Effect of intercropping peonies under walnut trees for growth and development of roots in two kinds of soil

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Abstract. To increase the economic benefit and to reduce the cost at early stages of walnut (Juglans regia L.) cultivation, the interaction of roots between walnut cultivar Zhongsong No. 1 and peony (Paeonia ostii T. Hong & J. X. Zhang) was studied. The growth rhythm per year and the vertical and horizontal distribution of roots were measured. Statistically evaluated data for five consecutive years of study using a random sampling method at yellow-brown and red soil types were presented. The results showed that walnut had three root growth peaks per year, whereas peony had two peaks. At vertical distribution of roots, walnut was concentrated under 40 cm and peony under 20 cm of soil. At vertical distribution of roots, walnut was concentrated 40-120 cm far from the tree, but peony was 20-60 cm. We are confident that the root growth and development when intercropping of peony was carried out under walnut trees was alternating and the negative influence was low. So, the cultivation model of “intercropping peony under walnut trees” was suitable at the Luoyang area, Henan province, but the cultivation distance ought to be at 120-180 cm.

1. Introduction
Walnut (Juglans regia L., Fam.: Juglandaceae) is one of the world’s four famous tree nuts (hazelnut, walnut, almond, cashew). It is classified as deciduous tree. In cultivation, large space between rows and low-density were required to meet the needs of plant growth and development. In the People’s Republic of China, the common planting distance of walnut cultivation is 4 - 6 m and the vacant land under walnut tree can be used to improve the land utilization efficiency by intercropping. This will increase the multiple cropping indexes and will also add farmers’ income. According to a statistical source, in 2016 the cultivation area of walnut was more than 80 million 667 m² in China [1]. The huge vacant land resulted under the cultivation field of walnut tree could not be used at all for effective development and utilization.

In intercropping, the selection of crops under forests is important. Inappropriate plant species may cause the opposite effect due to allelopathy and competitive growth [2,3]. However, using suitable crops can promote tree growth [4] and bring economic benefit through this type of cultivation processes. It was found that intercropping of different crops beside walnuts had different effects [5]. Walnuts
intercropped with peonies, woads and *Semen cassiae* were found beneficial for growth, development and yield for both the plants [6-10]. Otherwise, intercropping radish, cabbage and mung bean under walnut tree significantly hindered the growth of the latter [11-13]. Cropping maize and wheat together produced allelopathic stress which significantly affected the accumulation of walnut biomass [14,15]. Normally, intercropping affects overground biomass accumulation. But in case of walnuts even cropping with suitable kind of crops, the vertical and horizontal distribution of roots in soil are the main limiting factors for the growth and development for both the intercropped plants. Walnut is a deep-rooted plant, but the distribution of walnut roots is greatly affected by soil conditions and management procedures. Generally, fertile, loose and deep soils result in walnut roots developing much better. In walnut tree, the main root is vertically and deeply distributed while the lateral roots are distributed widely and horizontally. Under the condition of viscous and barren soil, the vertical distribution of walnut root is restricted by the bad soil characteristics. Whereby, the principal root grows shallower, but the lateral roots get distributed widely along horizontal direction. When irrigation is applied, the roots of walnut trees grew relatively shallow. On the other hand, under drought stress, the roots were deep in the vertical direction in order to ensure better soil water utilization [14,16,17]. Root growth of walnut is also species specific of the intercrops. For example, walnut trees intercropped with wheat, roots were seen to extend below the wheat roots in order to avoid competition with wheat for water and fertilizer. At this time, the roots of walnut were distributed in a deep longitudinal direction, but the density of roots in each layer of soil profile was lower than that of the purely cultivated walnut trees. The effect was positively correlated with the distance of intercropped wheat [18]. Therefore, it is necessary to select crops suitable for intercropping inside walnut forests.

The peony plant (*Paeonia ostii* and *P. suffruticosa*) could be used as ornamental and for pharmaceutical purposes, and as a source of oil. In the recent years, characteristic economic forests of peony were developed with high economic value [19]. Cultivation of this plant has a long history particularly in Yonglin county, Anhui province; Heze city, Shandong province; Luoyang city of Henan province and other places of China. In some previous studies it has been found that intercropping peony under walnut forest make reasonable use of the suitable shading and reduces the light inhibition of peonies, thereby promoting their growth and development [6]. In the meantime, intercropping peony and walnut significantly improved the quality of nuts and times of farming operation. This had ensured the peony and walnuts enough nutrient supply and shortened the growing period of the walnuts from juvenile to adult stage. At the same time, intercropping interactions stimulated the vegetative growth of underground parts of peony and the accumulation of biomass which increased yield [7]. The harvested peony seeds showed high quality with increased oil yield [8] and thus had brought remarkable economic benefits in cultivation processes. So, peony has been considered an ideal intercropping plant with walnut. However, the growth and underground distribution of roots under the intercropping system of both walnut and medicinal peony are not clear. Knowledge gap remains still regarding the selection of best models for such intercropping system. Information on suitable intercropping distance, time, density, etc. is not yet available. So, there is an urgent need of carrying out research to boost production of both walnut and peony. The objectives of the present research were to study the growth and distribution of roots in interplanting systems with peonies under walnut trees. So that a suitable cultivation mode of intercropping is developed and this will provide scientific guidance for obtaining higher economic yield in the walnut and peony cultivation processes.

