Chapter 3

MATERIALS AND METHODS

This research consists of two related parts, a field experiment and a field survey, both in Yunnan, the People's Republic of China.

3.1 Field Experiment

The experiment was conducted on 15-year-old Golden Delicious apple trees at Dabaiqiao Horticultural Farm in Kunming, Yunnan province. At this farm, the 20 year average temperature is 15 ^{0}C. January is the coolest month, with average temperature of 8 ^{0}C. July is the hottest month with an average temperature of 20 ^{0}C and has highest rainfall. Rainy season starts from May to September having monthly rainfall from 94 to 216 mm, respectively. The soil is a red earth with a pH of 5.5 and contains organic matter, total nitrogen (N), available phosphorus (P), exchangeable potassium (K), exchangeable calcium (Ca) and hot water soluble B concentration of 20.2×10^3, 600, 8.5, 85, 211 and 0.28 mg kg^{-1} soil, respectively. The spacing of experimental trees was 7 m × 7 m. Yield in 1995, the year preceding B treatment, was only 45-55 kg tree^{-1}.

At full bloom, (27 March, 1996), four levels of borax (11% B): 0 (B0), 40 (B40), 80 (B80) and 160 (B160) g tree^{-1} were applied on four replicate trees in a randomized complete block design (RCB). Each experimental unit consisted of one tree, with guard trees on four sides (Appendix -A). Borax was uniformly broadcasted on the soil surface

under the tree canopy and mixed well with soil. After B application, the trees were immediately sprinkle irrigated and irrigated every two weeks for four times. The basal fertilizers had been applied as urea, calcium superphosphate and potassium sulphate at rates of 1 kg N tree^{-1}, 0.5 kg P tree^{-1} and 0.5 kg K tree^{-1}, respectively, 20 days before supplying of borax. In addition, 150 kg cattle manure had been applied to each tree.

Five days after B application when new shoots were about 3 cm, ten new shoots and ten flower clusters were tagged on each tree. Length of tagged shoots, length and diameter of every fruit developing from tagged flower clusters were measured every 4 weeks from 4 to 20 weeks after full bloom (4W-20W).

The number of all flower clusters on each tree was counted 5 days after B application: in subsequent counts of fruit set and fruit drop, this number was assumed to represent the maximum possible number of fruit set. The number of dropped fruit was recorded during 3 fruit dropping stages: pea-size drop (1.5 cm fruit diameter), June drop (3.5 cm fruit diameter) and pre-harvest drop (5.5 cm fruit diameter). After June drop, fruit were thinned if necessary to a maximum of 25% of total fruit set.

For tissue B concentrations, 30 flower clusters, young shoots and young open leaves (YOL) with petioles were sampled 5 days before B application. Flower clusters were separated into central flowers and border flowers. Then, 30 of the youngest fully expanded leaves (YFEL) with petioles, young shoots and fruit were sampled at 4W, 8W,

12W, 16W and 20W. In addition, 30 dropped fruit were randomly sampled at 4W (pea-size drop), 8W (June drop) and 16W (pre-harvest drop).

When the skin color of 50% of the fruit surface changed from green to yellow, fruit of Golden Delicious apple ready for harvest. Fruit were harvested from each treatment when 50% of total fruit per tree had 50% of yellow color on their fruit shin area in B0. At harvest, 20W, 30 fruit were sampled from each treatment. Ten fruit were analyzed for B concentration and the rest were assessed for fruit physical firmness, soluble solids, total sugar, titratable acidity, vitamin C and number of seeds. Moreover, an additional 40 fruit were sampled from B160 trees for quality components. In addition, the number fruits tree^{-1} and fruit yield were recorded. Average fruit weight was obtained by dividing fruit yield by the number of fruit tree^{-1}.

The extra 40 fruit from B160 trees were equally separated into two equal groups kept under room temperature (20 ^{0}C) and low temperature (4 ^{0}C-5 ^{0}C) in a refrigerator, respectively for 25 days before assessment of physical firmness, soluble solids, total sugar, titratable acid and vitamin C.

