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# MALABSORPTION OF MINERAL NUTRIENTS AND EFFECTS OF FOLIAR FERTILIZATION ON CONTINUOUSLY CROPPED *CAPSICUM ANNUUM* L. VAR. *ANNUUM*

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## Abstract

Cayenne pepper (*C. annuum* var. *annuum*) cultivar known as line No. 5 was used to establish a reference baseline for fertilization experiments under conditions of continuous cropping versus crop rotation. The effects of continuous cropping on absorption of 11 essential nutrient elements and fruit yield were studied. Concurrently, we also examined the effects of foliar application of urea + $KH_2PO_4$ and Fe + B + Zn + Mn on nutrient absorption due to continuous cropping. The results showed that, compared with peppers grown in rotation soil, continuous cropping affected the uptake of eight elements (P, K, Mg, Fe, B, Zn, Mn, Cu) and transport of these elements to the aerial parts of the plant, although the element concentrations in continuous cropping soil were not lower than those in rotation soil. Continuous cropping caused a decline in fruit yield. The impact of continuous cropping on the uptake of trace elements was greater than it was for macro elements. Foliar application of urea + $KH_2PO_4$ significantly improved the P, Mg, Fe, and Mo content of continuously-cropped pepper plants, but did not significantly improve the content of N and K, and there was an antagonistic effect on Zn uptake. Foliar application of Fe + B + Zn + Mn, significantly increased the Fe, B, Zn, Mn, and P content in the plants; Ca uptake in the leaves and fruits was promoted to a certain degree, but there was obvious antagonism toward Mo and Cu uptake in the stems, leaves and fruits. Pepper fruit yields were significantly increased by foliar application of urea + $KH_2PO_4$ or foliar application of Fe + B + Zn + Mn. However the effects of foliar application of Fe + B + Zn + Mn on increased production were significantly better than the effects of foliar application of urea + $KH_2$ $PO_4$.

**Key words:** Pepper; Continuous cropping; Foliar fertilization; Mineral element.

## Introduction

*Capsicum annuum* belongs to the family Solanaceae. Its fruit, the chili pepper, is a worldwide condiment that is found in many regional foods due to its unique spicy taste. *C. annuum* L. var. *annuum* is a cultivar of the annual *C. annuum* species (Zhuang *et al.*, 1995) that features slim fruits (length 12-20 cm and diameter 1.0-1.4 cm) with intermediate to strong pungency and intense scent; these fruits are easily air-dried and preserved to produce chili powder and sauce. As such, *C. annuum* var. *annuum* is highly valued by consumers and is grown on an annual acreage of approximately 433,000 $hm^2$ in China (Zhao, 2008). *C. annuum* var. *annuum* is cultivated on a similarly large scale in India. In the most recent decade, the main *C. annuum* var. *annuum* producing areas in China (Shaanxi, Xinjiang, Sichuan, Chongqing and Hunan provinces) have not practiced reasonable crop rotation for a variety of reasons. Consequently, repeated cultivation in the same plot for many years has become the norm for *C. annuum* var. *annuum* production. As the number of years of continuous cropping increases, the disproportionality of the mineral nutrients in the soil (Lv *et al*., 2006), soil quality indicators (total microbial biomass, basal respiration, total organic carbon, active carbon, total nitrogen, aggregate stability, and particulate organic matter, etc.) and root vitality declined, compared to soils in which the crops were rotated (Aziz, *et al*., 2011; Aparicio *et al*., 2007; Hou *et al*., 2009). These changes affect the absorption of mineral nutrients and reduce the ability of the plants to transport these nutrients to their aerial parts (Wang *et al*, 2012), thereby compromising the growth and development of the pepper plants and the fruit yield under conditions of continuous cropping.

Foliar fertilization has been widely used in crop cultivation, and has been shown to relieve the effects of salt stress (Jabeen *et al*, 2011a; Jabeen *et al*., 2011b). Foliar fertilization may also be an effective way to relieve nutritional imbalances of mineral elements caused by continuous cropping. However, few studies have focused on the effects of continuous cropping on mineral nutrient absorption in *C. annuum*, or on the application of foliar fertilization to relieve mineral nutrient malabsorption. Therefore, in 2010, we studied the effect of continuous cropping on the major agronomic traits of *C. annuum* var. *annuum* by measuring the absorption of 11 mineral elements (N, P, K, Ca, Mg, Fe, B, Zn, Mn, Cu, and Mo) at 60 and 90 d after transplantation using modified pot experiments. Our results reveal that continuous cropping inhibits the uptake of P, K, Mg, Fe, B, Zn, Mn, and Cu in *C. annuum* var. *annuum* plants and significantly reduces plant growth and fruit yield (Zhou & Zhao, 2011). Because vacant plots in which *C. annuum* can be grown are difficult to find, rotation with other crops is generally practiced to alleviate the issues that result from continuous cultivation. Therefore, in 2011, we used rotation soil as a control soil in further investigations of the effects of continuous cropping on the fruit yield of *C. annuum* var. *annuum* and the absorption of 11 mineral nutrient elements at different growth stages and by different plant parts. We also examined the outcomes of using foliar application of urea+$KH_2PO_4$ or Fe+B+Zn+Mn to alleviate nutrient malabsorption resulting from continuous cropping, thereby providing reference information regarding fertilization of continuously cropped *C. annuum* var. *annuum*.

