Allelopathic Effects of *Bidens pilosa* L. var. *radiata* Sch. Bip. on the Tuber Sprouting and Seedling Growth of *Cyperus rotundus* L.

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**Abstract:** *Bidens pilosa* L. var. *radiata* Sch. Bip. (*BPr*) had been found capable of excluding *Cyperus rotundus* L. (*CR*) from its vegetation in fallow fields. Both allelopathy and competition of *BPr* were able to limit the growth of *CR*, but this has not been extensively investigated. To verify the two effects of *BPr* on *CR* management, density-dependent experiments and interspecies competitions with the application of activated carbon were conducted. The effects of *BPr* soil and its residues on the reproduction of *CR* were also evaluated. The results showed that the residues of *BPr* reduced the growth (54–61% of control) and tuber number (58–71% of control) of *CR* in the 3 plants pot−1 treatment but not in higher density treatments. In the interspecies competition, *BPr* exhibited an allelopathic but not competitive effect on *CR* when activated carbon was absent. *CR* tuber sprouting was significantly suppressed when sowed in the *BPr* soil. Likewise, *BPr* residue mulch inhibited the *CR* plant density by 87% as compared to natural-occurring *CR* residue mulch in the field. This study revealed that *BPr* might have potential for use as a cover plant and allelopathic mulch to control *CR* in the agroecosystem.

**Keywords:** *Bidens pilosa* L. var. *radiata* Sch. Bip. (*BPr*); *Cyperus rotundus* L. (*CR*); allelopathy; weed control; interspecies competition; density-dependent phytotoxicity

1. Introduction

*Cyperus rotundus* L. (*CR*), the most noxious weed on Earth, is a perennial herb in the Cyperaceae family possessing a C4 photosynthetic pathway [1,2]. It was reported that *CR* reproduces rarely by seed but mainly by tubers [3,4]. After sprouting, *CR* can form new tubers within 4–6 weeks and tuber chains in 10 weeks when it grows in a suitable environment [1]. The tuber sprouting within the tuber chain may be inhibited by apical dominance and remains dormant until the rhizomes or chains are cut off [3–5]. They then sprout and grow when the aerial parts die due to conventional agricultural practices such as mowing, plowing or herbicide application. Such a superior reproduction ability makes this species invasive and harmful, especially in upland agricultural production systems. Reports have indicated that *CR* can cause a 35–89% reduction in the yields of cabbage, tomato, cucumber, green bean, carrot, okra, bell pepper, onion, garlic and many other crops [6–9]. According to a study by Bendixen and Nandihalli [2], *CR* is disseminated in over 92 tropical and subtropical countries around the world and causes harvest losses in more than 50 crops.

Plant allelopathy is defined as the direct or indirect effects (positive and/or negative) on plants through the production of allelochemicals. However, the definition of the negative effect is more commonly used in allelopathy research [10,11]. Plant allelopathy has been considered as a herbicide reduction and a labor-saving weed management strategy (compared to hand weeding) in agricultural production systems [12,13]. Viji and Chinnamuthu [14,15] pointed out that the *CR* tuber dormancy...
could be induced by phenolics such as vanillic acid. Babu and Kandasamy [16] found that the tuber sprouting percentage of CR could be inhibited significantly by a water extract of fresh leaves of Eucalyptus globulus Labill. Cheema et al. [17] reported that both sorgaab (a sorghum water extract) and sorghum residue mulch could be applied to reducing plant density and shoot dry weight of CR when growing maize. Intercropping of sorghum, soybean and sesame for 2 years reduced the CR density (70–96%) and dry matter production (71–97%) [18]. Although studies on the application of plant allelopathy to control CR are rarely found, the aforementioned results show the potential of using such allelopathy to control the troublesome CR weed.

Bidens pilosa L. var. radiata Sch. Bip. (BPr) was studied for its significant allelopathic inhibition effects on many plant species [19–22]. Xuan et al. [23] indicated that 301 compounds (including polyacetylenes, flavonoids, phenolic acids, terpenes, fatty acids, etc.) have been identified from different parts of B. pilosa (BP). Among these compounds, the allelochemicals responsible for the phytotoxicity were mainly polyacetylenes and phenolics [19,20]. Campbell et al. [19] found that the phenylheptatriyne (PHT) extracted from the leaves of BP significantly inhibited the seedling growth of Asclepias syriaca L., Chenopodium album L., Phleum pratense L., and Trifolium pratense L. Deba et al. [20] revealed that phenolics (e.g., pyrocatechin, salicylic acid, p-vinylguaiacol, dimethoxyphenol, eugenol, 4-ethyl-1,2-benzenediol, iso-vanillin, 2-hydroxy-6-methylbenzaldehyde, vanillin, vanillic acid, p-hydroxybenzoic acid, protocatechuic acid, p-coumaric acid, ferulic acid, and caffeic acid) in BPr might play important roles in suppressing the germination and seedling growth of Echinochloa crus-galli (L.) P. Beauv. and Raphanus sativus L. The results of paddy field experiments also demonstrated that phenolic compounds released from the BPr residues might cause the death of weeds [24]. On the other hand, it has been reported that BPr was capable of exhibiting competitiveness and reducing the crop yields of soybean and corn in both additive and substitutive competition experiments [25]. Wang et al. [26] also indicated that BPr significantly suppressed the growth of Medicago sativa L. and Trifolium repens L. in substitutive competition experiments. It was demonstrated that BPr may possess inhibition on the target plants through allelopathy, competition or both. According to our previous experiences, BPr was observed being capable of competing with CR in areas invaded significantly by CR and became dominant afterward. This phenomenon suggested the potential of using BPr to control CR in an agricultural system. However, to the best of our knowledge, the interaction behind the interspecies interference between BPr and CR has not yet been clarified. Studies using BPr as a cover plant and allelopathic mulch to control CR in the agroecosystem are also very scarce. In the present study, it was hypothesized that both allelopathy and competition of BPr might be involved in limiting the growth and tuber sprouting of CR.

