SPACING AND MICRONUTRIENT EFFECTS ON YIELD AND PUNGENCY OF CHILE PEPPERS (*CAPSICUM ANNUUM* L.)

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TABLE OF CONTENTS

LIST OF TABLES

Table		Page

CHAPTER 1

INTRODUCTION

Hot pepper, *Capsicum annuum L*, also known as chile, red pepper, cayenee or bird pepper, depending upon the type and the way in which it is used, belongs to the Solanacea family which also includes eggplants, tomato and potato. The genus *Capsicum* contains well over 200 varieties ranging from the very pungent serrano to the very mild or sweet bell peppers (14, 36). Due to their many uses as spice, preservatives, pharmaceutical formulations, food coloring, etc., peppers are considered to be one of the most important crops in the world today and are increasingly in demand by the spice and food industries all over the world (31).

Peppers are widely grown throughout the tropical and subtropical areas of the world like Africa, Asia and Southeast Asia and are, in terms of the scale of production, the most important of all spices. However, due to several factors such as increasing cost of production, political upheavals and natural disasters in the major pepper exporting countries in Africa, Asia and others, imports to the United States are decreasing. This, on the other hand, is stimulating interest in commercial acreage in the United States, and especially in Oklahoma and Texas.

Because of the great interest being shown by the spice industry, pepper production is likely to attract additional growers in Oklahoma. The spice industry, due to reasons mentioned above, is very eager to have as many domestic sources as possible and is trying to encourage producers in Oklahoma and other parts of the country to go into the business of spice pepper production (48).

Although there is a great potential for pepper production in Oklahoma in the near future, not much has been done on research mainly because pepper is a relatively new crop to the state. There are many questions being asked by farmers as well as researchers, such as optimum plant population, color quality, capsaicin content, pest control, etc. The objective of this study was to answer some of these questions. Furthermore, when a crop is being introduced into the area, modifications of standard cultural practices may be necessary to adapt to climate, soil, topography and current farming practices of that area. Taking some of these problems into account, the following two experiments were proposed and conducted in the summer and fall of 1983.

Plant density study: Two separate locations, the Bixby Vegetable Research Farm and a grower cooperator farm near Hinton, Oklahoma were selected to conduct this study. These two sites are about 272 km apart and both have silty loam soil. Bixby is 183 m in elevation while Hinton is 427 m above sea level. Both locations have similar latitude and temperatures. Average rainfall is 96cm at Bixby and 81 cm at Hinton. Lake evaporation, on the other hand, is 157 cm at Hinton and 132 cm at Bixby due to lower relative humidity and greater wind speed at Hinton. The purpose of this study was to determine the optimum in-row spacing and number of plants/site.

Pungency study: This was a greenhouse study conducted in Stillwater at the Horticultural Research Greenhouse in the fall and winter of 1983. The purpose of this study was to determine whether foliar applications of micronutrients could increase the pungency of Bahamian Hot Chiles.

CHAPTER II

LITERATURE REVIEW

Plant Density Studies

Many factors are recognized as limiting crop growth and productivity. Some factors such as water, nutrients, insects, and diseases are subject to a measure of control, and most crop management practices are directed at balancing the levels of control to attain maximum economic return. When such controls are successful and when these factors are not limiting, maximum productivity depends primarily on rates of light interception and carbon dioxide assimilation by the crop surface which could be affected by how far apart or how close plants are spaced (24). Carbon compounds derived from photosynthesis are responsible for 90 to 95% of the total dry matter of plants. The amount of light available has an impact on total photosynthesis and hence yield (41).

Several studies in plant arrangement patterns have been conducted with crops such as soybeans and corn to determine the effect of equidistant (square or hexagonal) planting pattern over wide rows and narrow plant spacing at corresponding plant populations (11, 19, 38). Some of these studies indicate that the increase in yield may not be due to any particular arrangement of plants (38). However, another study showed that equidistant spacings yielded 12 and 13% higher than rectangular spacings at equal populations indicating that planting pattern was a factor for better production (26).

The use of improved machinery and the introduction of herbicides have now made it unnecessary for the wide row spacings used for crops like cotton, soybean and corn. Increasing plant population has been recommended to increase yields in these three crops (8, 22).

Numerous researchers also claim that peppers in general yield higher under narrower row spacings than under wider spacings. It was reported that among the various spacings examined, the most narrow spacing of 30x30cm gave the highest yield in all four varieties of hot peppers used in the trial (30). Also in another experiment, it was indicated that of all three spacings studied with a hot pepper cultivar, Jwala, the closest spacing of 45x30cm gave the highest yield (3).

In one experiment, two capsicum cultivars were tested and both cultivars yielded greatest under the highest plant density of 6 plants/m^2 (19). In a similar experiment, capsicums were grown at densities of 8, 12 or 24 plants/m^2 and the 24 plants/m^2 density gave the highest mean fruit yield (25). Still in another experiment, a study on cultural systems and plant spacings in autumn capsicums, it was reported that the highest yields were obtained from plants with two stems/plant spaced at the closest spacing of 30x80cm. It was also suggested that there was no significant difference between yields of plants grown with single or double stems/plant at the higher spacings (47).

In trials with capsicum cv. MDU-I, the number of shoots and fruits/plant and the weight of 100 fruits generally increased with rising N rates but decreased with plant density. The highest yield of dry fruits was obtained from plots with plants at the closest spacing of 30x20cm (33).

In one experiment where the effect of cultivar and plant density on the yield of mechanically harvested paprika was studied, it was reported that increasing plant density resulted in less lateral branching, making the fruits easier to harvest mechanically without affecting total yields (22).

There were, in general, significant differences reported in dry matter production due to both spacing and population from a study on soybeans. Dry matter production increased at high populations. This increase was reported to be due to efficient light interception by the greatest total canopy surface of the cultivars studied during the growth cycle (39). Increasing the number of plants/ha increased the assimilation area and increased total dry weight and hence economic yield (38). The study conducted by Szepsky on capsicums (44) also showed that the highest proportion of crop suitable for processing was produced at the highest plant population. This study also suggested that dry matter production and pigment contents were mainly determined by the cultivar and season and not by plant density.