## Materials and Methods

The experiments were performed in the Yangling Xintiandi test field of Northwest A&F University between March and September 2011. The field features a flat terrain and experiences a continental monsoon climate with an average annual temperature of 11-13°C. In the period between the planting and harvest of *C. annuum* var. *annuum*, the minimum temperature was 14°C, the maximum temperature was 37°C, and the annual precipitation was 500-700 mm.

**Test materials and soil:** The test material was a breeding line of *C. annuum* var. *annuum*, No. 5, provided by the *C. annuum* group of the College of Horticulture, Northwest A&F University. The potting soil (loam) was obtained from the Yangling Xintiandi test field. The control soil (referred to as rotation soil) was from the topsoil (0-30 cm) of a plot in which *C. annuum* had not been grown within the preceding three years; the previous crop was Chinese cabbage. The continuously cropped soil was obtained from the topsoil (0-30 cm) of a plot in which *C. annuum* var. *annuum* had been cultivated for each of the preceding four years.

**Experimental design:** The experiment began on March 6th, when a seedbed nursery was started in a solar greenhouse. Pot planting was performed on April 30th. Each pot was a round plastic barrel 26 cm high and 30 cm in diameter. The purpose of the potting experiment was to minimize the environmental differences between the treatments. To reduce temperature and humidity differences between the test soils and the field during the experiment, the pots were buried in the test field so that the top edge was level with the ground and the distance between the centers of neighboring pots was 28 cm. Before planting, the test soil sample was cleaned of gravel and other debris, air-dried and then sifted to generate fine-grained particles, before being fully mixed and used to fill pots (22 kg/pot). Double the amounts of N and P that would be used in a conventional fertilization regimen in fields (300 kg/ha N and 225 kg/ha $P_2O_5$) were used in this study; that is, 10.52 g urea (0.22 g N/kg dry soil) and 31.17 g calcium superphosphate (0.17 g $P_2O_5$/kg dry soil) were applied to each pot. The four following treatments were used for the four experimental groups: rotation cropping (RC); continuous cropping (CC); CC combined with foliar application of urea (46.70% N) and $KH_2PO_4$ (52.17% $P_2O_5$, 34.53% $K_2O$) (CCT1); and CC combined with foliar application of Fe, B, Zn and Mn (CCT2). Three plots of 15 pots each (four plants per pot) made up each treatment group. After planting, the test plants were fully watered to boost root growth, and precautions were taken to avoid soil compaction. Foliar fertilization was performed on June 1st, July 1st and August 1st, when application of one element was followed by another 24 h later. Urea was applied at a concentration of 0.3%, $KH_2PO_4$ at 0.2%, B (borax) at 0.2%, Fe (sulfate Fe) at 0.02%, Mn (sulfuric Mn) at 0.05% and Zn (sulfuric Zn) at 0.03%. All fertilizers were combined with 50 mg/L of tributyl phosphate (TBP) as a surfactant and applied until the leaves began to drip. The same volume of distilled water was applied to the plants of the other two treatment groups, thereby maintaining consistent humidity and temperature conditions among the treatments. Other conventional field management procedures were also employed.

The fertilizer concentrations described above were determined in a preliminary field experiment in which five gradient concentrations of each element were used. The solutions were applied on May 19th and May 23rd, and the outcomes were evaluated. Specifically, concentrations that led to toxicity (e.g., the curling of new leaves, darkening of leaf edges and appearance of black spots on the leaves) or were ineffective were excluded. The concentrations that did not produce toxicity and were associated with the most robust growth were used in the potting experiment.

### Determination indicators and methods

**Determination of the mineral element contents of soil and *C. annuum* var. *annuum* plants:** Before the experiment, a five-point sampling technique was utilized to collect 1 kg of topsoil samples from five of the RC and CC group sites. The topsoil samples were air-dried and sifted to enable the analysis of 11 mineral nutrients. At 60 and 90 d after planting *C. annuum* var. *annuum*, 6 plants were randomly selected from each treatment group, and the roots, stems, functional leaves and fruits were immediately harvested. These tissues were taken to the laboratory, where they were immediately washed with deionized water, heated at 105°C for 15 min in an oven, and then desiccated at 70°C until the weight remained constant. All desiccated samples were pulverized and sifted to determine the 11 mineral element contents.

The total soil N and the available soil N, as well as the total N content in various parts of the *C. annuum* var. *annuum* plants were determined using the Kjeldahl method (FOSS-2300 Azotometer, Foss A/S, and Denmark). The available soil P was determined using a method involving 0.5 $mol \cdot L^{-1}$ $NaHCO_3$ that is suitable for neutral to slightly alkaline soil. The P contents of various parts of the *C. annuum* var. *annuum* plants were determined using an ammonium molybdate colorimetric assay. Soil levels of K, Ca, Mg, Fe, Cu, Mn, and Zn and the K, Ca, Mg, and Fe contents of *C. annuum* var. *annuum* plants were determined using flame atomic absorption spectrometry (Solaar M6 atomic absorption spectrometer, Thermo Fisher Scientific, USA). The Mn, B, Zn, Mo, and Cu contents of the *C. annuum* var. *annuum* plants were determined using plasma mass spectrometry (ICP-MS, 820-MS spectrometer, Varian Medical Systems, Inc., USA). The methods employed have been described in detail in the dissertation of Zhou & Zhao (2011).

