

Effects of seed wasps and seedless fruits on fruit and oil yields of *Pistacia chinensis* as a biofuel tree

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Abstract: *Pistacia chinensis* Bunge (Anacardiaceae) produces high-oil fruits and is being evaluated as an important biofuel source through establishing large plantations in China. However, harvest of fruit and oil crops from this tree may be limited by the damage from the seed wasp (*Eurytoma plotnikovi* Nikolskaya as the specialist seed predator) and seedless fruits (*i. e.*, those parthenocarpic and aborted). In order to assess the impact of predator damage and seedless fruits on fruit and oil yields of this prospective biofuel tree, we quantified crop reduction caused by seedless fruits and seed wasps by estimating fruit and oil production among individual plants of three *P. chinensis* populations in Mt. Taihang Shan in Jiyuan City, Henan province and also measured fruit oil content for each fate category (*i. e.*, sound, seedless and insect-infested). The results indicated that fruit conditions varied considerably both among individual plants and among populations with the average 53.0% exhibiting seedless fruits and 22.6% showing insect-infested fruits. The dry mass of either seedless (30.7 mg/fruit) or insect-infested (33.1 mg/fruit) fruits was less than half of sound fruits (67.4 mg/fruit), and the oil content was similar between deceptive (3.9%) and insect-infested (3.8%) fruits, but up to ten times lower than that (39.9%) of sound fruits. Based on dry mass and oil content for each fate category, the observed fruit yield (2.9 kg/tree) was reduced up to 50% of the expected (4.7 kg/tree), and the observed oil yield (0.6 kg/tree) was less than one third of the expected (1.9 kg/tree), and fruit and oil yields were similar among populations. Our study indicates that *P. chinensis* faces serious challenges posed by seedless and insect-infested fruits that need to be overcome before adopting this tree as a major biofuel source.

Key words: *Pistacia chinensis*; biofuel; biofuel tree; seed wasp; *Eurytoma plotnikovi*; seedless fruits; fruit yield; oil yield

1 INTRODUCTION

Energy security has become a world-wide problem. Many countries are searching and developing renewable energy sources such as energy plants and their products to replace fossil fuels (Wang, 2005; Field *et al.*, 2008; Adler *et al.*, 2009). These energy plants and their products are considered to be important biofuel sources to supply sustainable energy, and to reduce greenhouse gases and acid rain. However, whether sufficient yields can be sustained from these energy plants is one key bottleneck to limit rapid development of related biofuel industries. In China, several endemic trees such as *Pistacia chinensis* Bunge (Anacardiaceae),

Xanthoceras sorbifolia Bunge (Sapindaceae), *Euphorbia lathyris* Linn. (Euphorbiaceae), *Jatropha curcas* Linn. (Euphorbiaceae), *Aluertes fordii* Hemsli. (Euphorbiaceae) and *Cornus wilsoniana* Wanger (Cornaceae) produce high-oil fruits/seeds and have been proposed as long-term biofuel sources to meet the growing energy needs (Wang, 2005; Li *et al.*, 2007). However, one important step prior to establishing large-area plantations is to assess potential factors that may limit the quantity and quality of their fruit/seed crops under commercial conditions.

P. chinensis has many attributes that recommend it as a biofuel tree: (1) high oil content (35%–40%) in fruits (Chen *et al.*, 2009; Wu *et al.*, 2009); (2) wide distribution across China with

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over 1 000 000 ha (Wang, 2005; Fu *et al.*, 2009; Hou *et al.*, 2010); (3) annual estimated oil production of ca. 200 thousand tons of each tree (Wang, 2005); (4) an ability to grow in barren hills especially where water is limited and other food/oil trees do not survive well; (5) potential for range expansion to other large barren areas in China (over millions ha); (6) oil is of high quality with a high proportion of unsaturated fatty acids (FA), including myristic acid, trace, palmitic acid, octadecanoic acid, gaidie acid, oleic acid, linolenic acid and linoleic acid (Hu *et al.*, 2007; Chen *et al.*, 2009; Qian *et al.*, 2009; Wu *et al.*, 2009); (7) local people along Mt. Taihang Shan have a long history of use of the oil for cooking (Qian *et al.*, 2009; personal investigation); and (8) large-area plantation of *P. chinensis* and/or other energy trees can effectively protect soil erosion and improve ecological and social sustainability (Wang, 2005; Li *et al.*, 2007).