There are also several researchers who reported that spacing did not have much effect on yield and total dry matter. In fact, some workers have indicated a reduction of yield as plant population increased. Fowler (5), reported that as plant density in cotton increased, stem diameter, number of branches, plant height and plant dry weight decreased resulting in smaller plants. He also showed that lower population levels enhanced earliness more than high population levels. Increasing the number of plants per unit land area also increased competition within the crop for space, light, CO_2, H_2O and nutrients (4, 10). Consideration of competition between plants is very important because both too high or too low densities will result in yield reduction. However, plants like cotton, generally, can adapt to a relatively wide range of populations with only slight effects on yield (4). As plant density increases in cotton a linear decrease is observed in stalk diameter, plant height, size of branches, size of bolls, number of branches per plant and bolls per plant. No significant yield difference was observed in cotton due to high populations (10). Although seed yields of soybean tended to be higher at the narrow spacing between rows, the effects of spacing within row were variable and seeds were lighter in weight as spacing decreased and the number of seeds per plant decreased (18).

Spacing is also believed to have an effect on plant lodging. It was reported that lodging on soybeans increased as population increased. Pods per plant significantly decreased as population increased. Significantly higher yield and less lodging was obtained at the lower population (12).

<u>Greenhouse Pungency Study</u>

Hot pepper contains a group of unique alkaloids, of which capsaicin and dihydrocapsaicin are the most important components (5). The pungency and the effect on the touch receptors is due to capsaicin, which is a fat soluble, flavorless, odorless and colorless compound (14, 32).

Capsaicin (trans-8-methyl-N-vanillyl-6-non-amide) is the major principle of chile pepper and paprika, and is known for its irritant properties. Capsaicin is believed to produce a number of physiological effects like increased salvation and sweating, altered respiration and blood pressure and decreased intestinal transport. It may also contribute to the etiology of liver cancer, particularly in areas where protein resources are limited (2).

With regard to the pungent principles of red pepper, at least five compounds have been reported. The list of these compounds includes capsaicin (CAP), dihydrocapsaicin (DHC), homocapsaicin (HC), homodihydrocapsaicin (HDC), and nordihydrocapsaicin (NDC) (15, 25, 43). Among these, CAP and DHC are the major analogues occupying more than 90% of the total capsaicinoids. On the other hand, HDC, HC, and NDC are considered to be minor analogues (6). Among the two major analogues, CAP is the most important component occupying 60% of the total capsaicinoid (16). All analogues, however, are biosynthesized from L-phenylalanine and L-valine, or L-phenylalanine and L-leucine in the placenta of capsicum fruits (19). Capsaicin synthetase is believed to be responsible for catalyzing reactions in these processes (5). The term 'capsaicinoid(s)' has been used to represent all these analogues of capsaicin. The pungent principle, capsaicinoids, have been widely used as spices, food additives, and also as drugs (15). The structure of the pungent principle, capsaicinoid, is the acid-amides of vanillylamine and C_9 to C_{11} isotype fatty acid (6).

It was Thresh who, in 1876, crystallized the pungent principle of capsicum spices and came up with the name capsaicin. The structure of capsaicin was then later shown by Dawson and Nelson, in 1923, to be the vanillylamide of nodicyclenic acid (7, 45).

Several methods have been in use for determination of the capsaicin in <u>Capsicum</u> spices. In the United States, the most common means of estimating the pungency of <u>Capsicum</u> spices is an organoleptic procedure introduced by Scoville in 1912 (8). However, the accuracy of this method is limited and often exhibits poor reproducibility between laboratories. A number of ultraviolet and colorimetric spectrophotometric procedures have also been reported for determination of capsaicin. The instrumental procedures have their problems in that they do not generally differentiate between capsaicin and its synthetic analogues. Furthermore, these procedures involve lengthy isolation steps. According to some researchers, the gas-liquid-chromatography procedure is the better alternative for the determination of capsaicin (9, 45, 46).

It is generally agreed that the distribution of capsaicin within the fruit is not uniform. According to one study, the pericarp, which is 40% of the chile dry weight, contains 89% of the capsaicin, the seeds, which are 54% of the chile, contain 11% of the total capsaicin (14). But according to another study, the seeds actually do not contain any capsaicin. It was suggested that the capsaicin which is detected on the seeds is mainly due to surface contact contamination resulting during separation of seeds from the remainder of the fruit (14). Pungency values of 0 in the seeds to a mean of 121.34 ng/g in the whole fresh pepper have been reported in other experiments (14, 36). In another experiment, it was suggested that the greatest concentration of capsaicin is found in the cross wall portion of the pepper pod (14).

Contradictory results have been reported as to the specific period of maximum capsaicin production. According to one study (16), capsaicinoid was detected 20 days after flowering both in placenta and pericarp and reached maximal level about 40 days after flowering. It was reported that the capsaicinoid started decreasing significantly on the

50th day. Because the capsaicinoid content reached the maximum level while the fruits were still alive, Iwai then concluded that the formation and accumulation of capsaicinoid might not necessarily be associated with senescence. Iwai attributed this result to degradation of capsaicinoids by an enzyme and to chemical decomposition of capscinoid by reactions such as photoxidation (16).

According to Ohta, the total capsaicinoid content remained constant after reaching maximal levels or kept on increasing until 60 days after flowering (29).

Several studies have been done to determine the major site of capsaicinoids formation. Most workers have reported that the major site of capsaicinoid formation and accumulation is the placenta because they found that the concentration of capsaicinoids in the placenta was much higher than that in the pericarp at any of the stages examined (15, 17, 32, 42). There are still other researchers who reported the major site to be the epidermal tissue of the placenta (47), the cross wall portion of the pepper pod (14) and the vacuole (7). Furthermore, there are others who claim or suggest the pericarp contains as high as 89% of the total capsaicinoid (36).

Factors such as variety, geographic location, growing and processing conditions, stage of maturity, location within the fruit, light, etc. have been reported to influence capsaicin content in peppers.

In one experiment where sweet pepper plants were grown under continuous light or under dark conditions, capsaicinoids have been detected after four days ripening under continuous light. After seven days ripening, the content of capsaicinoids in placenta increased 2 to 5 fold of that in pericarp in sweet peppers grown under continuous light, which originally lacked the hot taste. No capsaicinoid was detected in sweet peppers grown under dark conditions (15, 17).