**Determination of fruit yield and its component factors:** At 120 d after planting, 15 plants from each group (five plants/plot) were randomly selected, and the number of monoclonal commodity fruits (ripe, red fruits) was counted. In addition, two ripe, red fruits were harvested from the second and third branching points of each plant, and the average weight (g), natural length and transverse diameter in the middle of each fruit were measured. From 120 d after planting, ripe fruits were harvested weekly for three weeks to determine the dynamic yield. These data were then used to summarize the yields of the individual plots making up each group.

**Data analysis:** Data processing and plotting were performed using Excel. Analysis of significant differences was conducted using the SPSS statistical software package.

## Results

**pH and mineral nutrient status of the test soils:** Before the experiment, the pH values of the soils in the CC and RC groups were 7.71 and 7.64, respectively. Both soils were slightly alkaline, and the difference between the two was insignificant. Table 1 list the mineral element contents measured in the continuously cropped and rotation soils prior to the experiments. The total N, available K and available Zn were all significantly higher in the soils of the CC group than in the soils of the RC group ($p<0.05$). The levels of the remaining elements were comparable in the two soil types. According to the nutrient grading standards used in the second soil survey of Shaanxi Province (Guo *et al*., 1992), both soils contained sufficient available levels of the macronutrients N, P, K, Ca, and Mg, and rich available levels of the micronutrients Zn and Cu. Both soils, however, were relatively lacking in the available Mn and Mo contents, deficient in the available Fe content and extremely deficient in the available B content.

**The effect of continuous cropping on macronutrient uptake by *C. annuum* var. *annuum* roots:** The macronutrient contents of the *C. annuum* var. *annuum* plants in the different treatment groups are summarized in Table 2. In comparison with the RC group, the roots of the plants in the CC group exhibited a significantly higher level of N ($p<0.05$) and significantly lower levels of P and Mg during the full-fruit stage ($p<0.05$), significantly lower K levels during both the full-bloom and mature stages ($p<0.05$) and a markedly higher content of Ca during the full-bloom stage ($p<0.05$) (Table 2). These results are not completely consistent with those obtained recently by Zhou & Zhao (2011), in which the roots of continuously cropped *C. annuum* var. *annuum* exhibited inhibited N, P, K, Ca, and Mg uptakes and pronounced suppression of P absorption compared to the results obtained here. However, in the previous study by Zhou & Zhao (2011), the levels of effective N and P in the continuously cropped soil were significantly lower than those in the normal cropping soil (previously fallow soil), which may explain the observed differences. If this explanation is correct, these findings suggest that macronutrient uptake by the roots of continuously cropped *C. annuum* var. *annuum* is associated with the effective soil N and P contents. Specifically, if adequate levels of N, P, K, Ca, and Mg exist in the soil, then continuous cropping can still suppress P, K, and Mg uptake (although the level of suppression will be reduced), but the absorption of N and Ca will be not compromised.

**Table 1. Mineral element contents measured in the continuously cropped and rotation soils prior to the experiments (mg/kg).**

| **Treatment group** | **Total N** | **Available N** | **Available P** | **Available K** | **Available Ca** | **Available Mg** |
|---|---|---|---|---|---|---|
| RC | 1,000Ab | 45.56Aa | 36.88Aa | 207.39Ab | 2,765.26Aa | 287.14Aa |
| CC | 1,100Aa | 42.85Aa | 34.46Aa | 228.05Aa | 2,825.18Aa | 283.59Aa |
| Treatment group | Available Fe | Available B | Available Zn | Available Mn | Available Cu | Available Mo |
| RC | 3.76Aa | 0.18Aa | 2.87Ab | 8.36Aa | 1.31Aa | 0.08Aa |
| CC | 3.84Aa | 0.17Aa | 3.17Aa | 8.59Aa | 1.39Aa | 0.09Aa |

RC and CC indicate the soils used for rotation cropping and continuous cropping, respectively. Values in the same column followed by different lowercase letters differ at a significance level of $p<0.05$. Values in the same column followed by different uppercase letters differ at a significance level of $p<0.01$.

**Table 2. Macronutrient contents of *C. annuum* var. *annuum* plants subjected to various treatments (g/kg).**

| **Reproductive stage** | **Treatment** | **N** | **P** | **K** | **Ca** | **Mg** |
|---|---|---|---|---|---|---|
| Full bloom | RC | 21.03Aa | 2.39Aa | 13.75Aa | 14.53Ab | 3.28Aa |
| | CC | 19.65Aab | 2.25Aab | 12.20Ab | 16.61Aa | 3.47Aa |
| Full fruit | RC | 17.35Ab | 3.03Aa | 10.63Aa | 16.99Aa | 4.29Aa |
| | CC | 19.72Aa | 2.59Ab | 10.58Aa | 15.82Aa | 3.81Ab |
| Mature | RC | 15.94Aa | 2.50Aa | 10.97Aa | 17.26Aa | 3.35Aa |
| | CC | 15.46Aa | 2.47Aa | 9.75Ab | 16.85Aa | 3.19Aa |

