variety of Red Delicious, the fruit drops in open pollinated flowers at Padola, Kalzar and Jarol were 30.36%, 29.99% and 31.47% respectively, whereas, in honeybees pollinated flowers of Red Delicious, the fruit drops were 25.04%, 26.81% and 28.20% respectively. The difference observed in the fruit drops of self-incompatible varieties between open and honeybees pollinated flowers was without any significant difference ($P>0.01$).

3.3 Quality improvement in the apple fruits due to insect pollination

Quality improvement in the apple fruit was observed in terms of increase in weight (gm), length and breadth (cm), volume (ml) and number of seeds per fruit. In apple orchard at Padola, the mean weights of apple fruit in honeybee pollinated Golden Delicious, Red Gold, Royal Delicious and Red Delicious were 190.28 ± 1.92 gm, 195.16 ± 3.12 gm, 188.66 ± 3.24 gm and 175.16 ± 7.23 gm respectively. Whereas, at Kalzar the mean weights in honeybees pollinated Golden Delicious, Red Gold, Royal Delicious and Red Delicious were 247.88 ± 0.46 gm, 206.75 ± 1.25 gm, 347.83 ± 2.92 gm and 256.84 ± 5.43 gm respectively. At Jarol, the mean weights of Golden Delicious, Red Gold, Royal Delicious and Red Delicious in honeybee pollinated fruits were 195.56 ± 1.90 gm, 180.75 ± 1.24 gm, 266.16 ± 2.12 gm and 216.46 ± 3.92 gm respectively (Table 3).

The mean lengths of Golden Delicious, Red Gold, Royal Delicious and Red Delicious under this set of experiment at Padola were 8.53 ± 0.40 cm, 7.68 ± 0.04 cm, 8.16 ± 0.42 cm and 7.17 ± 0.54 cm respectively, while at Kalzar the mean lengths of Golden Delicious, Red Gold, Royal Delicious and Red Delicious were 8.16 ± 0.17 cm, 7.99 ± 0.10 cm, 10.66 ± 0.38 cm and 8.17 ± 0.43 cm respectively. The mean lengths of fruit in honeybees pollinated flowers of Golden Delicious, Red Gold, Royal Delicious and Red Delicious were 8.82 ± 0.53 cm, 7.92 ± 0.41 cm, 9.59 ± 0.03 cm and 8.02 ± 0.19 cm respectively at Jarol (Table 3).

In apple orchard at Padola, the mean breadths of Golden Delicious, Red Gold, Royal Delicious and Red Delicious in

honeybee pollinated flowers were 8.39 ± 0.23 cm, 7.84 ± 0.14 cm, 8.49 ± 0.53 cm and 8.54 ± 0.23 cm respectively. The mean breadths of Golden Delicious, Red Gold, Royal Delicious and Red Delicious in Honeybees pollinated flowers were 8.91 ± 0.04 cm, 8.75 ± 0.09 cm, 9.74 ± 0.24 cm and 9.03 ± 0.92 cm respectively at Kalzar, whereas, the mean breadths were 8.84 ± 0.02 cm, 8.02 ± 0.14 cm, 9.21 ± 0.03 cm, and 8.46 ± 0.06 cm, in Golden delicious, Red Gold, Royal Delicious and Red Delicious respectively at Jarol. The mean volumes of Golden delicious, Red Gold, Royal delicious and Red Delicious were 161.88 ± 3.13 ml, 171.29 ± 4.82 ml, 165.29 ± 3.75 ml and 140.58 ± 8.20 ml respectively at Padola, whereas, 281.50 ± 3.26 ml, 200.88 ± 2.35 ml, 417.02 ± 3.51 ml and 242.59 ± 13.25 ml respectively at Kalzar and 173.96 ± 1.92 ml, 154.59 ± 1.85 ml, 230.04 ± 2.05 ml and 177.50 ± 2.97 ml respectively at Jarol (Table 3).

At Padola, the mean number of seeds per fruit in honeybee pollinated flowers of Golden Delicious, Red Gold, Royal Delicious and Red Delicious were 7.24 ± 0.10 , 7.80 ± 0.10 , 6.8 ± 0.10 and 6.44 ± 0.10 respectively whereas, the mean number of seeds per fruit were 10.29 ± 0.38 , 11.29 ± 2.05 , 8.89 ± 0.67 and 8.29 ± 1.67 , respectively at Kalzar and these were 10.20 ± 0.87 , 10.89 ± 1.05 , 8.62 ± 0.92 and 8.29 ± 1.02 respectively at Jarol (Table 3).

In self-incompatible varieties like Royal Delicious and Red Delicious there was not any fruit set in control experiment but in self-compatible varieties like Golden Delicious and Red Gold the quality of apple fruit was noticed. It was noticed that weight, length, breadth, volume and number of seeds per fruit in Golden Delicious and Red Gold at all the three orchards were significantly maximum ($P<0.01$) in fruits which developed from honeybees pollinated flowers and minimum in fruits under control experimental conditions, whereas, in Royal Delicious and Red Delicious the weight, length, breadth, volume and number of seeds per fruit were significantly more ($P<0.01$) in fruits from honeybees pollinated flowers than in fruits from open pollinated flowers.