The price that a grower receives is, in most cases, determined by the color and pungency of red peppers. Results from one experiment show that maturity, drying procedures, and handling methods are important factors influencing initial color, color retention and pungency in peppers. In that experiment, peppers dried at 65.5°C had a significantly higher pungency level than peppers dried at lower or higher temperatures (20).

Previous studies also indicated that application of micronutrients as soil treatment or foliar application has an effect on quality, color, yield and capsaicin content of peppers. Although their report did not mention anything about pungency, the work by Navarot and Levin (26) indicates that the application of B, Cu and Zn or B + Cu + Zn significantly increased the yield and color quality of pepper fruits in a greenhouse experiment. However, under field conditions, B applied as borax was suggested to reduce yield. Moreover, the application of Cu + Zn gave the lowest percentage of unmarketable or cull fruits while Cu helped to attain higher coloration.

In a different experiment, where the influence of increased doses of micronutrients on the yield and capsaicin content was studied, it was suggested that Cu and Mn applied alone or in combination with other micronutrients in 10-fold increased proportion greatly increased capsaicin content of peppers (28).

There are also chances to increase the yield and pungency of peppers by the use of major nutrients like N-P-K. A study conducted to assess the effect of N-P-K on the yield and capsaicin content of peppers indicated that the capsaicin content of the ripe pods was significantly influenced by the various N-P-K rates, being reduced in particular by the absence of K (30). On the other hand, a study conducted on the effect of growth regulators combined with foliar applied N-P-K on the yield and capsaicin content of pepper fruits suggested that there was no influence of growth regulators and foliar-applied N-P-K noted on capsaicin content and dry weight of fruits although the highest yield of fruits and capsaicin was obtained from plants sprayed with growth regulators together with N-P-K foliar application (27).

According to Sardar (37), foliar applications of B, Mn or Zn and N sidedress application in a field study did not influence pungency of KSB 7 and KSB 10 pepper fruits.

Soil pH is believed to have an effect on pungency of pepper fruits (37). According to one study, the optimum soil pH for capsaicinoid formation, determined in several different buffer systems was found to be around 9.0 (6).

Among the many environmental factors, the effects of plant spacing, degree of irrigation, planting time and harvesting time on pungency were studied (32). The results suggest that the maximum capsaicin content was obtained from early planting and early harvesting. Crossing pungent and non-pungent cultivars is also believed to help increase pungency of peppers. In one experiment where pungent and non-pungent cultivars were crossed, some of the F_2 plants were found to be more pungent than the parents while still some were less pungent than the parents (29).

CHAPTER III

MATERIALS AND METHODS

Plant Density Study

Pepper seedling, cv. Bahamian Hot Chile, selection KSB 6-2, were obtained from Speedling Incorporated in Sun City, Florida. The greenhouse grown seedlings were size 080A. Each seedling was grown in an inverted pyramid cell that was 2.03 x 2.03 cm at the top and 4.45 cm deep. Cells tapered to a hole at the bottom allowing air root pruning.

Field plots were laid out in a 2 x 5 factorial design. Plots were made up of a central treatment row bordered on each side by a guard row to create the desired row width and within the row effect. Plots were 5 m long and 91.5 cm wide. This study had five different in-row spacings of 30, 35, 40, 45 and 50 cm and single or double plants per site. Between-row spacing was constant at 91.5 cm. The experiment was replicated four times.

Seedlings were transplanted by hand. Immediately after transplanting, each plant site received 225 ml of complete starter fertilizer solution. Single or double plant/site received the same amount of the starter fertilizer solution which was prepared by mixing 1 kg of 15-13-12 with 139 l of water.

Transplanting was done at Bixby on May 6 and at Hinton, Oklahoma on May 7, 1983. In general, everything was the same in both locations. The only difference was that the study at Hinton, Oklahoma was conducted on a farmer cooperator field unlike Bixby which was conducted on a research farm. Silt loam soils were used in both locations.

One week after transplanting, missing plants were replaced. Recommended cultural practices, such as insect and disease control, weeding, etc. were followed at both locations until the end of the study.

Plots at Bixby were harvested by hand on November 18, 1983. Harvested plants were placed in burlap sacks and dried for 24 hours at 65.5^{o}C. In harvesting, only plants in the middle row of each 3-row plot were harvested by cutting at the soil level. The guard rows on both sides of the middle rows were not harvested. The length of each harvested row was 4 m.

Plots at Hinton were harvested in the same manner on November 19, 1983 and allowed to dry in the same driers for 24 hours. After drying, all the plants were stored for further evaluation.

Pods, leaves and stems were separated in each sample by hand and weighed. From these data, percent pod and pod yields were calculated.

To determine whether plant spacing had an effect on pungency of peppers, pod samples were sent to KALSEC Inc., Kalamazoo, Michigan. Due to the large number of samples the company was not in a position to analyze all the samples. Therefore, only samples of single plants from the 30, 40 and 50 cm spacings from Hinton were analyzed. Samples from the 40cm spacing with single plants from Hinton were analyzed for pungency on different pod colors. These samples were separated into red, orange and green pod colors before the analysis.

To evaluate treatment effects on crop maturity 300 pods were taken at random from each plot at both locations and separated into three colors (green, orange and red). Number and weight of pods in each color group were determined. From these data average pod weight and percent green, orange and red pods were calculated.

Capsaicin content, expressed in Scoville Units, was determined by using the spectrophotometric method of capsaicin determination described by Palacio (34) first in 1977 and modified in 1979 (35). The method extracts capsaicin from ground peppers with ethyl acetate and then develops color with the addition of ethyl acetate solution of vandium oxytrichloride ($VOCl_3$) just before reading the extract at 720 nm.

Data were evaluated statistically using analysis of variance and means compared using LSD (23).

<u>Greenhouse Pungency Study</u>

Pepper seeds, cv. Bahamian Hot Chile, Selection KSB

6-2, were sown on a greenhouse seedling flat on September 16, 1983 in the greenhouse. After about four weeks, two seedlings were spotted over (transplanted) to 8-inch pots containing peat and vermiculite medium. After two weeks, each pot was thinned to one plant/pot by cutting one of the two plants at the soil level.