The relationship between plant allelopathy and competition is difficult to separate and/or isolate due to the complexity of the natural environment [27]. Inderjit and del Moral [10] indicated that co-linearities among environmental factors (for example, allelochemical concentration, soil pH, soil moisture, soil organic matter, soil nutrients, and so on) make it difficult to separate the two mechanisms from each other. Moreover, the effort to separate the two mechanisms might induce conditions that would never occur in a natural environment. Therefore, Weidenhamer [27] suggested that to distinguish the two mechanisms is more important and realistic than to separate them. Weidenhamer et al. [28] indicated that resource availability did not alter the predicted slope of the log weight-log density line due to the universal of -1 law in plant ecology, but the phytotoxin such as herbicides and allelochemicals did. Therefore, the density-dependent experiment could be used to distinguish the effect of allelochemicals from the intraspecies competition of the target plant. Besides, due to the strong adsorption capacity for organic chemicals (e.g., phenolic compounds), activated carbon addition was also considered as a feasible approach to distinguish plant allelopathy from the competition without influencing the plant growth [29–31].

Accordingly, to test our hypothesis, two pot experiments of density-dependent phytotoxicity and activated carbon addition were conducted to distinguish the allelopathy from the competition between these two investigated species in the semi-natural conditions. Additionally, to assess the potential of using BPr as a cover plant and allelopathic mulch to control CR in the field, two
experiments of the influence of the BPr soil and its residues on the tuber sprouting potential and tillers reproduction of CR were also conducted in the field.

2. Results

2.1. Experiment 1: Density-dependent phytotoxicity

The inhibition of CR seedling growth was observed at a BPr residue application rate of 0.1 kg m\(^{-2}\) for the treatment of 3 plants pot\(^{-1}\), and the shoot, root and total dry weights of CR were 72, 51 and 61% of the control (0 kg m\(^{-2}\) treatment), respectively (please refer to Table 1 for details). Meanwhile, the inhibition increased with an increasing residue application rate in the 3 plants pot\(^{-1}\) treatment. For the treatment of 6 plants pot\(^{-1}\), the seedling growth was only inhibited significantly at the application rate of 0.3 kg m\(^{-2}\) and the shoot, root and total dry weights of CR were 76, 66 and 70% of the control, respectively. There was no significant weight difference among the residue application rates for the 9 plants pot\(^{-1}\) treatment. It was noted that the root dry weight per plant in the treatment of 9 plants pot\(^{-1}\) was higher than those in the 3 and 6 plants pot\(^{-1}\) treatment, implying that the phytotoxicity of the BPr residue was diluted due to the higher plant density. In another word, the average phytotoxin absorbed per plant was less at 9-plant treatment than that at lower density treatments.

Table 1. Shoot, root and total dry weight per plant of C. rotundus (CR) growing in pot treated with the residue of B. pilosa var. radiata (BPr) at four different application rates.

| Application rate, residue of BPr (kg m\(^{-2}\)) | 3 plants pot\(^{-1}\) | 6 plants pot\(^{-1}\) | 9 plants pot\(^{-1}\) |
|-----------------------------------------------|----------------------|----------------------|----------------------|
| Shoot dry weight per plant (mg)               |                      |                      |                      |
| 0                                             | 451.11 ± 9.09\(^{\text{Aa}}\) | 253.89 ± 17.01\(^{\text{Ba}}\) | 194.44 ± 15.76\(^{\text{Ca}}\) |
| (100)                                         | (100)                | (100)                | (100)                |
| 0.1                                           | 325.00 ± 9.08\(^{\text{Ab}}\) | 230.00 ± 14.24\(^{\text{Bab}}\) | 193.33 ± 25.00\(^{\text{Ba}}\) |
| (72)                                          | (91)                 | (99)                 |                      |
| 0.2                                           | 305.56 ± 84.71\(^{\text{Ab}}\) | 217.78 ± 20.21\(^{\text{Ab}}\) | 209.26 ± 3.29\(^{\text{Ba}}\) |
| (68)                                          | (86)                 | (108)                |                      |
| 0.3                                           | 291.11 ± 53.48\(^{\text{Ab}}\) | 192.78 ± 16.84\(^{\text{Ab}}\) | 192.78 ± 7.61\(^{\text{Ba}}\) |
| (65)                                          | (76)                 | (99)                 |                      |
| Root dry weight per plant (mg)                 |                      |                      |                      |
| 0                                             | 512.22 ± 59.92\(^{\text{Aa}}\) | 330.00 ± 6.94\(^{\text{Ba}}\) | 314.44 ± 22.33\(^{\text{Ba}}\) |
| (100)                                         | (100)                | (100)                | (100)                |
| 0.1                                           | 261.67 ± 14.56\(^{\text{Ab}}\) | 305.56 ± 28.81\(^{\text{Ab}}\) | 313.33 ± 45.03\(^{\text{Aa}}\) |
| (51)                                          | (93)                 | (100)                |                      |
| 0.2                                           | 243.33 ± 85.05\(^{\text{Ab}}\) | 255.00 ± 26.03\(^{\text{Ab}}\) | 269.26 ± 9.86\(^{\text{Aa}}\) |
| (48)                                          | (77)                 | (86)                 |                      |
| 0.3                                           | 224.44 ± 42.92\(^{\text{Ab}}\) | 218.33 ± 15.28\(^{\text{Ac}}\) | 265.83 ± 18.93\(^{\text{Aa}}\) |
| (44)                                          | (66)                 | (85)                 |                      |
| Total dry weight per plant (mg)                |                      |                      |                      |
| 0                                             | 963.33 ± 67.41\(^{\text{Aa}}\) | 583.89 ± 19.35\(^{\text{Ba}}\) | 508.89 ± 37.74\(^{\text{Ba}}\) |
| (100)                                         | (100)                | (100)                | (100)                |
| 0.1                                           | 586.67 ± 20.14\(^{\text{Ab}}\) | 535.56 ± 39.84\(^{\text{Ab}}\) | 506.67 ± 69.15\(^{\text{Aa}}\) |
| (61)                                          | (92)                 | (100)                |                      |
| 0.2                                           | 548.89 ± 168.88\(^{\text{Ab}}\) | 472.78 ± 22.96\(^{\text{Ab}}\) | 478.52 ± 7.52\(^{\text{Aa}}\) |
| (57)                                          | (81)                 | (94)                 |                      |
| 0.3                                           | 515.56 ± 83.23\(^{\text{Ab}}\) | 411.11 ± 24.95\(^{\text{Ac}}\) | 458.61 ± 24.97\(^{\text{Aa}}\) |
| (54)                                          | (70)                 | (90)                 |                      |

For each variable, mean ± standard error (\(n = 4\)) within a row (in superscript capital letter) and within a column (in superscript small letter) followed by the same letter(s) are not significantly different at \(p < 0.05\) by LSD test. Data in the parenthesis are percentages of the control (0 kg m\(^{-2}\)).