However, *P. chinensis* presents some challenges to be overcome before it is to be successfully exploited as a biofuel tree. Little is known about its basic biology (*e.g.*, population growth, pollination and fruiting biology). Previous studies indicate that within the genus *Pistacia*, parthenocarpy, the production of seedless fruits without fertilization, is very common and can affect more than 50% of the crops, *e.g.*, *P. terebinthus* (Traveset, 1993a, 1993b), *P. lentiscus* (Jordano, 1989; Verdú and García-Fayos, 1998, 2001) and *P. vera* (Polito, 1999). Like other *Pistacia* species, parthenocarpy and abortion are also common for *P. chinensis* fruits (Copeland, 1955; personal observation). Traveset (1993b) reported that for *P. terebinthus*, the fresh mass of mature, green (sound) fruits is about twice that of nonviable red fruits (*i.e.*, seedless or insect-infested), and the former also have much more oil content in their pulp (35.83%) than the later (3.92%). In addition, the seed wasp *Eurytoma plotnikovi* Nikolskaya is found as a specialist seed predator to reduce over half or more of fruit yield of *P. chinensis* (Tian *et al.*, 1994; Chai and Zhang, 2004; Chai *et al.*, 2006). In 2007 and 2008, all fruiting trees of *P. chinensis* were attacked by *E. plotnikovi* with over 95% of fruits damaged in Shunping county, Hebei Province (Qi *et al.*, 2009). So both wasp-damaged and seedless fruits (*i.e.*, parthenocarpic and aborted) may substantially reduce fruit and oil yields. Therefore, it is necessary to assess the impact of predator damage and seedless fruits on fruit and oil yields of this biofuel tree.

In this study we quantified crop reduction caused by seed wasps and seedless fruits by estimating fruit and oil production from individual plants of three *P. chinensis* populations in Mt. Taihang Shan in Jiyuan City, Henan province. We also measured fruit oil content for each category (*i.e.*, sound, seedless and insect-infested). In addition, we propose some corresponding management approaches for this biofuel tree prior to establishing large-area plantations.

2 MATERIALS AND METHODS

2.1 Study species

Pistacia chinensis is a dioecious deciduous tree. Flowering occurs during March to late April. Male plants often initiate flowering two weeks before the females (Li *et al.*, 2007; Xu, 2009; Personal observation). Inflorescences of anemophilous flowers are always grouped. After pollination, the fruit develops within 1–2 weeks to the size similar to the mature fruit, but the zygote remains dormant until late July. Like other *Pistacia* species, immature *P. chinensis* fruits are red, but after maturation their color is highly correlated with seed viability: bluish green fruits often have viable seeds, but red fruits have nonviable seeds and include parthenocarpic, aborted and insect-infested fruits (*i.e.*, seed wasps) (Tian *et al.*, 1994; Liu *et al.*, 1999; Chai *et al.*, 2006; Li *et al.*, 2009; Personal observation). The mature *P. chinensis* fruit is an egg-shaped globular drupe and contains a single seed with a hard endocarp. Previous studies (Tian *et al.*, 1994; Chai and Xie, 2003; Chai *et al.*, 2006; Li *et al.*, 2009) and our ongoing investigation showed that adult females of *E. plotnikovi* lay 1–3 eggs at the inner wall of young fruits in late May to early July; the larvae hatch and the 1st star larvae remain undeveloped until the seed embryo begins to develop. In most cases, only one larva reaches final size in the seeds and stays in the seeds for 1–3 years before commencing pupation followed by adult emergence.