This study had eight foliar micronutrient treatments and was replicated six times. The treatments were B, Cu, Mn, BCu, BMn, MnCu, BMnCu and none. The concentration of each micronutrient foliar spray was 500ppm for B and 1500ppm for Cu, and for Mn. Salts supplying the micronutrients were $Na_2BO_3.10H_2O$ (11.34% B), $MnSO_4.H_2O$(32.51% Mn), and $CuSO_4.5H_2O$(34.22% Cu).

Spraying with micronutrients started on November 17, 1983, and was done every two weeks until April 20, 1984 for a total of 12 sprays. At each spraying, plants were sprayed until runoff. All nutrient solutions included one ml/l Surfking surfactant.

During each foliar spray period, plants in the same treatments were grouped together but separated from each treatment group by about 3 m to avoid foliar spray drift from one treatment to another. Those plants which required spraying with more than one micronutrient were first sprayed with one micronutrient, left to dry for about 15-20 minutes, and then sprayed again with the second micronutrient and so on. After each spraying, all pots were placed back on a table at their original site. This study used a randomized complete block design.

Spraying was discontinued after April 20, 1984, when some of the pods started turning red. The plants were then left in the greenhouse for about 7 weeks until most of the pods turned red.

Harvesting was done by hand on June 15, 1984 and only the red pods were harvested. After harvesting, pods were placed in labeled paper bags, fresh weights taken, and left on a table in the greenhouse for six weeks to air dry. On July 27, 1984, dry weights were recorded.

From the harvested and dried pods, 5 g samples were taken from each bag and used for nutrient content analysis. These analyses were done using the procedures explained by Horwitz (13) and by Smith and Storey (40). The rest of the pods were sent to KALSEC, Inc., Kalamazoo, Michigan, for pungency analysis.

CHAPTER IV

RESULTS

Plant Density Study

Results from the plant density studies indicated that in-row spacing and number of plants/site did not significantly influence total top dry weight at Hinton, Oklahoma. The main effects of in-row spacing and plants/site were also not significant at Bixby (Table I). However, there was a significant interaction in total top dry weight between in-row spacing and number of plants/site at Bixby. The two closest in-row spacing (30 and 35 cm) gave significantly higher top dry weight yields with one plant/site than with two plants/site compared to the 50 cm spacing.

The main effects of in-row spacing and plants/site did not significantly influence pod dry weight at Hinton although there was significant interaction between the main effects (Table II). At the 40 cm in-row spacing, one plant/site gave a significantly higher pod yield than two plants/site. With other in-row spacings number of plants/site did not significantly influence pod yield. At Bixby, there was a significant difference between plants/site treatments. One plant/site produced significantly higher pod yield than two plants/site. There

TABLE I

THE EFFECT OF IN-ROW SPACING AND NUMBER OF PLANTS/SITE ON TOTAL TOP DRY WEIGHT (G/PLOT) AT TWO LOCATIONS

No. of plants/site	In-row spacing (cm) 30	35	40	45	50	Mean
			Hinton			
1	$2989a_1^z$	$3140a_1$	$3147a_1$	$3051a_1$	$3124a_1$	3090_1
2	$2996a_1$	$3125a_1$	$2891a_1$	$2894a_1$	$3187a_1$	3019_1
Mean	2992a	3133a	3019a	2972a	3155a	
CV = 11.2%						
			Bixby			
1	$3082a_1^z$	$2864a_1$	$2791a_1$	$2918a_1$	$2287b_1$	2767_1
2	$2481a_2$	$2199a_2$	$2471a_1$	$2743a_1$	$2758a_1$	2560_1
Mean	2782a	2531a	2631a	2777a	2522a	
CV = 14.9%						

[z] Means followed by the same letter in each row or by the same number in each column are not significantly different at the 5% level.

Mean separation by LSD.

TABLE II

THE EFFECT OF IN-ROW SPACING AND NUMBER OF PLANTS/SITE ON POD DRY WEIGHT (G/PLOT) AT TWO LOCATIONS

No. of plants/site	In-row spacings (cm) 30	35	40	45	50	Mean
			Hinton			
1	1332a$_1$[z]	1402a$_1$	1559a$_1$	1429a$_1$	1497a$_1$	1444$_1$
2	1440a$_1$	1473a$_1$	1286a$_2$	1269a$_1$	1492a$_1$	1392$_1$
Mean	1386a	1437a	1323a	1349a	1495a	
CV = 12.6%						
			Bixby			
1	1656a$_1$[z]	1506a$_1$	1490a$_1$	1610a$_1$	1329a$_1$	1518$_1$
2	1236ab$_2$	1121b$_2$	1262a$_1$	1524a$_1$	1453a$_1$	1319$_2$
Mean	1446a	1313a	1376a	1567a	1392a	
CV = 18.0%						

[z] Means followed by the same letter in each row or by the same number in each column are not significantly different at the 5% level.

Mean separation by LSD.

was no significant difference between in-row spacings. There was, however, a significant interaction in pod dry weight between main effects. At the 30 cm and 35 cm in-row spacings, one plant/site gave a significantly higher pod yield than two plants/site. There was no significant difference between any of the in-row spacings with one plant/site. With two plants/site, the 45 cm produced significantly higher pod dry weight than the 35 cm in-row spacing.

Leaf weight was not significantly influenced by main effects at Bixby or Hinton (Table III). At Bixby, however, there was a significant interaction. The 50 cm in-row spacing produced a significantly higher leaf yield with two plants/site compared to one plant/site. With two plants/site spacing did not influence leaf weight. With one plant/site the 50 cm spacing had less leaf weight than other in-row spacings except the 45 cm spacing.

At Bixby and Hinton stem weight was not significantly effected by main effects (Table IV). At Bixby, stem weight was significantly higher at the 30 and 35 cm in-row spacings with one plant/site. However, there was no significant difference between plants/site treatments at these spacings. At the 50 cm in-row spacing two plants/site produced greater stem weight.