2. Materials and methods

2.1. Growth condition of samples

According to the growth conditions of principal production areas of peony in Henan province, two different soil types were selected. Those were situated in Mengjin county and Luoyang city (table 1). The test site is located in the mountainous area but beset with shallow hills in the west of Yu River, near the Yellow River. Warm and continental monsoon (semi-humid) affects obviously the monsoon circulation. Four distinct seasons do prevail in the area. The spring is dry and windy, the summer enjoys
heat abundant rainfall, autumn is cool and rainy and winter is also cold but with less snow. The annual average temperature is 13.7°C, and the highest mean occurs in July (26.2°C). The average minimum temperature, 0.5°C, is experienced in January. The annual average frost-free period is counted as 235 d. Among other climatic factors, sunshine hours are 2270.1 h/year. Annual average rainfall is 650.2 mm, with precipitations unevenly distributed: 50% are concentrated in the flood season, e.g., in July, August and September. The highest rainfall occurs in July (an average of 164.1 mm). In January, the average rainfall is 6.9 mm. The average annual evaporation capacity for a number of years approached 1599 mm which is generally strong from April to June mostly around 200 mm with a minimum of less than 100 mm. This condition is typical and representative of the shallow-hilly area between Yellow River and Huai River.

Table 1. Growth condition of samples in this study.

| Number | Acreage (hm²) | Agrotype           | pH          | Organic matter (g·kg⁻¹) | Content of rapidly available nutrients (mg·kg⁻¹) |
|--------|--------------|-------------------|-------------|-------------------------|-----------------------------------------------|
|        |              |                   |             |                         | nitrogen | phosphorus | potassium |
| Block1 | 7.5          | Red-clay          | 7.3±0.2     | 9.8±0.5                 | 61.5±3.2 | 12.9±0.8   | 149.5±11.5 |
| Block2 | 10.0         | Yellow-brown      | 7.5±0.1     | 11.7±0.4                | 59.1±1.9 | 13.2±0.6   | 151.3±9.8  |

2.2. Experimental design
In the middle of March 2011, walnut (cultivar: Zhongsong No. 1) seedlings were planted, with 'Xiangling' as the pollination variety following a density of 3×6m. In the middle of October, 2011, 2 year-old family seedlings of medicinal phoenix peony (Fengdan white, *Paeonia ostii* T. Hong & J. X. Zhang, Fam.: Paeoniaceae) hereinafter called peony, were planted with a planting distance of 2 m away from the walnut tree and a planting density of 0.4×0.5 m following the conventional field management technique. Each treatment area was 200 m² and prepared in 3 replicates. Two control treatments, one in each of walnut and peony planting gardens were used. The growth dynamics and distribution of walnut and peony roots in the experimental fields and the controls were analyzed by random sampling method for 5 consecutive years.

2.3. Sampling method
On the vertical section, the roots of walnuts were dug with a spade at the following depths: 0-20 cm, 21-40 cm, 41-60 cm, 61-80 cm and 81-100 cm. On the horizontal section, the root system of walnut was excavated at different distances ranging from 0-40 cm, 41-80 cm, 81-120 cm, 121-160 cm and 161-200 cm away from the tree. The distribution of peony roots and its intersection with walnut roots were investigated by the whole plant random sampling method.

Five walnut trees and peonies were randomly selected for root biomass measurement. The sampling time started from the falling leaves of walnut and the root distribution and growth were measured once a year. During the rooting season of walnuts and peonies, roots were dug up every 10 days for the observation of new roots. The changes of root weight ratio, the time of new rooting and quantity of dry roots in different soil layers were calculated.

2.4. Data analysis
The new roots were counted first in the field and then taken back to the laboratory for further processing. In the laboratory, the sample of roots were rinsed off with clear water and then dried in Kraft paper bags at 105°C to constant weight. Office Excel 2010 was used for data entry, preliminary processing and statistical analysis. DPS v6.50 was used for the measured sets of data to carry out significant variance analysis and Duncan multiple comparisons. Differences were considered statistically significant at p≤0.05.