For each residue application treatment, the ratios of shoot, root and total dry weight to the control were increased from low density (3 plants pot\(^{-1}\)) to high density (9 plants pot\(^{-1}\)), and the
growth inhibitions were higher at low than at high plant density treatments. The result also implied the presence of phytotoxin in the residue of BPr and showed that the response of CR seedling growth to the residue of BPr was density-dependent.

The shoot to root ratios of CR in the 3 plants pot⁻¹ treatment with BPr residue were significantly higher compared to the control. The ratios were 1.25, 1.32 and 1.36 of the application rates of 0.1, 0.2 and 0.3 kg m⁻² treatments, respectively (as shown in Table 2). The ratio greater than 1 indicated that the root growth was more suppressed by the residue of BPr than the shoot growth, and the biomass allocation pattern was altered. For the 6 and 9 plants pot⁻¹ treatments, the shoot to root ratios of CR at the application rates of 0.2 and 0.3 kg m⁻² were higher than that of the control but these ratios were smaller than 1. The influence of the residue of BPr on the shoot to root ratio of CR also showed a density-dependent phytotoxic effect. The ratios decreased with an increase in plant density for all the investigated application rates, also showing that the phytotoxin was diluted by the increasing density.

Table 2. The shoot to root ratio of CR growing in pot treated with the residue of BPr at four different application rates.

| Application rate, residue of BPr (kg m⁻²) | 3 plants pot⁻¹ | 6 plants pot⁻¹ | 9 plants pot⁻¹ |
|-----------------------------------------|---------------|---------------|---------------|
| 0                                      | 0.90 ± 0.09 Ab | 0.77 ± 0.05 Aa | 0.62 ± 0.01 Bb |
| 0.1                                    | 1.25 ± 0.06 Aab | 0.76 ± 0.06 Aa | 0.62 ± 0.03 Bb |
| 0.2                                    | 1.32 ± 0.10 Aa | 0.88 ± 0.14 Aa | 0.78 ± 0.04 Aa |
| 0.3                                    | 1.36 ± 0.23 Aa | 0.89 ± 0.09 Aa | 0.73 ± 0.04 Aa |

Mean ± standard error (n = 4) within a row (in superscript capital letter) and within a column (in superscript small letter) followed by the same letter(s) are not significantly different at p < 0.05 by LSD test. Data were log-transformed prior to analysis.

Table 3. The tuber and tiller numbers per plant of CR growing in pot treated with the residue of BPr at four different application rates.

| Application rate, residue of BPr (kg m⁻²) | 3 plants pot⁻¹ | 6 plants pot⁻¹ | 9 plants pot⁻¹ |
|-----------------------------------------|---------------|---------------|---------------|
| Tuber numbers per plant                 |               |               |               |
| 0                                      | 5.33 ± 0.69 Aa | 3.06 ± 0.20 Aa | 2.86 ± 0.16 Aa |
| (100)                                  | (100)         | (100)         |               |
| 0.1                                    | 3.67 ± 0.14 Ab | 2.94 ± 0.24 Aa | 2.85 ± 0.16 Aa |
| (69)                                   | (96)          | (100)         |               |
| 0.2                                    | 3.11 ± 0.73 Ab | 2.94 ± 0.20 Aa | 2.37 ± 0.07 Ab |
| (58)                                   | (96)          | (83)          |               |
| 0.3                                    | 3.78 ± 0.40 Aab | 2.72 ± 0.20 Ab | 2.36 ± 0.08 Bb |
| (71)                                   | (89)          | (83)          |               |

Tiller numbers per plant

| Application rate, residue of BPr (kg m⁻²) | 3 plants pot⁻¹ | 6 plants pot⁻¹ | 9 plants pot⁻¹ |
|-----------------------------------------|---------------|---------------|---------------|
| 0                                      | 2.78 ± 0.29 Aa | 1.83 ± 0.10 Aa | 1.75 ± 0.05 Aab |
| (100)                                  | (100)         | (100)         |               |
| 0.1                                    | 2.33 ± 0.14 Aa | 1.83 ± 0.00 Aa | 1.56 ± 0.06 Ab |
| (84)                                   | (100)         | (89)          |               |
| 0.2                                    | 2.00 ± 0.33 Aa | 2.17 ± 0.25 Aa | 1.93 ± 0.20 Aa |
| (72)                                   | (118)         | (110)         |               |
| 0.3                                    | 2.44 ± 0.40 Aa | 1.94 ± 0.36 Aa | 1.67 ± 0.08 Aab |
| (88)                                   | (106)         | (95)          |               |

For each variable, mean ± standard error (n = 4) within a row (in superscript capital letter) and within a column (in superscript small letter) followed by the same letter(s) are not significantly different at p < 0.05 by LSD test. Data in the parenthesis are percentages of the control (0 kg m⁻²).

The residue of BPr also demonstrated significant inhibition on the CR tuber numbers per plant at the low-density treatment (i.e., 3 plants pot⁻¹). The tuber numbers per pot were 69, 58 and 71% of the control at the 0.1, 0.2 and 0.3 kg m⁻² residue application rates, respectively (Table 3). For the higher
density treatments (i.e., 6 and 9 plants pot$^{-1}$), the inhibition effect of the residue on the tuber number per plant was reduced. Hence, the inhibition of tuber number by the phytotoxin in the residue was density-dependent. However, the tiller numbers per plant of CR were slightly reduced by the increasing residue application rate, indicating that the residue had less inhibition effect on the tuber sprouting than that of the density effect.