In honeybee pollinated flowers the fruits were larger in size than fruits from open pollinated flowers which were in turn larger than the fruits from control experiment. Observations on fruit quality were made by some earlier workers (Singh, 1962; Morse, 1976) who reported that honeybee pollination increased quality and quantity of apple fruits. The better pollinating efficiency of honeybees help in the fertilization of maximum number of ovules and thereby more number of seeds are formed in this way maximum amount of auxin (a growth hormone) is produced which results in better size of fruits. Fruits obtained by self pollination had a lower average weight, size and poor colour and contained a small number of seeds as compared to the fruits obtained from cross pollination (Murneek and Schowengerdt, 1935). It was reported that greater number of seeds depended upon greater number of bees involved in cross pollination (4). There was positive relation between honeybee pollination and quality of apple crop (Dulta, 1986; Sihag, 1997; Belavadi *et al.*, 2005). Fruit set in peach (*Prunus persica* L.) was maximum (6.5 fruit/branch) with placement of honeybee colonies at the closet distance of 20 m (5). Similar results on fruit quality

and yield due to honeybee pollination was also observed in different crops (Potts *et al.*, 2010b; Raj *et al.*, 2012).

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4. Acknowledgement

Table 1: Percentage of fruit set in three different experimental designs

Sr. No.	Variety	Padola (2200 m)			Kalzar (2514 m)			Jarol (2648 m)		
		Honeybees pollinated flowers (H)	Open pollinated flowers (O)	No insect pollinator (Control) (C)	Honeybees pollinated flowers (H)	Open pollinated flowers (O)	No insect pollinator (Control) (C)	Honeybees pollinated flowers (H)	Open pollinated flowers (O)	No insect pollinator (Control) (C)
1.	Golden delicious	31.33	20.77	17.53	29.74	18.51	16.89	22.58	19.82	17.92
2.	Red Gold	23.69	17.91	15.74	22.82	16.33	15.93	21.71	20.04	17.37
3.	Royal Delicious	22.52	15.71	0	21.61	15.57	0	17.39	8.74	0
4.	Red Delicious	21.02	13.50	0	20.51	11.95	0	16.62	8.63	0

For honeybee pollination two colonies, one *Apis mellifera* and one *Apis cerana* with 6 frames each were placed inside the net.

Each percentage is an average of twelve observations. S.E. = Standard error about the mean.

Table 2: Percentage of fruit drop in three different experimental designs

Sr. No.	Variety	Padola (2200 m)			Kalzar (2514 m)			Jarol (2648 m)		
		Honeybees pollinated flowers (H)	Open pollinated flowers (O)	No insect pollinator (Control) (C)	Honeybees pollinated flowers (H)	Open pollinated flowers (O)	No insect pollinator (Control) (C)	Honeybees pollinated flowers (H)	Open pollinated flowers (O)	No insect pollinator (Control) (C)
1.	Golden delicious	25.74	28.31	37.73	27.21	28.73	39.20	28.92	30.99	40.17
2.	Red Gold	24.69	30.88	37.33	26.47	32.89	38.90	28.27	31.99	37.52
3.	Royal Delicious	26.20	30.20	0	26.47	30.93	0	27.58	31.32	0
4.	Red Delicious	25.04	30.36	0	26.81	29.99	0	28.20	31.47	0

For honeybee pollination two colonies, one *Apis mellifera* and one *Apis cerana* with 6 frames each were placed inside the net.

Each percentage is an average of twelve observations. S.E. = Standard error about the mean. For fruit drop: $H < O < C$ ($P < 0.01$).

Table 3: Effect of insect pollinators on the quality of apple fruit in terms of weight (gm), length (cm), breadth (cm), volume (ml) and number of seeds per fruit