The main effects of in-row spacing and plants/site at Hinton on percent pod were not significant. Interaction between main effects indicated that the 40 cm in-row spacing

TABLE III

THE EFFECT OF IN-ROW SPACING AND NUMBER OF PLANTS/SITE ON LEAF DRY WEIGHT (G/PLOT) AT TWO LOCATIONS

No. of plants/site	In-row spacings (cm) 30	35	40	45	50	Mean
			Hinton			
1	752a$_1$[z]	695a$_1$	748a$_1$	783a$_1$	750a$_1$	746$_1$
2	672a$_1$	747a$_1$	718a$_1$	743a$_1$	736a$_1$	723$_1$
Mean	712a	721a	733a	763a	743a	
CV = 11/5%						
			Bixby			
1	593a$_1$[z]	542a$_1$	547a$_1$	472a$_1$	350b$_2$	501$_1$
2	470a$_1$	425a$_1$	467a$_1$	485a$_1$	508a$_1$	471$_1$
Mean	531a	483a	507a	479a	429a	
CV = 20.0%						

[z] Means followed by the same letter in each row or by the same number in each column are not significantly different at the 5% level.

Mean separation by LSD.

TABLE IV

THE EFFECT OF IN-ROW SPACING AND NUMBER OF PLANTS/SITE ON STEM DRY WEIGHT (G/PLOT) AT TWO LOCATIONS

No. of plants/site	In-row spacing (cm) 30	35	40	45	50	Mean
			Hinton			
1	$905a_1$[z]	$1044a_1$	$841a_1$	$839a_1$	$877a_1$	903_1
2	$855a_1$	$907a_1$	$885a_1$	$882a_1$	$959a_1$	901_1
Mean	895a	975a	863a	861a	918a	
CV = 18.5%						
			Bixby			
1	$834a_1$[z]	$817a_1$	$755b_1$	$730b_1$	$608b_2$	749_1
2	$775a_1$	$653a_1$	$742a_1$	$734a_1$	$796a_1$	740_1
Mean	804a	735a	748a	732a	702a	
CV = 17.0%						

[z] Means followed by the same letter in each row or by the same number in each column are not significantly different at the 5% level.

Mean separation by LSD.

with one plant/site produced the highest percent pods in total top dry weight (Table V) with two plants/site in-row spacings were not significant. Percent pod was significantly influenced by the main effect of plants/site at Bixby. One plant/site significantly produced greater percent pods than two plants/site. Significant interactions did not occur.

Consistent results were obtained from both locations concerning percent red, orange and green pods by weight. There were no significant main effects or interactions for percent red pods by weight (Table VI), percent orange pods by weight (Table VII) and percent green pods by weight (Table VIII) at either location.

Average pod weight was significantly influenced by the main effect of in-row spacing at Bixby (Table IX). At Bixby the 45 cm in-row spacing produced a significantly higher average pod weight than the 35 cm in-row spacing, but no significant in-row spacing effect was observed at Hinton. The main effect of plants/site did not significantly influence average pod weight at either location.

Average weight of red pods was not significantly influenced by in-row spacing or plants/site at Hinton but was significantly influenced at Bixby (Table X) where the 45 cm in-row spacing exceeded the red pod weight at the 35 cm in-row spacing. Although number of plants/site did not influence average weight of red pods at any in-row spacing, a significant interaction shows the 45 cm and 50 cm in-row spacing produced significantly higher yield with one

TABLE V

THE EFFECT OF IN-ROW SPACING AND NUMBER OF PLANTS/SITE ON PERCENT PODS IN TOP DRY WEIGHT AT TWO LOCATIONS

No. of plants/site	In-row spacings (cm) 30	35	40	45	50	Mean
			Hinton			
1	$44b_1$[z]	$45b_1$	$50a_1$	$46b_1$	$48b_1$	47_1
2	$48a_1$	$47a_1$	$45a_2$	$44a_1$	$47a_1$	46_1
Mean	46a	46a	47a	45a	47a	
CV = 6.8%						
			Bixby			
1	$54a_1$[z]	$53a_1$	$53a_1$	$57a_1$	$58a_1$	55_1
2	$50a_1$	$51a_1$	$51a_1$	$55a_1$	$53a_1$	52_2
Mean	52b	52b	52b	56a	56a	
CV = 6.9%						

[z] Means followed by the same letter in each row or by the same number in each column are not significantly different at the 5% level.

Mean separation by LSD.

TABLE VI

THE EFFECT OF IN-ROW SPACING AND NUMBER OF PLANTS/SITE ON PERCENT RED PODS BY WEIGHT AT TWO LOCATIONS

	In-row spacings (cm)					
No. of plants/site	30	35	40	45	50	Mean
			Hinton			
1	60a$_1$[z]	60a$_1$	62a$_1$	59a$_1$	66a$_1$	61$_1$
2	64a$_1$	60a$_1$	61a$_1$	57a$_1$	61a$_1$	61$_1$
Mean	62a	60a	62a	58a	63a	
CV = 10.2%						
			Bixby			
1	79a$_1$[z]	77a$_1$	80a$_1$	80a$_1$	80a$_1$	79$_1$
2	84a$_1$	80a$_1$	84a$_1$	82a$_1$	78a$_1$	82$_1$
Mean	82a	78a	82a	81a	79a	
CV = 7.4%						

[z] Means followed by the same letter in each row or by the same number in each column are not significantly different at the 5% level.

Mean separation by LSD.

TABLE VII

THE EFFECT OF IN-ROW SPACING AND NUMBER OF PLANTS/SITE ON PERCENT ORANGE PODS BY WEIGHT AT TWO LOCATIONS

	In-row spacings (cm)					
No. of plants/site	30	35	40	45	50	Mean
			Hinton			
1	$20a_1$[z]	$21a_1$	$19a_1$	$20a_1$	$20a_1$	21_1
2	$19a_1$	$21a_1$	$22a_1$	$23a_1$	$20a_1$	20_1
Mean	20a	21a	21a	22a	20a	
CV = 19.3%						
			Bixby			
1	$11a_1$[z]	$13a_1$	$9a_1$	$9a_1$	$9a_1$	10_1
2	$7a_1$	$10a_1$	$8a_1$	$8a_1$	$12a_1$	9_1
Mean	9a	11a	9a	8a	11a	
CV = 39.0%						

[z] Means followed by the same letter in each row or by the same number in each column are not significantly different at the 5% level.

Mean separation by LSD.