2.2 Experimental design

This study was carried out in Mt. Taihang Shan (35°03'N, 112°29'E; 400–500 m) in Jiyuan City of Henan Province, North China. *P. chinensis* is common with large populations along Mt. Taihang Shan (Wang, 2005; Fu *et al.*, 2009; Hou *et al.*, 2009). The climate at the study site is temperate zone continental monsoon type with mean temperature of 13.2–14.0°C and annual mean precipitation of 695 mm (Song and Qu, 1996).

During late September in 2009, we collected fruits at maturity from 40 fruiting female trees in three separate populations of *P. chinensis*: 18 trees in Dagouhe, 16 trees in Shaoyuan and 6 trees in Yugong. Before collecting fruits, we also estimated fruit crops for all sampled trees by counting the number of size-similar branches for each tree, and then counting infructescences from each of four branches and all fruits from each of 10 – 20 infructescences for each tree. For collected fruits, we randomly selected 100 or 200 fruits and then individually dissected them to check their condition measured in three categories: sound, seedless (including aborted and parthenocarpic) and insect-infested.

We randomly selected fruits from five of the sampled trees to measure their dry mass (0.1 mg) after oven-dried at 60°C for 72 h. For each tree, at least 30 fruits were included for each category, *i. e.*, sound, seedless and insect-infested, respectively. In addition, we also measured fruit oil content (%) for each category from these five trees using Soxhlet method (Manirakiza *et al.*, 2001). In this study, we used fruit dry mass and its oil content to estimate observed and expected yields because oil was extracted from fruits when they were harvested.

2.3 Indexes calculated

2.3.1 Fruit crop per tree: We estimated fruit crop (N_c) for each tree based on the following formula: $N_c = N_b * N_i * N_f$, where N_b is the number of all size-similar branches for a given tree, N_i is the average infructescence number per branch, and N_f is the average fruit number per infructescence.

2.3.2 Expected fruit and oil yields per tree: We estimated expected fruit and oil yield for each tree by initially assuming that all fruits were sound. The expected fruit yield (M_{fe} , kg/tree) was estimated by the following formula: $M_{fe} = N_c * M_s / 1\ 000\ 000$, where N_c is the fruit crop for a given tree and M_s is the mean dry mass (mg) of sound fruits. The expected oil yield (M_{oe} , kg/tree) was estimated by the following formula: $M_{oe} = M_{fe} * O_s / 1\ 000\ 000$, where O_s is the mean oil content for sound fruits.

2.3.3 Observed fruit and oil yields per tree: The observed fruit and oil yields included all fruit categories observed in this study. The observed fruit yield (M_{fo} , kg/tree) was estimated by the following formula: $M_{fo} = N_c * (P_s * M_s + P_d * M_d + P_i * M_i) / 1\ 000\ 000$, where P_s , P_d and P_i is the proportion of each category for each tree, *i. e.*, sound, seedless and insect-infested, respectively; M_s , M_d and M_i is the mean dry mass (mg) of each category for each tree, *i. e.*, sound, seedless and insect-infested,

respectively. The observed oil yield (M_{oo} , kg/tree) was estimated by the following formula: $M_{oo} = N_c * (P_s * M_s * O_s + P_d * M_d * O_d + P_i * M_i * O_i) / 1\ 000\ 000$, where O_s , O_d and O_i is the mean oil content of each category for each tree, *i. e.*, sound, seedless and insect-infested, respectively.

2.4 Statistical analysis

Peason's Chi-Square test was used to test the differences of the three fruit categories among the three populations. One-way ANOVA was used to test the difference of the dry mass or the oil content (arcsine square-root transformed) among the three fruit categories with LSD for pairwise comparison. One-way ANOVA was also used to test the differences of either expected or observed fruit and oil yields among different populations. Paired-sample *t* test were used to test the differences between expected and observed yields for either fruit or oil and for each and also of the three populations. All analyses were conducted by using SPSS 11.5.