3. Results
3.1. Annual trend of new root growth between walnut and peony intercropping

Walnuts exhibited evident growth peaks three times a year which, however, decreased in intensity with the growth stage (figure 1). Both intercropped and cultivated walnuts showed three significant growth peaks in a year, with the first and second growth peaks occurring in succession within a short period of time. New-root growth peaks appeared for the first time in the middle of May (May 12), then the new-root ratio gradually reduced in late June (June 22) (figure 1) to achieve the second growth peak; both growth peaks were nearly 40 days apart. This may be due to the heavy precipitation in the Yellow-Huai River basin in winter, which was conducive to the growth of the root system after the recovery of the ground temperature in spring. Later on, with the continuous rise of soil temperature, the amount of evaporation increases and the soil moisture content drops significantly, causing difficulties for growing the new root of walnut. By the end of June, the rainy season was advancing and precipitations increased, which was conducive to the growth of new walnut roots. Then, with the yearly peak in temperature, the occurrence of new roots was inhibited, resulting in a rapid decrease in the proportion of new roots. The third growth peak occurred in early September (about September 2), and by late October new roots gradually ceased to form. From the results, we are confident that the trend of growth of walnut new root is closely related to soil temperature and moisture. Therefore, water storage and conservation of walnut tree in hilly arid land is very important.

![Figure 1. Ratio of new roots in walnut trees in two soil types.](image)

In the walnut intercropping, different soil types had different effects on the generation of new roots (figure 1). High soil permeability was good for the development of walnut new roots. The proportion of new roots in yellow-brown soil was significantly higher than that in red soil (figure 1). Intercropping walnut significantly (p≤0.05) influenced the generation of new root. This trend in yellow-brown soil was significant (p≤0.05); the ratio of new roots in red soil was significantly lower than that in the yellow-brown soil in most cases.

In terms of biomass accumulation of walnut roots in different years, it has been seen that in the first year of seedlings, the roots of walnut were damaged in different degrees during transplanting. This had caused a later development of new root growth than usual in a typical year (data not shown). This was mainly related to the formation of callus tissue after root injury which caused new root development in
the following period. With the increase in the age of walnut trees, the root system reached the growth peak in the third year, which is also the rapid expansion period of the crown of walnut trees [16]. After the fourth year, the tree crown began to reduce and root growth gradually weakened.

The annual growth peak of new roots of peony had two significant peaks, and they occur alternately with the growth peak of walnut roots (figure 2). The first growth peak of peony appeared in early April (about April 3rd), and gradually stopped in early May (about May 3rd), that is 40 days later than the growth peaks of walnuts. The second growth peak started in late September, until mid-November peak and this is about 20 days later than that of walnut trees. The growth continued until the next year early January (about January 3rd), then entered physiological dormancy. Thus, it can be seen that there was no overlap between the annual growth peak of new roots in the intercropping of walnut and peony in Yellow-Huai River basin.

![Figure 2. Ratio of new roots of medicinal peony in two soil types.](image)

In the intercropping with walnuts, the generation of new roots of peony varied according to soil type (figure 2), whereas the generation of new roots in walnut was little affected. The yellow-brown soil with good soil permeability promoted the occurrence of new roots of peony. However, intercropped walnut significantly affects the occurrence of new roots of peony; this phenomenon was more significant in yellow-brown than in red soil \((p \leq 0.05)\) (figure 2).

3.2. Vertical distribution of new roots between walnut and peony during intercropping

In the vertical distribution of root system, the roots of walnut showed the trend of "low - high - low". The ratio of new roots peaked at 40 cm depth from the ground and the difference between treatments was significant \((p \leq 0.05)\) (figure 3). Different soil types and intercropped peony had significant effects on the vertical distribution of new roots in walnut \((p \leq 0.05)\) (figure 3). The new roots in red soil were significantly lower \((p \leq 0.05)\) than those in yellow-brown soil (figure 3), which may also be related to the clay of the red soil and the resistance of the new root growth. Intercropping walnut in red and yellow-brown soil showed that the ratio of new roots was less \((p \leq 0.05)\) than not-intercropped walnuts (figure 3). This proves that the vertical distribution the of root system of walnut suffers certain degree of inhibition when it is intercropped with peony.
Figure 3. Vertical distribution of roots of walnut in two soil types.

Figure 4. Vertical distribution of peony roots in two soil types.