The relationship of log total dry weight per plant vs. log plant density showed that the regression slopes of the investigated treatments of BPr residues deviated from that of the control and the deviation became more apparent as the residue application rate increased (Figure 1). The different reduction between the low and high density treatments showed again that the CR absorbed more phytotoxin per plant at the low density treatment (3 plants pot$^{-1}$) than at the high density treatments (6 and 9 plants pot$^{-1}$). Besides, the results also demonstrated that the plant growth might be stimulated at high density due to hormesis (stimulation at subtoxic concentration) at the 9 plants pot$^{-1}$ treatment [11].

![Figure 1. Relationship of log mean total dry weight and log plant density of C. rotundus (CR) for the residue of B. pilosa var. radiata (BPr) application at four different rates.](image)

2.2. Experiment 2: Interspecies competition between BPr and CR

In experiment 2, through the relative arrangement of aboveground and belowground partition coupled with the disposition of BPr and CR, four competition modes were obtained: (1) both the shoot and root of the two species were separated (NO competition), (2) only the root was separated (SHOOT competition), (3) only the shoot was separated (ROOT competition) and (4) neither the shoot nor root was separated (FULL competition). By comparison between the results of the NO and SHOOT competitions, it was found that the shoot dry weights of BPr and CR were higher with the activated carbon treatment (AC treatment) than without activated carbon (N treatment), but only the difference in SHOOT competition of CR was significant (Figures 2(a) and 2(b)). For the ROOT competition, the shoot dry weight of BPr was reduced by 32% at AC treatment as compared to N treatment. CR showed an adverse response to the activated carbon, and the shoot dry weight was enhanced by 44% at AC treatment as compared to N treatment. Compared to the other three competitive experiments, BPr exhibited the lowest shoot weight at the N (1.07 g pot$^{-1}$) and AC (0.79 g pot$^{-1}$) treatments in the FULL competition, while CR produced the highest shoot weight at the N (1.01 g pot$^{-1}$) and AC (1.2 g pot$^{-1}$) treatments in the FULL competition.
CR had a higher root growth than BPr for all tested competitions. Similar to the shoot growth, the root growth of CR was stimulated by the AC treatment in the NO and SHOOT competitions, and only the difference in SHOOT competition of CR was significant (Figures 2(c) and 2(d)). For the ROOT competition, the root dry weight of BPr was significantly reduced by 50% in AC treatment compared to N treatment. By contrast, the root growth of CR was 25% higher in AC treatment than in N treatment.

**Figure 2.** Shoot, root and total dry weight per pot of BPr and CR when grown with two activated carbon application treatments and subjected to four competition modes. (a) Shoot dry weight of BPr. (b) Shoot dry weight of CR. (c) Root dry weight of BPr. (d) Root dry weight of CR. (e) Total dry weight of BPr. (f) Total dry weight of CR. AC and N denoted pots added with and without activated carbon application, respectively. Error bars are the standard error of mean (n = 5). Means with the same letter(s) are not significantly different at 5% level by LSD test.
Comparing all the competitions showed that \( BPr \) had the lowest root dry weight (0.43 g pot\(^{-1} \) for N treatment and 2.27g pot\(^{-1} \) for AC treatment) in the FULL competition, whereas \( CR \) had the highest root dry weight for each N (3.57 g pot\(^{-1} \)) and AC (4.23 g pot\(^{-1} \)) treatment in the FULL competition.

After four weeks of growth, \( CR \) accumulated more total biomass than \( BPr \) in all the treatments (Figures 2(e) and 2(f)). However, for all the competitions except for the NO competition, the growth reduction of \( CR \) at N treatment implied that \( BPr \) might possess the allelopathic effect through shoot leachate and root exudation. Meanwhile, under the ROOT and FULL competitions, the total growth reduction of \( BPr \) at AC treatment might be caused by the aggressive resource competition of \( CR \) root.

Although both plants are perennial herbs, they had opposite biomass allocation patterns. The biomass distribution was facilitated firstly in shoot rather than in root for \( BPr \), but such distribution occurred in root rather than in shoot for \( CR \) at the growing stage. With the NO and SHOOT competitions, neither N nor AC treatment had an effect on the shoot to root ratios of \( BPr \) and \( CR \). The average shoot to root ratios of \( BPr \) and \( CR \) in these competitions were 1.5 and 0.28, respectively (Figure 3).

![Figure 3](image)

**Figure 3.** Shoot to root ratio of \( BPr \) and \( CR \) when grown with two activated carbon application treatments and subjected to four competition modes. (a) Shoot to root ratio of \( BPr \). (b) Shoot to root ratio of \( CR \). AC and N denoted pots added with and without activated carbon application, respectively. Error bars are the standard error of mean (\( n = 5 \)). Means with the same letter(s) are not significantly different at 5% level by LSD test. Data were log-transformed prior to analysis.
For the ROOT competition, the shoot to root ratios were significantly increased at AC treatment for both BPr and CR. The increased ratio of BPr was caused by the strong reduction of the root growth (Figures 2c and 3), while the ratio of CR increased due to the thriving growth of the shoot (Figures 2b and 3). Under the FULL competition, the shoot to root ratios of BPr were significantly higher at both activated carbon treatments than that in the control (NO competition), whereas the ratios of CR were shown no difference from that in the control. Similar to the ROOT competition, the reduction of BPr root growth under the FULL competition resulted in the increased ratios. However, the unchanged ratios of CR under the FULL competition might result from the thriving growth of both the shoot and root (Figures 2b, 2d and 3). The CR tuber proliferated greatly in all treatments after ten weeks of growth (Figure 4). Different from the responses of CR seedling growth to the BPr allelopathy, the N treatment showed no inhibition on the tuber proliferation in all the competitions when compared with the AC treatment.