Full-bloom stage: 60 days after transplanting; full-fruit stage: 90 days after transplanting; mature stage: 120 days after transplanting. RC indicates rotation cropping, and CC indicates continuous cropping. Values in the same column followed by different lowercase letters differ at a significance level of $p<0.05$. Values in the same column followed by different uppercase letters differ at a significance level of $p<0.01$

**The effect of continuous cropping on micronutrient uptake by *C. annuum* var. *annuum* roots:** The roots of plants in the CC group exhibited generally lower levels of Fe, B, Zn, Mn, and Cu than those in the RC group (Table 3). Specifically, the levels of B, Zn, and Cu were significantly lower or very significantly lower during the full-bloom stage ($p<0.05$ or $p<0.01$, respectively), the levels of Fe, Zn, Mn, and Cu were significantly lower during the full-fruit stage ($p<0.05$), and the levels of B, Zn, and Cu were significantly lower ($p<0.05$) or very significantly lower ($p<0.01$) at maturity. In addition, the plants in the CC treatment group exhibited a markedly higher level of Mn during the mature stage ($p<0.05$) and much higher levels of Mo from the full-bloom to mature stages ($p<0.01$). These results indicate that continuous cropping clearly inhibits Fe, B, Zn, Mn and Cu uptake by the roots of *C. annuum* var. *annuum*. The magnitude of this inhibition, in descending order, is B>Zn>Fe>Cu>Mn. The inhibition is primarily evident during the full-fruit and full-bloom stages and is present to a lesser extent during the mature stage. In addition, continuous cropping increased Mo absorption by the roots of *C. annuum* var. *annuum*, but this enhancement gradually diminished as the plant's reproductive development progressed. These findings are generally consistent with those from a recent study by Zhou & Zhao (2011).

**The effects of foliar fertilization and continuous cropping on the macronutrient contents of leaves and fruits of *C. annuum* var. *annuum*:** As shown in Table 4, continuous cropping affected the macronutrient levels in the roots of *C. annuum* var. *annuum* plants more significantly than those in the leaves. Compared to the plants in the RC group, the leaves of plants in the CC group presented significantly reduced P and K contents ($p<0.05$) during the full-bloom stage, and the levels of Ca and Mg were very significantly reduced ($p<0.01$) during the full-fruit stage. However, the contents of all five macronutrients in the leaves of the CC group were similar to those of the RC group during the mature stage. In addition, the levels of all five macronutrients in the commodity fruits (i.e., ripe, red fruits) from the plants in the CC group were less than those in the fruits from the RC group; in particular, the K and Mg contents were strongly reduced ($p<0.05$). Overall, the influence of continuous cropping on the macronutrient contents of *C. annuum* var. *annuum* leaves varied as the plants matured (in descending order of macronutrient content: full-bloom, full fruit stage, and mature stage).

Compared to the plants in the CC group, the leaves of the plants in the CCT1 group exhibited a significantly higher P content during the full-bloom stage ($p<0.05$) and a very significantly higher P content during the mature stage ($p<0.01$). In addition, foliar application of urea and $KH_2PO_4$ (CCT1) produced a significantly higher ($p<0.01$) P content in the fruits. Foliar application of urea and $KH_2PO_4$ not only increased the P contents of leaves and fruits but also played a positive role in Mg absorption in the continuously cropped group. However, this treatment had no apparent effects on N and K absorption.

Compared to the plants in the CC group, the leaves of the plants in the CCT2 group exhibited significantly a higher P content and a significantly lower Mg content ($p<0.05$) during the full-bloom stage; during the full fruit stage, the Ca content was significantly increased ($p<0.05$), and during the mature stage, the P content was significantly increased and the K content was significantly decreased ($p<0.05$). Moreover, the P content of fruits from the CCT2 was significantly higher ($p<0.05$) than the P content in the fruits of the CC group. Foliar application of Fe+B+Zn+Mn on the continuously cropped group of *C. annuum* var. *annuum* plants increased the P content in the leaves and the fruits and the Ca content in the leaves. However, the fertilizer exhibited antagonistic effects on the K and Mg contents in the leaves, although both effects were mild.

**Table 3. Micronutrient contents in the roots of *C. annuum* var. *annuum* plants in the CC treatment and RC treatment groups (mg/kg).**

| Reproductive stage | Treatment | Fe | B | Zn | Mn | Cu | Mo |
|---|---|---|---|---|---|---|---|
| Full bloom | RC | 2308.03Aa | 14.37Aa | 13.63Aa | 65.27Aa | 11.28Aa | 0.20Bb |
| | CC | 2376.78Aa | 11.06Bb | 12.40Ab | 67.43Aa | 9.86ABb | 0.31Aa |
| Full fruit | RC | 2327.20Aa | 12.65Aa | 8.87Aa | 55.77Aa | 10.92Aa | 0.21Bb |
| | CC | 2106.41Ab | 12.53Aa | 8.03Ab | 48.35Ab | 9.93Ab | 0.25Aa |
| Mature | RC | 1821.41Aa | 7.69Aa | 4.76Aa | 37.42Ab | 6.77Aa | 0.16Ab |
| | CC | 1759.67Aa | 6.57Ab | 3.37Bb | 41.87Aa | 6.03Ab | 0.19Aa |