TABLE VIII

THE EFFECT OF IN-ROW SPACING AND NUMBER OF PLANTS/SITE ON PERCENT GREEN PODS BY WEIGHT AT TWO LOCATIONS

	In-row spacings (cm)					
No. of plants/site	30	35	40	45	50	Mean
			Hinton			
1	$20a_1$[z]	$19a_1$	$18a_1$	$21a_1$	$15a_1$	19_1
2	$16a_1$	$19a_1$	$17a_1$	$19a_1$	$18a_1$	18_1
Mean	18a	19a	18a	20a	16a	
CV = 29.8%						
			Bixby			
1	$10a_1$[z]	$10a_1$	$11a_1$	$10a_1$	$9a_1$	10_1
2	$9a_1$	$10a_1$	$8a_1$	$10a_1$	$10a_1$	9_1
Mean	10a	10a	9a	10a	9a	
CV = 34.4%						

[z] Means followed by the same letter in each row or by the same number in each column are not significantly different at the 5% level.

Mean separation by LSD.

TABLE IX

THE EFFECT OF IN-ROW SPACING AND NUMBER OF PLANTS/SITE ON AVERAGE POD WEIGHT (MG) AT TWO LOCATIONS

	In-row spacings (cm)					
No. of plants/site	30	35	40	45	50	Mean
			Hinton			
1	$165a_1$ [z]	$166a_1$	$178a_1$	$175a_1$	$185a_1$	174_1
2	$179a_1$	$168a_1$	$170a_1$	$167a_1$	$177a_1$	172_1
Mean	172a	167a	174a	171a	181a	
CV = 9.6%						
			Bixby			
1	$240a_1$ [z]	$217a_1$	$223a_1$	$254a_1$	$247a_1$	236_1
2	$252a_1$	$235a_1$	$251a_1$	$256a_1$	$223a_1$	243_1
Mean	246ab	226b	237ab	255a	235ab	
CV = 10.2%						

[z] Means followed by the same letter in each row or by the same number in each column are not significantly different at the 5% level.

Mean separation by LSD.

TABLE X

THE EFFECT OF IN-ROW SPACING AND NUMBER OF PLANTS/SITE ON AVERAGE WEIGHT OF RED PODS (MG) AT TWO LOCATIONS

No. of plants/site	In-row spacings (cm) 30	35	40	45	50	Mean
			Hinton			
1	$205a_1$[z]	$193a_1$	$208a_1$	$201a_1$	$214a_1$	204_1
2	$209a_1$	$195a_1$	$196a_1$	$194a_1$	$208a_1$	200_1
Mean	207a	194a	202a	197a	211a	
CV = 8.4%						
			Bixby			
1	$268^{a}b_1$[z]	$244b_1$	$247b_1$	$284a_1$	$276a_1$	263_1
2	$282a_1$	$260a_1$	$279a_1$	$285a_1$	$253a_1$	273_1
Mean	275ab	252b	263ab	285a	266ab	
CV = 9.4%						

[z] Means followed by the same letter in each row or by the same number in each column are not significantly different at the 5% level

Mean separation by LSD.

plants/site when compared to the 35 and 40 cm spacing. Average weight of orange pods was not significantly influenced by the main effects (Table XI) at either location. However, there was a significant interaction at Hinton. With one plant/site the 40, 45 and 50 cm in-row spacings produced significantly higher average orange pod weight than the 30 cm in-row spacing. Number of plants/site did not significantly influence average weight of orange pods at any spacing.

Average weight of green pods was not significantly influenced by main effects and no interactions occurred at either location (Table XII).

Color and Heat:

Red, orange and green pods were separated from a 300 pod sample/plot and analyzed to determine whether pod color was related to pungency. The results indicated that orange and green pods were significantly more pungent than red pods (Table XIII).

Spacing and Heat:

To determine the effect of spacing on pungency, pod samples from the experiment at Hinton were analyzed from in-row spacings of 30, 40 and 50 cm. The results indicated that there were no significant differences in pungency due to in-row spacings (Table XIV).

TABLE XI

THE EFFECT OF IN-ROW SPACING AND NUMBER OF PLANTS/SITE ON AVERAGE WEIGHT OF ORANGE PODS (MG) AT TWO LOCATIONS

No. of plants/site	In-row spacings (cm) 30	35	40	45	50	Mean
			Hinton			
1	130b$_1$[z]	138ab$_1$	151a$_1$	155a$_1$	154a$_1$	146$_1$
2	141a$_1$	152a$_1$	144a$_1$	143a$_1$	147a$_1$	145$_1$
Mean	136a	145a	147a	149a	151a	
CV = 9.6%						
			Bixby			
1	171a$_1$[z]	162a$_1$	175a$_1$	185a$_1$	197a$_1$	178$_1$
2	184a$_1$	189a$_1$	177a$_1$	189a$_1$	169a$_1$	182$_1$
Mean	177a	176a	176a	187a	183a	
CV = 13.1%						

[z] Means followed by the same letter in each row or by the same number in each column are not significantly different at the 5% level.

Mean separation by LSD.

TABLE XII

THE EFFECT OF IN-ROW SPACING AND NUMBER OF PLANTS/SITE ON AVERAGE WEIGHT OF GREEN PODS (MG) AT TWO LOCATIONS

No. of plants/site	In-row spacings (cm) 30	35	40	45	50	Mean
			Hinton			
1	123a 1 [z]	134a 1	135a 1	139a 1	139a 1	136 1
2	139a 1	129a 1	137a 1	138a 1	138a 1	134 1
Mean	131a	131a	136a	139a	139a	
CV = 16.0%						
			Bixby			
1	159a 1 [z]	147a 1	155a 1	168a 1	158a 1	158 1
2	148a 1	151a 1	153a 1	163a 1	149a 1	153a 1
Mean	154a	149a	154a	166a	154a	
CV = 13.6%						

[z] Means followed by the same letter in each row or by the same number in each column are not significantly different at the 5% level.

Mean separation by LSD.