3 RESULTS

3.1 Proportion of three fruit categories from three populations

Fruit conditions varied considerably among either populations ($\chi^2 = 843.71$, $df = 4$, $P < 0.001$) or individual plants sampled ($\chi^2 = 3\ 680.03$, $df = 78$, $P < 0.001$). The proportion of sound fruits (40.9%) was the highest in the Dagouhe population, but more seedless fruits (62.9%) occurred in the Shaoyuan population and more insect-infested fruits (32.2%) in the Yugong population (Fig. 1). For the population mean, the proportion (mean \pm SE) of seedless fruits was 53.0% \pm 3.4%, which was more than twice that of sound fruits (24.4% \pm 3.4%), and the proportion of insect-infested fruits was similar to that of sound fruits (22.6% \pm 4.0%) (Fig. 1).

3.2 Dry mass and oil content of three fruit categories

The dry mass (mean \pm SE) of sound fruits was significantly higher (67.4 \pm 1.1 mg) than that of either seedless fruits (30.7 \pm 0.3 mg) or insect-infested fruits (33.1 \pm 0.4 mg), but that of insect-infested fruits was similar to that of seedless fruits ($F_{2,12} = 962.875$, $P < 0.001$) (Fig. 2: A). The oil content (mean \pm SE) of sound fruits (39.9% \pm 1.9%) was more than ten times higher than that of either seedless (3.89% \pm 0.3%) or insect-infested fruits (3.8% \pm 0.2%) ($\chi^2 = 9.38$, $df = 2$, $P = 0.009$) (Fig. 2: B).

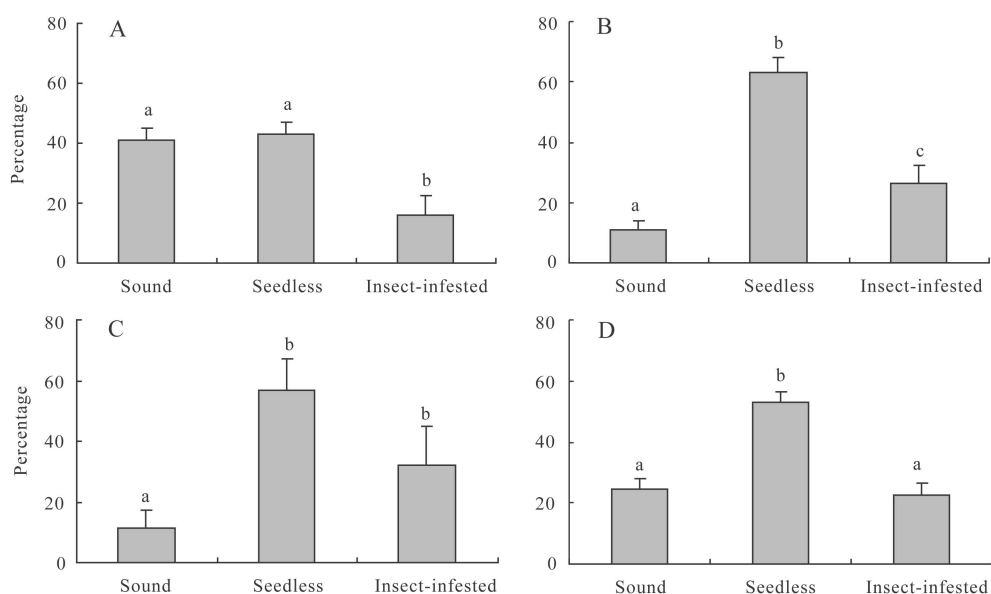


Fig. 1 Fates of *Pistacia chinensis* fruits among three populations: sound, seedless including parthenocarpic and aborted, and insect-infested

A: Dagouhe (N = 18 trees); B: Shaoyuan (N = 16 trees); C: Yugong (N = 6 trees); D: All trees (N = 40 trees) at Mt. Taihang Shan. Bars with different letters indicate a significant difference ($P < 0.05$).