![Figure 4. Tuber numbers per pot of CR when grown with two activated carbon application treatments and subjected to four competition modes with BPr. AC and N denoted pots with and without activated carbon application, respectively. Error bars are the standard error of mean (n = 5). Means with the same letter(s) are not significantly different at 5% level by LSD test.](image)

2.3. Experiment 3: The Tuber Sprouting of CR in the Field of Mature BPr Vegetation

The response of CR tuber sprouting to the presence of BPr showed that the highest tuber sprouting percentage, mean sprouts per quadrat and dry weight per sprout, occurred in the treatment mulched by the opaque plastic sheet (OP treatment), followed by sowing tubers in the BPr vegetation without (VN treatment) and with removing the shoots and litter (VS treatment) (Table 4). The OP treatment here was considered as a control measure because it was a common weed controlling manner in agricultural practice. It was observed that it had a high sprouting percentage of 81% with 30.75 sprouts per quadrat and a mean dry weight per sprout of 9.98 mg. The tuber sprouting percentage (52%), mean sprouts per quadrat (18 sprouts per quadrat) and dry weight per sprout (5.26 mg) were decreased when the tubers of CR were sowed in the BPr soil with removing shoot and litter (VN treatment). The results of VN treatment indicated that although the shoots and litter of BPr were removed, the allelochemicals remained in the soil and continued to inhibit the CR tuber sprouting. On the other hand, only one tuber with one sprout in total was found in VS treatment, demonstrating that the presence of the living BPr might continue to release allelochemicals to inhibit the tuber sprouting of C. rotundus.
Table 4. Comparison of tuber sprouting percentage, mean sprouts per quadrat and dry weight per sprout of CR when sowed in the BPr vegetation with (VS) or without (VN) removing the shoots and litter, and in the field (mulched with an opaque plastic sheet, OP) outside the BPr vegetation as control.

| Treatments | Tuber sprouting Percentage (%) | Mean sprouts per quadrat | Dry weight per Sprout (mg) |
|------------|--------------------------------|--------------------------|----------------------------|
| OP         | 81.00 ± 0.05 a                  | 30.75 ± 2.25 a           | 9.98 ± 1.76 a             |
| VN         | 52.00 ± 0.09 ab                 | 18.00 ± 3.03 ab         | 5.26 ± 0.76 ab            |
| VS         | 1.00 ± 0.01 b                   | 0.25 ± 0.25 b           | 1.50 ± 1.50 b            |

Within each column, mean ± standard error (n = 4) followed by the same letter(s) are not significantly different at p < 0.05 by Dunn’s nonparametric comparison for post hoc test after a Kruskal-Wallis test.

2.4. Experiment 4: The Effects of Vegetation and Residue Mulch of BPr on the Reproduction of CR

At the beginning of the experiment (0 days after sowing, 0 DAS), the tuber densities of the plots covered with BPr and CR were 55.51 and 60.44 tubers dm\(^{-2}\), respectively, and the dry weights per tuber were 98.41 and 101.12 mg tuber\(^{-1}\), respectively (Table 5). No significant difference was found in the tuber density and the dry weight per tuber between the two tested cover plants before starting the experiments by sowing with the seeds of BPr. After the seeds were sowed, it was observed that although most seeds germinated in the first week, CR reproduced and grew more rapidly than BPr seedlings at the early stage. The seedlings of CR continued growing even the canopy of BPr was closed. Therefore, the tuber density increased approximately two folds for both the cover plant treatments with BPr (92.5 tubers dm\(^{-2}\)) and CR (104.91 tubers dm\(^{-2}\)) on the 69 DAS and no significant difference was found between them. However, the dry weight per tuber at the cover plant of BPr (49.51 mg tuber\(^{-1}\)) was significantly lower than that of CR (63.31 mg tuber\(^{-1}\)), indicating that the shadow and/or allelopathy of BPr might reduce the tuber biomass accumulation.

Table 5. In the field severely invaded by CR, the influence of BPr as cover plants on the tuber density and dry weight per tuber of CR. The investigation was conducted on 0 and 69 days after sowing (DAS).

| Cover plants | Tuber density (tubers dm\(^{-2}\)) | Dry weight per tuber (mg tuber\(^{-1}\)) |
|--------------|-----------------------------------|-----------------------------------------|
| BPr          | 55.51 ± 5.30 *                    | 98.41 ± 6.68 *                          |
| CR           | 60.44 ± 6.90 *                    | 101.12 ± 7.81 *                         |
| 69 DAS (December 26, 2019) |                                  |                                          |

| Cover plants | Tuber density (tubers dm\(^{-2}\)) | Dry weight per tuber (mg tuber\(^{-1}\)) |
|--------------|-----------------------------------|-----------------------------------------|
| BPr          | 92.50 ± 9.59 *                    | 49.51 ± 4.69 b                          |
| CR           | 104.91 ± 9.38 *                   | 63.31 ± 4.94 a                          |

Within each column, mean ± standard error (n = 12) of different DAS followed by the same letter(s) are not significantly different at p < 0.05 by LSD test.

Both of the cover plant treatments were subject to mowing at 69 DAS and all the plant residues were left on their plots. Afterward, for the cover plant of BPr, half of the plots were mulched with the opaque plastic sheet (B-Py treatment) and another half were not (B-Pn). The same handling was conducted for CR. C-Py and C-Pn treatments denoted the plots mulched with and without the opaque plastic sheet, respectively. Two weeks afterward (i.e., 83 DAS), the investigation of the CR plant density showed that the highest CR density was found at C-Py treatment (764 plants m\(^{-2}\)), followed by C-Pn (524 plants m\(^{-2}\)), B-Py (118 plants m\(^{-2}\)) and B-Pn (65 plants m\(^{-2}\)) treatments (Figure 5). According to the results, the treatments grew with BPr and mulched with its residues (B-Py and B-Pn treatments) significantly inhibited the reproduction of CR as compared to that grew without BPr and mulched with the residues of CR (C-Py and C-Pn treatments). The results also further indicated that the reproduction of CR was suppressed by the allelopathy of BPr rather than the opaque plastic sheet. In short, the relationship between the residue dry weight and CR plant density illustrated that
the BPr residues could provide suppression on the density of CR even at a lower quantity of 2 ton ha\(^{-1}\) (Figure 6); meanwhile, the CR density was suppressed by 87% on average at the mean application rate of 4 ton ha\(^{-1}\) (Figures 5 and 6).