Variety	Honeybees pollinated flowers (H)					Open pollinated flowers (O)					No. insect pollinator (C) control					
	Weight WH	Length LH	Breadth BH	Volume VH	No. of Seeds SH	Weight WO	Length LO	Breadth BO	Volume VO	No. of Seeds SO	Weight WC	Length LC	Breadth BC	Volume VC	No. of Seeds SC	
Padola (2200 m)																
1.	Golden delicious	190.28 ±1.92	8.53 ±0.40	8.39 ±0.23	161.85 ±3.13	7.24 ±0.10	145.23 ±3.16	7.79 ±1.05	8.70 ±0.10	138.96 ±2.05	8.62 ±0.67	86.23 ±3.03	6.58 ±0.05	6.78 ±0.67	69.94 ±8.00	4.96 ±0.57
2.	Red Gold	195.16 ±3.12	7.68 ±0.04	7.84 ±0.14	671.29 ±4.82	7.20±0.10	280.32 ±2.91	6.52 ±0.07	6.64 ±0.07	65.50 ±0.96	7.29 ±0.78	47.96 ±2.05	5.80 ±0.08	6.20 ±0.45	32.83 ±1.95	62.5 ±0.47
3.	Royal Delicious	188.66 ±3.24	8.16±0.42	8.49±0.53	165.29 ±3.76	5.8 ±0.10	169.83 ±3.97	7.79 ±1.29	8.14 ±0.45	145.93 ±3.98	5.99±0.97	No fruit set				
4.	Red Delicious	175.16 ±7.23	7.17 ±0.54	8.54 ±0.23	140.58 ±8.21	5.44 ±0.10	130.24 ±7.90	6.80 ±1.11	6.78 ±0.76	113.75 ±12.37	6.29 ±0.80	No fruit set				
Kalzar (2514 m)																
1.	Golden delicious	247.88 ±0.46	8.16 ±0.17	8.91±0.04	281.50 ±3.26	10.29 ±0.38	221.56 ±1.19	8.71 ±0.02	8.39 ±0.54	203.96 ±0.74	9.29 ±1.04	105.86 ±0.75	7.67 ±0.10	7.91 ±0.67	100.00 ±4.00	6.69 ±1.05
2.	Red Gold	206.75 ±1.25	7.99 ±0.10	8.75 ±0.09	200.88 ±2.35	11.29 ±2.05	181.75 ±0.99	6.94 ±0.04	8.43 ±0.10	190.88 ±7.80	8.62 ±0.67	161.41 ±1.02	6.00 ±1.02	7.32 ±1.06	84.88 ±2.18	6.69 ±1.02
3.	Royal Delicious	347.83 ±2.92	10.66 ±0.28	9.74±0.24	417.50 ±3.51	8.89 ±0.67	244.90 ±6.75	8.96 ±1.03	8.67 ±0.56	232.90 ±3.97	6.29 ±0.67	No fruit set				
4.	Red Delicious	256.84 ±5.43	8.17±0.43	9.03 ±0.42	242.59 ±13.25	8.29 ±1.67	231.79 ±15.16	8.04 ±0.85	8.12 ±0.35	215.09 ±3.05	6.99 ±0.56	No fruit set				
Jarol (2648 m)																
1.	Golden delicious	195.56 ±1.90	8.82 ±0.53	8.84±0.02	173.96 ±1.92	10.20 ±0.87	153.89 ±2.36	8.27 ±0.68	8.24 ±0.05	161.59 ±6.44	8.79 ±0.35	125.89 ±3.87	9.96 ±0.67	6.89 ±0.07	84.78 ±3.10	6.39 ±0.91
2.	Red Gold	180.75 ±1.24	7.92 ±0.41	8.02 ±0.14	154.59 ±1.85	10.89 ±1.05	179.45 ±2.51	7.54 ±0.56	8.23 ±0.10	139.43 ±5.45	7.95 ±0.54	171.11 ±5.63	6.86 ±0.19	6.92 ±1.00	80.82 ±2.78	7.32 ±1.02

3.	Royal Delicious	266.16 ±2.12	9.59±0. 03	9.21 ±0.03	230.04 ±2.05	8.62 ±0.92	25.192 ±6.46	7.65 ±0.45	8.59 ±0.25	159.26 ±3.05	6.29 ±0.64	No fruit set
4.	Red Delicious	216.46 ±3.92	8.07 ±0.19	8.46 ±0.06	177.50 ±2.97	8.29 ±1.02	193.79 ±4.54	8.42 ±0.56	8.26 ±0.19	154.59 ±6.54	6.36 ±0.89	No fruit set

For honeybee pollination two colonies, one *Apis mellifera* and one *Apis cerana* with 6 frames each were placed inside the net.

Each percentage is an average of twelve observations.

S.E. = Standard error about the mean.

In all the orchards:

1W(H) > 1W(O) > 1W(C) : 1L(H) > 1L(O) > 1L(C) : 1B(H)

> 1B(O) > 1B(C) : 1V(H) > 1V(O) > 1V(C) : 1S(H) >

1S(O) > 1S(C) (P<0.01)

2W(H) > 2W(O) > 2W(C) : 2L(H) > 2L(O) > 2L(C) : 2B(H)

> 2B(O) > 2B(C) : 2V(H) > 2V(O) > 2V(C) : 2S(H) > 2S(O)

> 2S(C) (P<0.01)

3W(H) > 3W(O) : 3L(H) > 3L(O) : 3B(H) > 3B(O) :

3V(H) > 3V(O) : 3S(H) > 3S(C) (P<0.01)

4W(H) > 4W(O) : 4L(H) > 4L(O) : 4B(H) > 4B(O) :

4V(H) > 4V(O) : 4S(H) > 4S(C) (P<0.01)

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