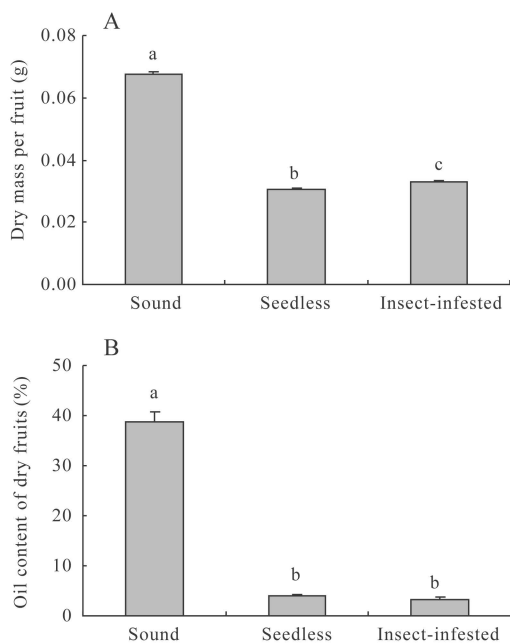


Fig. 2 Dry mass (g/fruit) (A) and oil content (%) (B) in sound, seedless (including parthenocarpic and aborted) and insect-infested fruits of *Pistacia chinensis*. Bars with different letters indicate a significant difference ($P < 0.05$).

3.3 Fruit and oil yields

Both observed and expected yields from either fruit or oil were similar among different populations (observed fruit yield, $F_{2,37} = 0.288$, $P = 0.752$; expected fruit yield, $F_{2,37} = 0.124$, $P = 0.884$; observed oil yield, $F_{2,37} = 1.602$, $P = 0.215$; expected oil yield, $F_{2,37} = 0.124$, $P = 0.844$), but observed yield from either fruit or oil was

significantly lower than the expected yield ($P < 0.001$) (Fig. 3: A, B). Based on dry mass, the observed fruit yield (2.9 ± 0.5 kg/tree, population mean) was less than 50% of the expected yield (4.7 ± 0.7 kg/tree) ($t = 6.720$, $df = 39$, $P < 0.001$) (Fig. 3: A). The observed oil yield (0.6 ± 0.1 kg/tree, population mean) was less than one-third of the expected yield (1.9 ± 0.3 kg/tree) ($t = 6.739$, $df = 39$, $P < 0.001$) (Fig. 3: B).

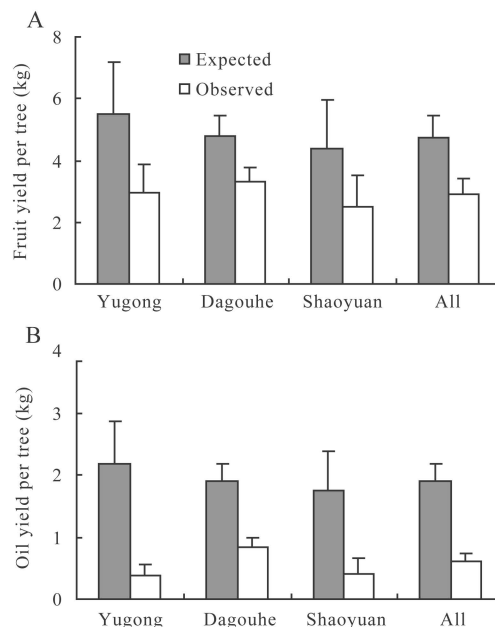


Fig. 3 Observed and expected yield (kg/tree) of dry fruits (A) and oil (B) among three populations of *Pistacia chinensis*. A: Fruit yield; B: Oil yield. (Dagouhe, N = 18 trees; Shaoyuan, N = 16 trees; Yugong, N = 6 trees; and all trees, N = 40 trees) at Mt. Taihang Shan.