Figure 5. The influence of BPr as a cover plant on the regeneration of CR. Plant density of CR was investigated two weeks after mowing (69 DAS). Error bars are the standard error of mean (n = 6). Means with the same letter(s) are not significantly different at 5% level by LSD test.

Figure 6. Relationship of plant density of CR (tillers m\(^{-2}\)) and residue dry weight (ton ha\(^{-1}\)). B-Py is BPr residue covered with the opaque plastic sheet, B-Pn is BPr residue covered without the opaque plastic sheet, C-Py is CR residue covered with the opaque plastic sheet and C-Pn is CR residue covered without the opaque plastic sheet.

3. Discussion

3.1. Distinguishing Allelopathy from Competition

Studies indicated that the leachate or residues of allelopathic plants might inhibit the growth of CR. For example, the leachate of *E. globulus*. fresh leaves significantly reduced both the shoot and root dry weight of CR [16]. Residues of *Helianthus annuus* L., *Sorghum bicolor* (L.) Moench and *Brassica*
campestris L. reduced the plant density and dry weight of CR \cite{32,33}. However, due to the complexity of the ecological environment, it is hard to distinguish the allelopathy of the leachate or residues from the competition (including interspecies or plant-microbial interactions) \cite{27,34}.

Weidenhammer et al. \cite{28} firstly provided experimental evidence that density-dependent phytotoxicity of allelochemicals could be used to distinguish allelopathy from the intraspecies competition (or other microbial activities). Allelochemicals could cause a greater growth reduction on the target plant at low density than that at high density due to the dilution of phytotoxicity of allelochemicals. Besides, Weidenhammer et al. \cite{27,28} also pointed out that the slope of log mean plant weight versus log mean density would be altered due to interactions between density and allelopathy, thus deviated from the expected log yield-density relationships of the -1 law of plant ecology \cite{27}. In the present study, the results of experiment 1 demonstrated that the phytotoxicity of BPr residues to the shoot and root (including tuber numbers) growth of CR seedlings was density-dependent (Table 1, 3). Residues exhibited the greatest phytotoxicity to the seedlings at the low density (3 plants pot$^{-1}$) and the reduction of growth decreased as the density increased. Simultaneously, the slopes of the log mean plant weight versus log mean plant density curves at the tested residue application rates obviously deviated from that of the control (Figure 1). This also provided evidence for the existence of density-dependent phytotoxicity in the residue of BPr.

Scavo et al. \cite{13} indicated that a two-way relationship might exist between soil characteristics and allelochemicals that affects the retention, transport and transformation processes of allelochemicals in soil. In a short-term pot experiment, although the soil organic matter content, available nitrogen, pH and EC were altered by adding allelochemical residues in the soil, the phytotoxicity of the Chenopodium murale L. residues possessed the main negative effects on the chickpea and pea \cite{35}. Based on the findings of these two studies, the soil organic matter content, available nitrogen and EC in our study were supposed to be raised with the increase in BPr residue application rate and stimulated the growth of CR. However, the negative growth response of CR demonstrated that the allelopathy of BPr residues might be the main cause of the influence of this experiment.

It has been reported that intercropping of allelopathic plants in cotton improved the cotton yield and reduced the CR population \cite{18}. However, little has been investigated regarding the effect of interspecies competition and allelopathy at the same time due to the difficulties of distinguishing them from each other under natural or semi-natural conditions. In experiment 2, activated carbon was added to four different competition modes to absorb the allelochemicals in the pots. The findings showed that under SHOOT competition, neither the growth of BPr nor CR was suppressed from each other with the addition of activated carbon (AC treatment) as compared to the AC treatment under NO competition. However, the shoot and root growth of CR was reduced at N treatment (Figures 2). It was presumed that the growth reduction of CR at N treatment under SHOOT competition might result from the phytotoxicity in the shoot leachate of BPr. Besides, notwithstanding that light is a considerable factor for shoot competition, no evidence supported that either BPr or CR suffered shading from the other species. Previous literature also indicated that light is not an important factor for shoot competition before plant canopies are capable of shading on another species \cite{36}.

Under ROOT and FULL competitions, the growth of CR was enhanced while BPr was decreased at AC treatment as compared with N treatment. Researches indicated that CR often demonstrated a predominant root competition with coexisting plants. Tuor and Froud-Williams \cite{37} showed that CR could significantly decrease the shoot dry weight and height of both maize and soybean under root and full competitions as compared to zero competition (the same as NO competition in this study). Horowitz \cite{38} indicated that the growth of citrus seedling was significantly inhibited by CR despite of fertilizing with nitrogen or not. He further supposed that the phytotoxic substances produced by CR might partly contribute to the competition with citrus. Although the previous studies demonstrated that CR could compete with coexisting plants through allelopathy, the allelopathic effects of CR on BPr were not observed in experiment 2 since the CR growth was suppressed by BPr when no activated carbon was applied.
The most conflicting results from experiments 1 and 2 were the comparison of the shoot to root ratios. The result of experiment 1 indicated that both the plant density and residue application rate of BPr altered the ratios but in opposite directions (Table 2). The ratios decreased as the plant density increased for a given residue application rate but increased with the increase of application rate. According to the prediction of optimal partitioning theory (OPT), plants tend to partition more biomass in root than in shoot when major nutrients are deficient and thus result in a lower shoot to root ratio [39]. A similar tendency reported by Williams et al. [40] showed that CR allocated more biomass to root rather than shoot when growing at high density. The effect of plant density on the ratios appeared that CR in the high density subjected to more resource competition and the nutrient supply for each plant was reduced. However, the response of shoot to root ratio was inverse in trend by the application of BPr residues in each given density treatment. It was presumed that the root nutrient absorption ability of CR was impaired due to the deleterious effects of BPr allelochemicals such as the phenolic acids thus resulted in increasing the shoot to root ratio [41,42].