Full-bloom stage: 60 days after transplanting; full-fruit stage: 90 days after transplanting; mature stage: 120 days after transplanting. RC represents rotation cropping, CC represents continuous cropping, CCT1 represents continuously cropped soil with foliar application of NPK, and CCT2 represents continuously cropped soil with foliar application of micronutrients. Values in the same column followed by different lowercase letters differ at a significance level of $p<0.05$. Values in the same column followed by different uppercase letters differ at a significance level of $p<0.01$

**Table 4. Macronutrient contents (mg/g) in the leaves and fruits of *C. annuum* var. *annuum* plants under different treatment regimens**

| Organ | Reproductive stage | Treatment | N | P | K | Ca | Mg |
|---|---|---|---|---|---|---|---|
| Leaf | Full-bloom | RC | 44.30Ab | 2.59Bb | 40.46Aa | 23.11Aa | 13.89Aa |
| | | CC | 47.02Aab | 2.25Bc | 34.55Ab | 21.96Aab | 13.81Aa |
| | | CCT1 | 48.78Aa | 3.33Aa | 37.27Ab | 20.89Ab | 12.94Aab |
| | | CCT2 | 47.21Aab | 3.29Aa | 36.82Ab | 22.19Aab | 12.10Ab |
| | Full fruit | RC | 39.10Aab | 3.39Aa | 23.68Ab | 29.90Aa | 16.72Aa |
| | | CC | 38.70Aab | 3.58Aa | 25.85Aab | 25.96Bb | 13.54Bbc |
| | | CCT1 | 41.39Aa | 3.29Aa | 26.08Aa | 25.74Bb | 12.35Bc |
| | | CCT2 | 37.42Ab | 3.31Aa | 24.87Ab | 28.31ABa | 14.66ABb |
| | Mature | RC | 35.90Aa | 3.36Bc | 19.74Aa | 34.14Aa | 16.21Aa |
| | | CC | 35.28Aa | 3.57Bc | 21.06Aa | 33.22Aa | 16.43Aa |
| | | CCT1 | 34.85Aa | 4.47Aa | 19.89Aa | 33.52Aa | 16.46Aa |
| | | CCT2 | 36.10Aa | 4.06Ab | 18.95Ab | 35.83Aa | 16.00Aa |
| Fruit | Red, ripe fruits at the mature stage | RC | 23.60Aab | 3.82Bb | 22.85Aa | 1.16Aa | 2.66Aa |
| | | CC | 23.20Aab | 3.74Bb | 20.44Ab | 1.11Aa | 2.12ABb |
| | | CCT1 | 24.90Aa | 4.46Aa | 22.09Aab | 1.13Aa | 2.20ABb |
| | | CCT2 | 22.50Ab | 4.53Aa | 22.42Aab | 1.18Aa | 2.05Bb |

Full-bloom stage: 60 days after transplanting; full-fruit stage: 90 days after transplanting; mature stage: 120 days after transplanting. RC represents rotation cropping, CC represents continuous cropping, CCT1 represents continuously cropped soil with foliar application of NPK, and CCT2 represents continuously cropped soil with foliar application of micronutrients. Values in the same column followed by different lowercase letters differ at a significance level of $p<0.05$. Values in the same column followed by different uppercase letters differ at a significance level of $p<0.01$

**The effect of foliar fertilization and continuous cropping on the micronutrient contents of leaves and fruits of *C. annuum* var. *annuum*:** As shown in Table 5, the Mo content was significantly higher in the leaves of the CC group than in those of the RC group during the full-bloom and mature stages ($p<0.01$), and the Mn content was significantly higher during the mature stage ($p<0.05$). However, the contents of the remaining micronutrients were all lower in the CC group. During the full-bloom stage, the Fe, B and Mn levels were significantly reduced ($p<0.05$), and the Zn and Cu contents were even more significantly reduced ($p<0.01$); during the full-fruit stage, the B and Cu levels were significantly reduced ($p<0.05$), and the Zn and Mn contents were even more significantly reduced ($p<0.01$); and during the mature stage, the B and Zn contents were significantly reduced ($p<0.05$). The results were similar in the leaves, fruits and roots, i.e., the use of continuously cropped soil suppressed the absorption of Fe, B, Zn, Mn, and Cu in the leaves of *C. annuum* var. *annuum* plants. The degree of suppression, in descending order, was Zn>B>Fe>Mn>Cu. In addition, the overall degree of inhibition persisted during the various growth stages as well: full-bloom stage>full fruit stage>maturity stage. Furthermore, continuous cropping conditions promoted Mo absorption in the leaves. The Fe, B, Zn, and Cu contents of the red, ripe fruits of the CC group were significantly reduced ($p<0.05$) compared with the fruits of the RC group.