TABLE XIII

MEAN VALUES OF PUNGENCY (SCOVILLE UNITS) IN THREE POD COLORS FROM 40 CM IN-ROW SPACINGS AND ONE PLANT/SITE AT HINTON, OKLAHOMA

Pod color	Pungency in Scoville Units
Orange	285938 A[z]
Green	273375 A
Red	212438 B

CV = 8.4%

z = mean separation by DMRT, α = 0.05

TABLE XIV

MEAN VALUES OF PUNGENCY (SCOVILLE UNITS) FROM THREE IN-ROW SPACINGS WITH ONE PLANT/SITE AT HINTON, OKLAHOMA

In-row spacing (cm)	Pungency In Scoville Unit
40	219750 A[z]
50	199500 A
30	173250 A

CV = 12.5%

z = mean separation by DMRT, α = 0.05

Greenhouse Pungency Study

Application of micronutrients significantly decreased dry weight yield of KSB 6-2 pepper pods. Pod yield reductions were greatest when combinations of micronutrients were sprayed on the plants. Pungency was significantly increased by Cu and MnCu foliar applications. The pungency was not significantly different for any of the other treatments. Scoville Unit Index (SUINDEX), a measure of SUX dry weight, was significantly lower than the control for all treatments except Cu and Mn (Table XV).

Pod mineral content was significantly influenced by micronutrient applications. Pod content of Cu was significantly increased by the application of Cu, BCu, MnCu and BMnCu.

Pod Mn content was also significantly influenced by micronutrient applications. Significant increases in the pod content of Mn were found with the application of Mn, BMn, BMnCu and MnCu. Spraying with Cu, BCu, and B did not significantly influence pod Mn content. Pod mineral content of both N and Zn were not significantly influenced by any foliar spray treatment. Results of the greenhouse pungency studies are summarized in Table XV.

Name: Agegnehu Sissay Date of Degree: December, 1984

Institution: Oklahoma State University

Location: Stillwater, Oklahoma

Title of Study: SPACING AND MICRONUTRIENT EFFECTS ON YIELD AND PUNGENCY OF CHILE PEPPERS (*CAPSICUM ANNUUM* L.)

Pages in Study: 52 Candidate for Degree of Master of Science

Major Field: Horticulture

Scope of Study: Two separate studies were conducted in the spring and fall of 1983. The first was a plant density study conducted at the Bixby Research Farm and at a grower cooperator's farm near Hinton, Oklahoma. The purpose of this study was to determine the optimum in-row spacing and number of plants/site for the KSB 6-2 Chile selection. The second study was a greenhouse pungency study conducted to determine if foliar applications of Cu, B, and Mn, alone or in combination, could increase the capsaicin content of KSB 6-2 chile selections.

Findings and Conclusions: Similar results were obtained concerning pod yield at both locations. Percent pod and average pod size was lower at Hinton than at Bixby. In-row spacing and number of plants/site did not influence pungency but pungency was related to pod color. The green and orange pods were significantly more pungent than red pods. Foliar applications of micronutrients significantly decreased dry pod yield. The application of Cu and MnCu significantly increased pungency.

ADVISER'S APPROVAL ______________________________

TABLE XV

THE EFFECT OF FOLIAR MICRONUTRIENT APPLICATION ON YIELD, PUNGENCY AND POD MINERAL CONTENT

Foliar treatments	N(%)	Zn(ppm)	Mn(ppm)	Cu(ppm)	Pod pungency in Scoville Units	Pod dry weight/plant	SU INDEX[x]
Control	2.40	14.8	35.6	3.93	159062	41.5	6.71
B	2.51NS[z]	13.5NS	35.4NS	5.82NS	181912NS	29.6***	5.32*
BCu	2.34NS	14.3NS	29.6NS	16.40***	183250NS	25.9***	4.79*
BMn	2.41NS	14.8NS	63.2***	4.68NS	171375NS	24.3***	4.11**
BMnCu	2.34NS	15.6NS	56.0***	9.90*	167000NS	17.3***	2.89***
Cu	2.42NS	14.3NS	30.4NS	26.30***	190187*	32.7**	6.19NS
Mn	2.46NS	15.1NS	105.0***	4.12NS	180625NS	34.1*	6.11NS
MnCu	2.32NS	15.2NS	53.2**	15.70***	207250*	24.9***	5.19*

[z] * = significant at 5.0%, ** = significant at 1.0%, *** = significant at 0.1%, NS = nonsignificant from no foliar applications

[x] = pod dry weight x Scoville Unit divided by 1,000,000

CHAPTER V

DISCUSSION

Plant Density Study

Pod yield was similar at Hinton and Bixby but total top dry weight was greater at Hinton than at Bixby. This is probably due to more environmental stress such as high winds and temperatures at Hinton. Under conditions of environmental stress, fewer pods set and more plant growth occurs, hence more leaf and stem weight was produced at Hinton.

Percent pods was lower at Hinton than at Bixby. This again is probably due to the same reason explained above. When leaf and stem growth is greater, there will be more light interception which could result in increased yield (24).

Although there were no significant differences on percent red pods by weight (Table VI), percent orange pods by weight (Table VII), and percent green pods by weight (Table VIII) at either location, percent red pods by weight was greater at Bixby and percent orange and green pods by weight were lower at Bixby. This is probably due to maturity differences. Although planting and harvesting were done at both locations at the same time, the crop at Bixby matured a little earlier than at Hinton possibly due to more pods setting earlier in the season at Bixby where climatic stress was lower.

Average pod weight was significantly higher at the 45 cm in-row spacing at Bixby but not at Hinton. When plants are crowded, seeds or pods tend to become smaller and lighter (10, 18). This may be the reason for the 45 cm in-row spacing to perform better than the two closer spacings at Bixby. However, if this was true, the 50 cm in-row spacing should also show the same result but it did not.

In general pods were lighter in weight at Hinton than at Bixby. Apart from environmental stress which reduces fertilization, management differences may have contributed to these results. The experiment at Bixby was conducted on a research field where there were trained technicians to manage the planting. On the other hand, the experiment at Hinton was conducted on a farmer cooperator field where there were no trained technicians and where more emphasis is given to production and not to research.

In general, in-row spacing, did not seem to significantly influence most of the variables measured at either location. Previous studies partially support this result. In-row spacing did not influence top dry weight or pod yields of other KSB chile selections (38). Plants/site significantly influenced few of the variables measured. The one plant/site gave significantly higher yields in some cases than the two plants/site or it was not significantly different than two plants/site in other cases at either location. These results suggest that KSB 6-2 selections have the ability to adjust to the in-row spacings and number of plants/site studied. There is no reason to have Speedling transplants grown as doubles from these results.