In experiment 2, under the competition including root interference (i.e., ROOT and FULL competitions), both BPr and CR had statistically-significant lower shoot to root ratios in the presence of allelopathy (N treatment) than that in the absence (AC treatment) except for the CR under FULL competition. The responses of shoot to root ratio of CR to the allelopathic effects of BPr roots did not increase as that influenced by the residues of BPr in experiment 1. Two possibilities were supposed for the difference between experiments 1 and 2. First, the allelochemicals in the residues and the root exudation might be different. Deba et al. [20] found that the main phenolic compounds in the leaves, stems and roots of BPr were similar in composition but different in content. Second, the allelopathic effects coupled with root competition caused a different inhibition mechanism in experiment 2. A similar result from Nilsson [30], who reported that Scots pine seedlings had a lower shoot to root ratios when in the presence of both allelopathy and interspecies root competition. Schenk [42] pointed out that plant roots exhibited different responses when overlapping with ‘self’ and ‘non-self’ roots, and the responses might further be altered by allelochemicals that inhibited root growth.

El-Rokiek [43] pointed out that the phenolics (ferulic acid, caffeic acid for example) in the mango leaves might inhibit the seedling growth and tuber sprouting of CR. Fifteen phenolic compounds isolated from BPr were also reported to possess the allelochemicals of phenolics (e.g., caffeic, ferulic, p-coumaric, p-hydroxybenzoic, salicylic acid and so on) [20,23]. Such phenolics were reported to cause deleterious damage to plant roots. For example, Einhellig [44] indicated that the salicylic acid appeared to cause damage to the membrane structure and permeability of the root cell while the caffeic acid decreased the nitrogen, phosphorus, potassium, iron and molybdenum in cowpea. Bergmark et al. [41] showed that ferulic acid inhibited the nitrogen uptake in the roots of maize seedling. Abenavoli et al. [45] also demonstrated that the trans-cinnamic, ferulic and p-coumaric acid reduced the net nitrogen uptake and plasma membrane H⁺-ATPase activity. PHT, a putative allelochemical of polyacetylene, was presumed to release phyto toxic radicals and this inhibitory mechanism could be enhanced by illuminating with sunlight or near-UV light. PHT was found to exist in the leaves of B. pilosa and was reported to suppress the seedling growth of A. syriaca, C. album, P. pratense and T. pratense with LC₅₀ of 0.66, 0.83, 2.88 and 1.43 ppm, respectively [19]. In experiments 1 and 2, BPr residues, shoot leachates and root exudates were supposed to release phenolics and PHT to the soil and interfered with the growth of CR. Besides, it was also reported that allelopathic plants exhibited higher inhibitory effects on the neighboring plants when growing under nutrient-deficient conditions [13]. Therefore, since no extra fertilizer was added in experiment 2 during the ten-week growing period, the nutrient-deficient conditions might also contribute to stimulating the allelochemical exudation of BPr when competing for nutrients with CR (under ROOT and FULL competitions).

3.2. The Influence of B. pilosa var. Radiata on the Reproduction of CR in the Field

The BPr soil exhibited a strong phytotoxicity effect on the tuber reproduction of CR. The tuber sprouting percentage, mean sprouts per quadrat and dry weight per sprout were lower in both the VN (with removing the shoots and litter) and VS treatments (without removing the shoots and litter)
than those in the OP treatment. Moreover, the VS treatment possessed higher suppression than VN treatments (Table 4). In a natural environment, the concentration, movement and persistence of allelochemicals determine the phytotoxic level of donor plants on the target plants [13]. The watersoluble allelochemicals such as the phenolics were considered to have a short residence time in the soil due to the rapid leaching and degradation [46,47]. In the present study, the results of experiments 1 and 2 illustrated that BPr might release allelochemicals through its residues, shoot leachates and root exudes. The removal of aboveground BPr and litter in the VN treatment interrupted the input of allelochemicals (e.g., phenolics and PHT) into the soil. The remaining phytotoxicity of BPr soil was expected to degrade soon when no allelochemical was continuously released from the aboveground plant and litter to the quadrat. Meanwhile, the replenishment of allelochemicals from BPr plants outside the quadrat was also limited due to the slow rates of chemical diffusion in soil, sorption of the soil particles and organic matter, and microbial degradation [48].

In the field severely invaded by CR, the tubers proliferated obviously in both cover plant treatments during the experimental period (Table 5). It was supposed that the apical dominance in tubers was broken off when the tuber chains were cut off due to the plowing before the experiment [4,5], and a large number of dormant tubers in chains started sprouting. Likewise, with the characteristics of the C4 photosynthetic pathway [1], the sprouts of CR grew faster than the seedlings of BPr. Hence, the tuber numbers increased in all treatments before mowing.

The inhibition effects of the allelopathic residues on the reproduction of CR depended on plant species and approaches used. Mahmood and Cheema [49] reported that soil-incorporated sorghum stalks (15 ton ha⁻¹) had less inhibitory effect on the density of CR than the surface-applied treatment (15 ton ha⁻¹). Khalqi et al. [32] further indicated that the residue combination of sorghum, sunflower and brassica (each at 7.5 ton ha⁻¹) reduced the CR plant density by 87% as compared to the control. On the contrary, the soil-incorporated allelopathic straws of wheat and rye could efficiently decrease the weed densities of Portulaca oleracea L., Amaranthus retroflexus L. and Echinochloa colonum L. but failed to inhibit the emergence of CR [50]. In the present study, despite the fact that the tubers proliferated before the cover plants were mowed, the plant density of CR was significantly reduced in the presence of BPr residue mulch and decreased with the increasing residue dry weight. In addition, it was also found that the CR plant density was higher in both cover plant species in the presence of opaque plastic sheet treatment as compared to that in the absence of the opaque plastic sheet. Although soils covered with the plastic sheet was observed to elevate the temperature in the air space (between the sheet and soil surface) and the upper soil [51], it was found that no negative effect on the tuber reproduction unless the upper soil temperature was elevated up to 45°C and persisted for more than 7 h per day [52]. Moreover, studies indicated that phenolic compounds released from apple residues decreased with the elevating soil temperature [53]. Meanwhile, due to the characteristics of photosensitization and high activity, the phytotoxicity of polyacetylenes such as PHT might be reduced in the darkness and the temperature higher than 30 °C [19,54]. Therefore, in this experiment, the allelochemicals of BPr might degrade more rapidly in the B-Py and C-Py treatments than in the B-Pn and C-Pn treatments due to the elevating temperature under the sheet.