Vertical distribution of peony roots showed a continuous declining trend. The proportion of new roots in the soil layer reached the peak at 20 cm depth which decreases further with an increasing depth of soil layer. The difference between treatments reached a significant level ($p \leq 0.05$) (figure 4). Vertical distribution of new roots of peony when intercropped showed significant effects with different soil types. The new roots in red soil were significantly lower than those in yellow-brown soil ($p \leq 0.05$) (figure 4). This may still be related to the clay of red soil. In the intercropping model, the new roots of peony were less than that of not-intercropped which indicated that intercropping can inhibit the peony new root generation through vertical soil profile (figure 4).
3.3. Horizontal distribution of new root between walnut and peony during intercropping

The roots of walnut showed a pattern of increasing abundance up to 40 cm from the trunk, and then a decreasing trend. The generation of new roots was still abundant at 80 cm distance from the main stem. Different soil types and intercropped peony had significant effects on the horizontal distribution of new walnut roots ($p \leq 0.05$) (figure 5). The number of new roots in the range of 0-80 cm in red soil was lower than that in yellow-brown soil ($p \leq 0.05$). In intercropping, walnut roots within 0 -120 cm in yellow brown soil were significantly ($p \leq 0.05$) more developed than those in red soil. However, in the 160 -200 cm range, an opposite trend was found.

![Figure 5. Horizontal distribution of walnut roots in two soil types.](image1)

![Figure 6. Horizontal distribution of peony roots in two soil types.](image2)
Regarding the horizontal distribution of peony roots, a trend similar to that found for walnut was found (figures 5 and 6). The distribution of new roots peaked at 40 cm from the main stem (figure 6). Different soil types and intercropping had significant effects on the horizontal distribution of new roots of peony. The number of new roots in yellow-brown soil in the range of 0-40 cm was significantly higher than that in red soil. However, it showed an opposite trend, i.e. the proportion of new roots in red soil was higher ($p \leq 0.05$) than that in yellow-brown soil. In the intercropping model, the roots of walnut within 0-40 cm deep in red soil and yellow-brown soil were significantly less than in CK ($p \leq 0.05$), but the difference was not obvious at 60-80 cm soil depth (figure 6).

4. Discussion
The trend of annual growth pattern of walnut roots in the Yellow-Huaihe River basin can be divided into three phases. In the first phase, the growth peak occurred between the middle of April and end of May. The second one took place from the middle of June to the beginning of July while the third growth peak occurred between the beginning of September and mid-July. All the three growth peaks followed a decline over time. However, growth peak in peony occurred twice in a year. The first peak occurred between late March and mid-April. The second and late growth peak started in late September and continued till December. This result was consistent with Liu et al [20]. Considering the time period, it could be said that the first growth peak of peony occurred 20 days or so earlier than that of walnut trees. The second peak of peony occurred at 40 days later than walnuts’ third growth peak. But in the last year of the experiment the growth peak of peony was significantly longer compared the growth peaks of walnut. This indicated that in the intercropping of walnut and peony the new roots growth peak of both occurred alternately, so the mutual influence was small. Unlike walnut, peony had two significant growth peaks, first growth peak occurred 40 days later than walnut, the second growth peak occurred 20 days later than walnut’s third growth peak. So, basically peonies and walnut trees in intercropping systems did not overlap in their periods of new-root growth. From the perspective of soil type, both horizontal and vertical directions, the red soil contains high $K^+$ content which is conducive to the growth and development of nucleoli [16]. However, compared with the yellow-brown soil with good permeability, it is still not conducive to the development of new roots of walnuts and peonies. In terms of horizontal distribution of roots, new roots of walnut were mainly distributed at 40-120 cm far from the main trunk. But in peony, root distribution in the horizontal direction occurs at 20-60 cm distance. This indicates that peony actually takes a longer time for optimum root growth. This view is also supported by medicinal peony, in which 4 years from planting to harvesting are usually necessary to reach the medical specifications [19]. The intercropping distance between walnut and peony should be kept at 120-180 cm. Too close a distance will result in competitive stress growth and may have adverse effects on the occurrence of new roots of both the species. In summary, we are confident that the intercropping of walnuts and peony in the Yellow-Huaihe River basin was one of the effective modes to improve the multiple crop indexes. Last but not least; the planting distance between the two crops should be kept above 120-180 cm, so as to reduce the horizontal competition of water and nutrients. This will facilitate both the species to obtain a large growth space. In turn it will also be convenient for the effective implementation of field management measures to ensure good production. Planting peony among peach trees has been an important measure to compensate for the economic deficit in the early stage, as intercropping not only reduced the occurrence of photoinhibition [6], but also made full use of land resources. Unified field management measures reduced the input of repeated labour incentives in the walnut garden. Moreover, the results of peony planting in the third year have been getting high economic value [21]. Therefore, the intercropping pattern of “walnut + medicinal peony” may be regarded as an appropriate intercropping measure in the Yellow-Huaihe River basin. The present research provides some recommendations that could be popularized for rising production. In combination, it is necessary to study the mechanism of mutual growth and development to explore the optimal intercropping management method.

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