During the full-fruit and mature stages, the Fe content in the leaves of the CCT1 group was significantly elevated ($p<0.05$) in comparison with the leaves of the CC group. The Mo content was significantly elevated ($p<0.05$) during the full-fruit stage and very significantly elevated ($p<0.01$) in the mature stage. However, the Zn content was significantly decreased in both stages ($p<0.05$). In addition, the Zn content was very significantly reduced ($p<0.01$) in the fruits of the CCT1 group during these two growth stages. These findings indicate that the foliar application of urea+ $KH_2PO_4$ promotes the uptake of Fe and Mo in the leaves but exerts an antagonistic effect on the absorption of Zn in the leaves and fruits. Both effects grew stronger when as the number of foliar applications increased, but no effects on the absorption of Mn or Cu were observed.

Comparing the leaves of plants in the CCT2 group to those in the CC group, the B and Mn contents were significantly increased during the full-bloom stage, and the Mo content was significantly decreased. During the full-fruit stage, the Fe content was significantly increased ($p<0.05$) and the B, Zn, and Mn contents were very significantly increased ($p<0.01$), but the Mo content was significantly decreased. Finally, during the mature stage, the Fe, B, Zn, and Mn contents were all significantly increased, and the Cu and Mo contents were significantly decreased, with the latter being below the molybdenum deficiency threshold (0.10 mg/kg dry weight, Liu, 2002a). In addition, the Fe, B, and Mn contents were significantly higher in the fruits of the CCT2 group compared to the fruits of the CC group, and the Mo content was significantly lower. Overall, these results illustrate that the foliar application of Fe+B+Zn+Mn on *C. annuum* var. *annuum* plants can significantly increase the contents of Fe, B, Zn, and Mn and promote the transfer of Fe, B, and Mn into the fruits. However, this approach also exerts antagonistic effects on the absorption of Cu and Mo. As the number of applications of Fe+B+Zn+Mn increased, the magnitudes of the increases and antagonistic effects were enhanced.

**Table 5. Micronutrient contents (mg/g) in the leaves and fruits of *C. annuum* var. *annuum* plants under the studied treatment conditions.**

| Organ | Reproductive stage | Treatment group | Fe | B | Zn | Mn | Cu | Mo |
|---|---|---|---|---|---|---|---|---|
| Leaf | Full-bloom | RC | 278.63Aa | 55.82Bb | 40.58Aa | 57.20ABb | 6.02Aa | 0.20Bc |
| | | CC | 247.33Ab | 50.78Bc | 29.47Bb | 51.76Bc | 5.82Bb | 0.31Aa |
| | | CCT1 | 256.47Ab | 48.72Bc | 27.44Bb | 48.74Bc | 5.42Bb | 0.32Aa |
| | | CCT2 | 239.05Ab | 67.96Aa | 28.45Bb | 62.32Aa | 5.53Bb | 0.22Bb |
| | Full fruit | RC | 385.31Bb | 46.52Bb | 23.63Bb | 49.52Bb | 5.74Aa | 0.14Bc |
| | | CC | 365.13Bb | 40.44BCc | 18.50Cc | 40.39Cc | 5.45Ab | 0.17Ab |
| | | CCT1 | 421.10Aa | 40.15Cc | 14.24Dd | 42.56 Cc | 5.23Ab | 0.20Aa |
| | | CCT2 | 424.52Aa | 87.02Aa | 28.61Aa | 63.92Aa | 5.17Ab | 0.12Bc |
| | Mature | RC | 363.86Bb | 38.62Bb | 16.45Bb | 28.97Bc | 5.25Aa | 0.09BCc |
| | | CC | 374.54Bb | 34.89Bc | 14.44Bc | 31.05Bb | 5.15Aa | 0.11Bb |
| | | CCT1 | 412.79ABa | 36.18Bbc | 11.72Cd | 29.88Bb | 5.20Aa | 0.17Aa |
| | | CCT2 | 438.02Aa | 60.87Aa | 20.73Aa | 47.80Aa | 3.97Bb | 0.07Cc |
| Fruit | Red, ripe fruits at the mature stage | RC | 78.33Aa | 10.33Aa | 6.53Aa | 8.72Ab | 4.18Aa | 0.07Aa |
| | | CC | 70.99Ab | 8.93Ab | 5.91Ab | 8.77Ab | 3.98Ab | 0.08Aa |
| | | CCT1 | 72.68Ab | 8.53Ab | 4.15Bc | 8.84Ab | 3.85Ab | 0.07Aa |
| | | CCT2 | 78.21Aa | 10.03Aa | 5.90Ab | 9.83Aa | 3.90Ab | 0.05Ab |

Full-bloom stage: 60 days after transplanting; full-fruit stage: 90 days after transplanting; mature stage: 120 days after transplanting. RC represents rotation cropping, CC represents continuous cropping, CCT1 represents continuously cropped soil with foliar application of NPK, and CCT2 represents continuously cropped soil with foliar application of micronutrients. Values in the same column followed by different lowercase letters differ at a significance level of $p<0.05$. Values in the same column followed by different uppercase letters differ at a significance level of $p<0.01$

**Table 6. Fruit yield and its component factors in *C. annuum* var. *annuum* plants under different treatment regimens.**

| Treatment group | Fruit length (cm) | Fruit diameter (cm) | Fruit weight (g) | Fruit number per plant | Number of commodity fruits per plant | Yield of fresh fruits per group (kg /180 plant) |
|---|---|---|---|---|---|---|
| RC | 17.46Aa | 1.34Aa | 7.29Aa | 44.76Aab | 35.81ABa | 44.47Aa |
| CC | 15.60Ab | 1.29Aa | 6.38Ab | 41.41Ac | 32.30Bb | 34.85Bc |
| CCT1 | 16.88Aa | 1.33Aa | 7.06Aa | 43.15Abc | 34.32ABb | 39.78ABb |
| CCT2 | 16.34Aab | 1.30Aa | 6.64Ab | 46.73Aa | 37.85Aa | 42.25Aa |