4 DISCUSSION

4.1 Impacts of predator damage and seedless fruits on fruit and oil yields

Our study showed that both seedless and insect-infested fruits contribute to a high reduction in fruit and oil yields among the three populations of *P. chinensis*. We found that fruit fates varied both among individual plants and among populations. This may be caused by intrinsic factors (*e. g.*, abundance and floral sex ratios in reproductive populations and the characteristics of female trees) and/or extrinsic factors (*e. g.*, pollination, climate change, and water availability). But how these factors work on different populations needs to be further studied. Our results showed that 53.0% of seedless fruits and 22.6% of insect-infested fruits occurred in *P. chinensis*. This further confirms that a high proportion of seedless fruits is produced and thus it largely limits reproductive outputs in *Pistacia* plants (Grundwag, 1975, 1976; Jordao, 1989; Traveset, 1993a, 1993b, 1994; Verdú and García-Fayos, 1998; Polito, 1999). Other studies also showed that fruit abortion and insect seed predation are major causes to reduce reproductive outputs, *e. g.*, *Roupala montana* (Proteaceae) (Raimúndez-Urrutia, 2008) and *Bursera moreletensis* (Burseraceae) (Ramos-Ordoñez *et al.*, 2008). For *P. chinensis*, artificial pollination of female trees through spraying a mixture of pollen and water can increase sound fruits by 59.7% and sound seeds by 49.6% compared to those exposed to natural pollination (Lv and Xie, 2009). Thus, pollen limitation and/or pollination failure may be important causes for high proportions of seedless fruits in *P. chinensis*. However, our study found less than the 50% – 100% damage reported previously from seed wasps (Tian *et al.*, 1994; Chai *et al.*, 2006; Qi *et al.*, 2009). It is possible that previous studies may not consider the impacts of seedless fruits in the calculations. Fruit abortion and parthenocarpy in *Pistacia* and other plants are hypothesized as a decoy mechanism to reduce predator damage (Jordao, 1989; Traveset, 1993a, b, 1994; Fuentes and Schupp, 1998; Verdú and García-Fayos, 1998). In this study, it is unclear whether seedless fruits in *P. chinensis* have the potential to reduce predation of viable seeds by seed wasps.

We also found that in *P. chinensis*, both seedless and insect-infested fruits suffered a heavy reduction in both dry mass (50%) and oil content (90%) compared to sound fruits. Oil content is

higher for seed kernels (44.81% – 55.97%) but some lower for sound fruits (29.61% – 38.61%) (Chen *et al.*, 2009; Wu *et al.*, 2009). Due to the high incidence of seedless and insect-infested fruits, we found that the observed fruit yield (2.9 kg/tree) was reduced less than 50% of the expected yield (4.7 kg/tree), and the observed oil yield (0.6 kg/tree) was less than one third of the expected yield (1.9 kg/tree), but there was no difference of either fruit or oil yield among different populations. This indicates that *P. chinensis* faces serious challenges posed by seedless and insect-infested fruits that need to be overcome before adopting this tree as a major source of biofuels.

4.2 Implications for management

P. chinensis does have the intrinsic potential to produce high-oil fruits and high crops if they are not limited by seedless and insect-infested fruits. Our work suggests that there is a need for detailed information on reproductive biology and why *P. chinensis* (and maybe other dioecious species) produce lots of seedless fruits that constitute more than half of their expected crops (Traveset, 1994; Verdú and García-Fayos, 1998; Polito, 1999). Since fruit abortion and parthenocarpy are common in dioecious plants (Schwabe and Mills, 1981; Sutherland, 1986), intrinsic factors including floral sex ratios in reproductive populations, pollination biology and fruiting biology should be well studied to fully understand how these factors contribute to reproductive outputs. It is suggested that 6 – 8:1 to 20:1 of female to male and the even distribution of male plants can increase the percentage of sound fruits (Li *et al.*, 2009; Xu, 2009). In addition, genetic improvement and cultivation should also be considered as a means to decrease the incidence of unsound fruits; modern molecular technologies such as genetically modified approaches have also been advanced (Li *et al.*, 2007).