The flower and leaf samples for B analysis were dried in a ventilated oven at 80 ^{0}C for 2 days and the shoot samples were dried for 4 days. The leaf samples were washed with distilled water before drying. The fruit sampled during fruit development were crosscut into 0.2 cm slices and seeds and fruit stems were removed before drying at 65 ^{0}C for 7 days. Thirty mature fruit at harvest were dissected into four longitudinal

wedges. One pair of opposing sections were further crosscut into 0.2 cm slices. The rest were further dissected into skin (0.1 cm), outer cortex (half radius from skin), inner cortex (half radius from core), and core tissue without seed (1.5 cm diameter). These fruit parts were dried at 65 ^{0}C for 10 days. Then, dried plant parts were ground before analysis for B concentration.

Dry-ashing was used to extract B from plant tissue. Extracted solutions were colored for B determination by the Azomethine-H method (Basson, *et al.*, 1969). Two samples of standard reference material containing 12 ± 1 (mean ± standard error) mg B kg^{-1} dry wt. from Chiang Mai University were included with each batch of samples. Detail of sample preparation and B analysis is presented in Appendix -D.

Flesh firmness of fruit with skin and soluble solids were measured by using a Magness-Taylor penetrometer and a hand refractometer, respectively. Total sugar was determined by using Feiling's reagent titratable method (Helrich, 1990).

For titratable acidity (% malic acid), fruit juice pH was brought to 8.1 by using 0.1 N NaOH. Then percentage of malic acid in fresh weight was calculated by using the following formular:

$$\text{Malic acid (\%)} = \frac{V * N * 0.067}{Y} \times 100 \quad \text{(Helrich, 1990).}$$

N = Normality of NaOH

V = Volume of NaOH (ml)

Y = Fresh weight of sample (g)

0.067 is equivalent to the weight of malic acid

For vitamin C, 10 g cut fresh fruit was ground in 5-10 ml 2% (v/v) HCl. Extracted solution was transferred to a brown volumetric flask and volume was adjusted to 100 ml with 2% HCl. Zero point five ml 10% KI, 1 ml 0.5% starch and 5 ml distilled water were added to 10 ml of diluted solution before titrating with 0.001N KIO$_3$. Then, vitamin C content was calculated by using the following equation:

$$Vita\,min.C\,(mg\,/\,100g) = \frac{V*0.088}{B} \times \frac{b}{a} \times 100 \qquad \text{(Helrich, 1990)}.$$

V = KIO$_3$ volume (ml)
B = Adequat sample solution volume (ml)
b = Total diluted solution volume (ml)
a = Sample weight (g)
0.088 is equivalent to 1 ml 0.001N KIO$_3$ of vitamin C

Costs of labor, irrigation management, pesticide fertilizer and the sale price of apple of each treatment were recorded. Returns on investment in borax were obtained by dividing increased net benefit by cost of borax.

3.2 Field Survey

The field survey was undertaken in three major apple production areas of the temperate highlands of Yunnan: Lijiang (26^0 N 100^0 E, 2393 MSL), Zhaotong (27^0 N 104^0 E, 1950 MSL) and Kunming (25^0 N 109^0 E, 1900 MSL). Twenty orchards of Golden Delicious apple in each production area were surveyed. In Lijiang, 10 orchards

was located at Taian and 10 orchards was located in Baisha; in Zhaotong, 10 orchards was located at Ludian and 10 orchards was located in Shayi; in Kunming, 11 orchards was located at Guaidu and 9 orchards was located Xishan. In each orchard, type and amount of used fertilizer were recorded.

At fruit harvest, samples of leaf with petioles and fruit were taken from each of 60 orchards for B analysis. Each leaf and fruit samples consisted of 30 leaves and 30 fruit respectively from 6 trees,. Thirty fruit were separated into 2 groups. In the first 20 fruit, each fruit was weighed and its diameter, length, firmness, soluble solids, and seed number were measured. The rest of the fruit were analyzed for B concentration. Each fruit was crosscut into 0.2 cm slices. Seeds and fruit stem were removed and dried in a ventilated oven at 65 ^{0}C for 10 days and milled. The procedures for leaf sampling, preparation and B analysis were similar as that in the field experiment.

3.3 Data Analysis

Statistix software was used to analyze variance, least significant difference (LSD) and regression of data.