The coefficient of variation (CV) calculated for some of the variables ranges from 20% for leaf weight to 39% for percent weight of orange pods. These are not extremely large for field studies, however, larger plots or more replicates would have been desirable (37).

Yield did not decline at any in-row spacing. This suggests that both narrower and wider in-row spacings should have been included in the study to determine the maximum and minimum in-row spacing.

The relationship of color and pungency was also studied. The results indicated that orange and green pods had a significantly higher capsaicin content than the red pods (Table XIII). This shows that capsaicin increases for a certain period of time and then declines. From this study it is not possible to determine the exact time when the capsaicin content started declining. However, the result obtained in this experiment seems to be in agreement with results obtained by other researchers (36). In one experiment it was reported that capsaicin was detected 20 days after flowering and reached maximal level about 40 days after flowering and started decreasing significantly on the 50th day (16). This is probably the reason for the decrease in pungency for red pods. The analysis was done about 75 days after flowering.

Table XVI compares the increase in pod dry weight and the increase in pod capsaicin content as pod growth and maturity occurs. It appears from data in this study that capsaicin percentage reaches a peak in orange pods and although pod weight increases as pods become red there is very little increase in the capsaicin content per pod. Therefore, it appears that the decrease in Scoville value reported for mature pods is due to an increase in pod dry weight while capsaicin content changes very little. The pungent constituent is diluted out with pod maturity.

TABLE XVI

RELATIONSHIP BETWEEN POD COLOR, POD WEIGHT AND CAPSAICIN CONTENT AND THE RELATIVE CHANGE IN WEIGHT AND PUNGENCY WITH POD MATURITY

Pod color	Pod weight (mg)	Increase in pod Weight (%)	Capsaicin[y] (%)	capsaicin[x] content (Mg/pod)	increase in capsaicin content (%)
Green	135	--	1.82	2.46	--
Orange	151	11.8	1.91	2.88	17.1
Red	208	27.4	1.42	2.95	2.4

[z] Data from Tables X, XI and XII; Hinton location, one plant/site, 40cm in-row spacing.

[y] Percent capsaicin is Scoville Units divided by 15 million (32).

[x] From Table XIII. Capsaicin content calculated from percent capsaicin X pod weight.

Although not all spacings were included in the analysis, pungency analysis was done on spacings of 30, 40 and 50 cm with single plants/site at Hinton to determine whether spacing had an effect on pungency. The results indicated that there were no significant differences observed between the treatments (Table XIV). The results might have been different if the pungency analysis was done on all the treatments but this is doubtful. Previous research results suggest that pungency is not affected by spacing (32).

Greenhouse Pungency Study

Dry pod yield of KSB 6-2 selection was not significantly increased by the application of micronutrients as compared to the control. In fact, dry pod yield was decreased significantly by foliar application of each micronutrient alone or in combination with one or two other micronutrients (Table XV). This was probably due to toxicity effects (49). During the first four foliar applications, most of the plants in all the treatments except the control showed toxicity effects such as leaf burns. Symptoms of toxicity had been more severe with the application of B alone or in combination with other micronutrients. Previous studies also suggested that B application increased pungency, but also reduced pod yield under field conditions (26). Of all the treatments which affected pod yield, the most important one is the treatment

BMnCu. This treatment caused the most severe toxicity effects during the early periods of the experiment.

Capsaicin content was significantly affected by the application of Cu or MnCu. Pungency was increased significantly by foliar application of these micronutrients as compared with the control. The rest of the micronutrients did not have any effect on pungency. Similar results have been achieved previously. It was reported that the application of Cu and Mn alone or in combination with other micronutrients greatly increased capsaicin content of chiles in the greenhouse (26). However, according to Sardar (37), two foliar applications of B, Mn or Zn and N sidedress applications in a field study did not influence pungency of KSB 7 and KSB 10 pepper pods.

Scoville Unit Index (SUINDEX) was significantly decreased by foliar application of all micronutrients except Cu and Mn. The decrease in SUINDEX was attributed to toxicity effect. Due to the low pod yield, the high values of pungency or SU obtained by foliar application of CU and Mn are offset. However, having highly pungent pods with less pod yield might still be beneficial if the spice industry is ready to pay more for more pungent pods. A greater quantity of capsaicin can be extracted from a smaller quantity of pepper pods when pungency is higher (37).

Pod Cu content of the KSB 6-2 selection was significantly influenced by the application of Cu and other micronutrients applied in combination with Cu. This trend was repeated with Mn pod content. This apparently shows that these two micronutrients were taken up by the plants through their leaves. However, pod mineral content of N and Zn were not influenced by any of the treatments. On the other hand, the high pod mineral content of Cu and Mn could also be due to surface contamination.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Similar results were obtained concerning pod yield at both locations. However, percent pod and average size was lower at Hinton than at Bixby probably due to more environmental stress at Hinton. The inconsistency of the results on in-row spacings from both locations makes it more difficult to come up with a specific recommendation. However, some facts are clear. The 50cm in-row spacing and one plant/site should be used until better information is obtained. This will help to reduce disease problems and cost of planting. At the 30cm in-row spacing about 35,400 pepper seedlings are required to plant a hectare. However, at 50cm, the requirement is 38% less or 21,800 plants. Two plants/site didn't influence yield.

In this study, it was not possible to determine where yield would decline due to in-row spacing. The KSB 6-2 selection did not show these effects at the closest or widest in-row spacing used at either location. A further study using both closer and wider spacings should lead to more definite conclusions.

Although in-row spacing did not significantly influence pungency, pod color had a significant effect. This is probably due to maturity. When pods matured (turned to red), pod weight increased but pungency decreased.

Application of micronutrients significantly decreased yield when compared with the control but the foliar application of Cu and MnCu increased pungency. Pod mineral content of Mn and Cu was influenced by the application of Mn and Cu containing micronutrients but pod mineral content of N and Zn were not influenced by these micronutrients.

Future studies to increase pungency should also give emphasis on increasing pod yield. Timing, number of applications and types of micronutrients are some of the factors which need consideration (1). More emphasis should be given to Cu and Mn. Twelve applications were excessive.

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