RC represents rotation cropping, CC represents continuous cropping, CCT1 represents continuously cropped soil with foliar application of NPK, and CCT2 represents continuously cropped soil with foliar application of micronutrients. Values in the same column followed by different lowercase letters differ at a significance level of $p<0.05$. Values in the same column followed by different uppercase letters differ at a significance level of $p<0.01$

**The effect of foliar fertilization and continuous cropping on the yield and constituent factors of *C. annuum* var. *annuum*:** As shown in Table 6, the fruit length, unit weight, number of fruits per plant and number of commodity fruits per plant of the CC group were reduced by 10.56%, 12.41%, 7.71% and 9.81%, respectively, compared to the RC group. These differences were significant ($p<0.05$). Moreover, the plot yield of the CC group was 21.63% lower than that of the RC group, which is highly significant ($p<0.01$). However, the diameter of fruits from the CC group was only 3.73% smaller than that of fruits of the RC group, and this difference was insignificant. These results demonstrate that CC conditions decreased the quality of the single fruits, primarily by reducing the fruit length, and affected the total yield by reducing both the mass of single fruits and the number of commodity fruits per plant.

The fruit diameter and the numbers of fruits and commodity fruits per plant were not significantly different in the CC and CCT1 groups. However, the fruit length, fruit weight and yield of fresh fruits in the CCT1 group significantly increased by 8.21%, 10.66%, and 14.51%, respectively ($p<0.05$) (see Table 6). Therefore, foliar application of urea and $KH_2PO_4$ can increase the fruit yield, primarily by increasing the length and weight of single fruits. However, the improvement was limited, and the overall yield was still lower than that of the RC group.

Likewise, the fruit length, fruit diameter, and unit fruit weight per plants were not significantly different in the CCT2 group and the CC group. However, the numbers of fruits and commodity fruits per plant and the yield of the CCT2 group were significantly or very significantly increased by 12.85%, 17.18% and 21.23%, respectively (Table 6). Hence, the foliar application of micronutrients primarily increases the yield by raising the number of commodity fruits per plant, and this yield improvement was much larger than that produced by foliar application of urea+$KH_2PO_4$. The yield of the CCT2 group was near that of the RC group.

## Discussion

**The effects of the continuous cropping treatment on the nutrient absorption and yield of *C. annuum* var. *annuum*:** This study shows that in the presence of sufficient N, P, K, Ca, and Mg in the soil, the continuous cropping treatment affected the absorption of P, K, and Mg in the roots and the subsequent transport of these nutrients to aerial plant parts during some of the growth and development stages. The results are in agreement with those obtained in studies of soybeans by Ruan *et al*., (2003) and Du *et al*., (2003). Overall, the continuous cropping treatment exhibited more pronounced effects on micronutrient uptake than on macronutrient uptake. Specifically, the continuous cropping treatment significantly inhibited the absorption of Fe, B, Zn, Mn, and Cu in the roots and markedly decreased the contents of these micronutrients in the leaves and fruits. Conversely, the continuous cropping treatment increased the absorption of Mo by the roots and significantly elevated its level in the leaves. Remarkably, the trends for all micronutrients examined in this study match well with the results of Zhou and Zhao (2011). Hence, these data indicate that the continuous cropping treatment apparently inhibits the absorption of Fe, B, Zn, Mn and Cu by the roots of *C. annuum* var. *annuum* when compared to either normal cropping with previously fallow soil or with rotation soil previously used to grow Chinese cabbage.

The decreased nutrient uptake of *C. annuum* var. *annuum* plants caused by planting in continuously cropped soil drastically reduced the fruit length, the unit fruit weight, the number of commodity fruits per plant and the fresh fruit yield. This result is consistent with results obtained in a corresponding study by Zhou & Zhao (2011), in which the authors examined the same cultivar of *C. annuum* var. *annuum* and used fallow soil as the control soil. In addition, our results also generally matched those obtained in a study by Hou *et al*., (2009), who investigated *C. annuum* var. *conoides* and used rotation cropping soil as the control soil.

**The effects of foliar application of NPK on mineral nutrient absorption and yield in *C. annuum* var. *annuum*:** Foliar application of urea+$KH_2PO_4$ (the CCT1 group) significantly increased the P content in the leaves and fruits of the CC plants but produced no apparent increase in the N and K levels. Hence, our results indicate the following possibilities. First, although the continuous cropping soil was rich in N, P and K and sufficient N and P fertilizers had been applied before the experiment, continuous cropping inhibited the absorption of P by the roots, leading to a P nutritional deficiency in the plants. Second, N uptake by the roots was not affected by CC, and K uptake was only mildly affected, and the *C. annuum* var. *annuum* plants thus did not lack N or K during their growth. The foliar application of urea and $KH_2PO_4$ significantly increased the P contents of the leaves and fruits but did not appreciably increase the levels of N and K. A previous study has shown that the foliar application of P fertilizer to P-deficient crops results in the leaves providing double the P absorption of the roots under conditions of sufficient P supply (Noack *et al*., 2011).