Extrinsic factors such as environmental conditions (*e. g.*, water availability) and predator damage may also affect fruit quantity and quality. Several studies suggest that water may be one important limiting resource that leads to the occurrence of parthenocarpic fruits (Verdú and García-Fayos, 1998). Verdú and García-Fayos (1998) found that the irrigated females of *P. lentiscus* significantly increased the percentage of sound seeds compared to those of nonirrigated females. In China, most *P. chinensis* trees grow in barren areas where rainfall is often very low and unpredictable, *e. g.*, Mt. Taihang Shan in China (Fu *et al.*, 2009; Hou *et al.*, 2010). However, it is

not clear whether water shortage increases the percentage of seedless fruits in *P. chinensis*. In addition, we found that ca. one-fourth of the fruit crop of *P. chinensis* was damaged by seed wasps, and subsequently this also reduces fruit and oil yields. Thus, effective methods used to control this seed predator should also be considered. One is to select insect-resistant cultivars if some *P. chinensis* cultivars can effectively avoid wasp damage (Yang, 2009). Another control method is sanitation to remove all wasp-infested fruits for 2–3 consecutive fruiting years, but this is labor-consuming and costly. In addition, spraying pesticides can reduce wasp damage at the peak of adult emergence and oviposition, but it may not be practical because *P. chinensis* trees are relatively high (5–10 m) and grow in barren hills (Li *et al.*, 2009; Wang *et al.*, 2009; Wu *et al.*, 2009). Moreover, biological control including natural enemies, sex pheromones and bacteria, virus and fungi should also be considered. There is no information available for specialist natural enemies of the seed wasp, but general natural enemies such as spiders and ants are common (Chai and Xie, 2003; Wu *et al.*, 2009).

4.3 Conclusion

In conclusion, our study showed that both seedless and insect-infested fruits reduce fruit and oil yields of *P. chinensis*. Though *P. chinensis* produces high-oil fruits and high crops on some fruiting plants, exploitation of this biofuel tree on a commercial scale faces serious challenges. More knowledge of both intrinsic and extrinsic factors contributing to or reducing fruit and oil yields of this biofuel tree is needed; and such knowledge will be essential for developing the renewable energy sources in the future.

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种子小蜂和空壳果实对生物能源树种黄连木果实和油产量的影响

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摘要: 黄连木 *Pistacia chinensis* Bunge 因果实含油量高, 被作为生物能源树种在我国进行大面积栽培推广和能源开发利用。然而, 专性寄生害虫(主要为黄连木广肩小蜂 *Eurytoma plotnikovi* Nikolskaya)的危害及由单性结实和败育所造成的空壳果实可能影响黄连木的果实产量和质量。为了评价种子害虫和空壳果实对黄连木果实产量和质量的影响, 我们测定了河南省济源市 3 个黄连木种群 40 株结果雌树的果实产量和油产量, 同时测定了完好果实、空壳果实和虫蛀果实等 3 类果实的干重和含油量。结果表明:(1) 虫害率和空壳率分别达 22.6% 和 53.0%, 且不同个体和种群间均存在显著差异($P < 0.05$)。(2) 空壳果实(30.7 mg/果)和虫蛀果实(33.1 mg/果)的干重均不到完好果实(67.4 mg/果)的一半; 空壳果实(3.9%)和虫蛀果实(3.8%)的含油量均显著低于完好果实(39.9%)。(3) 实测的果实产量(2.9 kg/树)和油产量(0.6 kg/树)分别仅为期望产量的 50% (4.7 kg/树)和 33% (1.9 kg/树), 但在 3 个种群之间无显著差异。本研究显示小蜂危害和空壳果实对黄连木果实产量和质量能造成严重影响, 在将其作为生物能源树种利用时对此应加以克服。

关键词: 黄连木; 生物能源; 生物能源树种; 种子小蜂; 黄连木广肩小蜂; 空壳果实; 果实产量; 油产量

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