In the CCT1 group, the Mg, Fe and Mo uptakes were enhanced, but the Zn absorption decreased, echoing the results of previous studies. It has been reported that P exhibits synergistic interactions with Mg, Fe and Mo, i.e., appropriate P application can significantly promote the uptake of Mg, Fe and Mo by plants (Fan & Wang, 2012; Liu, 2002a). Conversely, P and Zn interact antagonistically (Gianquinto *et al*., 2000; Zhu *et al*., 2001). In this study, the fresh fruit yield was significantly higher in the CCT1 group than in the CC group (by 14.15%), indicating that the positive effects of higher P, Fe and Mo contents in the leaves and fruits outweighed the negative effects of reduced Zn levels. Nevertheless, the fruit yield of the CCT1 group remained lower than those of the RC (by 10.55%) and CCT2 (by 5.85%) groups, demonstrating that the positive effect was limited.

**The effects of foliar application of micronutrients on mineral nutrient absorption and yield of *C. annuum* var. *annuum*:** Foliar application of Fe+B+Zn+Mn to *C. annuum* var. *annuum* plants in the CCT2 group not only significantly enhanced the contents of Fe, B, Zn and Mn in the leaves and those of Fe, B and Mn in the fruits but also markedly promoted P absorption by the leaves and fruits. Moreover, this treatment produced a considerable improvement in Ca uptake by the leaves, though it also resulted in obvious antagonistic effects on Mo and Cu absorption. Many studies have revealed synergistic interactions between B and P, B and K, B and Mo, Mo and K, and Mo and Mg in plants (Sahin, 2012; Liu & Yang, 2000 Liu, 2002a; Liu, 2002b), and more or less antagonistic interactions between Fe and Mo, Zn and Mo, Mn and Mo, B and Cu, Zn and Cu, and B and Mg (Liu, 2002a; Liu, 2002b; Lopez-Lefebre *et al*., 2002). A synergistic interaction has also been reported between Zn and Cu (Aref, 2011). In this study, Fe, B, Zn, and Mn were successively applied. The element(s) that generates antagonism towards the uptake of Mo and Cu remains to be investigated.

Foliar application of Fe+B+Zn+Mn to *C. annuum* var. *annuum* plants in the CCT2 group significantly increased the fruit yield compared to the CC group (by 21.23%). These findings suggested that the positive consequences of the increased Fe, B, Zn, Mn and P contents greatly outweigh the negative effects of the decreased Mo and Cu contents. Importantly, the yield enhancement of the CCT2 group was 7.08% higher than that of the CCT1 group, indicating that under our experimental conditions, micronutrient deficiency is the primary factor limiting the yield of *C. annuum* var. *annuum*. In the CCT1 group, the increased yield was attributable to increases in the fruit length and unit fruit weight, but these increases were not large, and the increase in fruit yield was limited. In comparison, in the CCT2 group, the increased yield was attributable primarily to an increase in the number of commodity fruits per plant, which was even higher than the corresponding number in the RC group. The B content in the leaves and fruits was significantly increased in the CCT2 group, which may explain these results as B plays a prominent role in the differentiation of flower buds, pollen germination and pollen tube elongation. It has been shown that application of B increases fruit set and yield in several fruit and nut crops, including almond (*Prunus dulcis*), Persian walnut (*Juglans regia*) and olive (*Olea europaea* L.) (Kizildemir, 2013; Nezami, 2012; Keshavarz, 2011; Perica *et al*., 2001). Interestingly, in many of these experiments, foliar B applications were effective on trees that presented no obvious vegetative symptoms of B deficiency. Although the *Capsicum* plants in the RC and CC groups exhibited no apparent symptoms of B deficiency in the present study, foliar application of B produced an impressive yield enhancement. This enhancement may have been due to the low levels of available B in the soils (0.20 mg/kg in the continuous cropping soil and 0.19 mg/kg in the rotation cropping soil), which were lower than a previously proposed threshold value of available B in the soil (0.25 mg/kg, Guo *et al*., 1992). The inhibition of B absorption by continuous cropping may have also contributed to the enhancement. Nevertheless, whether the application of B can increase the fruiting rate of *C. annuum* has not been reported. Therefore, further studies are needed to elucidate whether the increased fruit number in *C. annuum* var. *annuum* plants in the CCT2 group is directly related to the increased B content in the plants.

## Conclusions

The results of this experiment showed that pepper plants grown in soil that had been continuously cropped exhibited reduced root uptake of eight elements (P, K, Mg, Fe, B, Zn, Mn, and Cu), and also reduced transport of these elements to aerial parts in comparison with control plants. Foliar application of urea and $KH_2PO_4$ significantly boosted the P, Mg, Fe, and Mo contents in pepper plants. Foliar application of Fe+B+Zn+Mn significantly enhanced the Fe, B, Zn, Mn, and P contents of pepper plants. The continuously-cropped plants consequently exhibited a much lower yield than the control plants. Both foliar application regimens significantly increased fruit yield in *C. annuum* var. *annuum*.

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