Plant species that possess great competitiveness and invasive capacity with crops or native species usually have a strong allelopathic capacity [55]. BP, especially its variety BPr, was found to be highly invasive in subtropical and tropical regions [23]. Field competition studies conducted by Ng et al. [56] showed that BPr was dominant in groups consisting of Poaceae and C3 plants in competition with monoculture and polyculture groups of sixteen species. Likewise, BP and BPr have been found to possess phytotoxic effects on its sympatric plant species, such as Bidens bipinnata L. and Pteris multifida Poir., in the ecosystems [21,22]. In southeast Asia, BP and BPr have been investigated for their capability in paddy weed management. For example, Hong et al. [57] evaluated ten allelopathic species for paddy weed control in Vietnam and found that BP was an effective allelopathic species to eradicate 80% more of weeds and increase rice yield more than 20%. Krumsri et al. [58] examined the phytotoxic effects of BP residue on E. crus-galli under various conditions, and found that fresh BP residues exerted more phytotoxicity than the dried residues. Meanwhile, both soil mulching and incorporating with BP residues significantly decreased the density of E. crus-galli
when using the BP plants harvested at a 60-day growth stage. In Thailand, Poonpaiboonpipat and Poolkum [24] indicated that the most effective application rate of BPr residue in the paddy field was 4 ton ha\(^{-1}\) which inhibited the weed growth by 86.73% and increased the rice yield by 81.03%. These findings support the fact that the residues of BP and BPr have been successfully used as natural herbicides in the weed management of paddy fields and provided additional benefits to rice yields. In this study, the allelopathic effects of BPr in the upland agricultural system were studied in the open fields. Given the results of field experiments, with the contribution of phytotoxicity of allelochemicals, BPr exerted great competitiveness on the noxious weed of CR in the field. B-Pn and B-Py treatments attained 87% inhibition of CR plant density as compared to the results of C-Pn and C-Py treatments.

Additionally, some research further showed the benefits of preventive weed control measures by introducing allelopathic species into the crop rotation. Scavo et al. [59] indicated that the field rotated with an allelopathic crop of Cynara cardunculus L. for three years showed a significant decrease (34–50%) in the amount of soil weed seeds when compared to the traditional wheat/faba bean rotation. In the present study, the results showed that soils in a three-year-old BPr vegetation exerted strong allelopathic effects on CR tuber sprouting, especially in VS treatment.

In addition to an invasive weed, BP is also an edible and medicinal herb in many countries and has been extensively investigated for the potential of pharmacological use [23]. The good agricultural practice of BP was established because of its frequent pharmacology application in Taiwan [60]. Hence, this species should not only be regarded as an invasive weed but also a medicinal crop. However, for further introducing BPr into the crop rotation systems or using its residue as allelopathic mulch in weed managements, its allelopathic effects on the subsequent or the standing crops require additional consideration. Ng et al. [56] reported that some legume species such as Phaseolus radiatus L. Mimosa pudica L. and Sesbania cannabina (Retz.) Poir. were observed to be more resistant to BPr invasion than Ipomoea aquatica Forssk. and Zea mays L. Wang et al. [26] indicated that the forage legume, Vicia villosa Roth, exhibited a greater competitive ability against BP than M. sativa and T. repens in the competition experiment. Although Tembo et al. [61] indicated that BP extracts exerted little benefit for pest control in three legume crops, no growth or yield inhibition was observed. These findings indicated that the inhibitory effects of BPr should be species-dependent, and some legumes might be the proper candidate species in the crop rotation with BP. Our results of experiment 3 also indicated that the phytotoxicity decreased obviously after the aboveground plant materials were removed for two weeks. Therefore, it was confirmed (1) the field allelopathic potential of BPr in monoculture for medicinal herb cultivation, regardless of its growth in a field severely invaded by CR and (2) the possibility of introducing BPr within an upland crop rotation for the weed control and reduction in herbicide utilization. However, the applications of BPr as a rotation crop and/or allelopathic mulch in the agricultural practices deserve another future study.

4. Materials and Methods

4.1 Plant Material Preparation

Seeds and plant residues (including leaves, stem, flower and seeds) of BPr were collected from the plants grown in the experimental field of Taitung District of Agriculture Research and Extension Station (Taitung DARES, located at 22°4′52″ N, 121°8′59″ E). Seeds were stored in a paper bag at room temperature before use. The plant residues of the bloom stage used were harvested and dried at ambient temperature for 15 days. The dried residues were crushed into small pieces (<2 cm) with an electric cutter, and stored at −20°C before use. Tubers of CR were collected from the experimental field of Taitung DARES. Tubers with a diameter of 0.5–1.0 cm were chosen and washed for use on the same day when the experiment started.

4.2 General Experimental Design

The present study consisted of four experiments. Experiment 1 aimed to distinguish the BPr residue allelopathy from the intraspecies competition of CR by evaluating the density-dependent
phytotoxicity. Experiment 2 was designed to explore the presence of allelopathy by adding activated carbon and the competitiveness of the two investigated species. In experiment 3, the CR tubers were sowed in a three-year-old BPr field with or without removing the aboveground plant materials. The results of experiment 3 helped to recognize if the phytotoxicity of BPr changed after mowing. Experiment 4 assessed the effects of using BPr as a cover plant and mulch on the reproduction of CR.

The first two experiments were performed in semi-natural conditions to provide evidence of the presence of allelopathy of BPr. The last two experiments conducted in the field helped to assess the potential of using BPr to control CR in the field. The results from the four experiments were compared to show the difference in observation between greenhouse and field experiments.

4.3 Experiment 1: Density-Dependent Phytotoxicity

Soil collected from the vegetable farmland invaded severely by CR in Taitung DARES was air-dried, sieved through 2-mm mesh to remove large plant debris. Soils of 1.2 kg mixed thoroughly with four application rates of BPr residues (0, 1.4, 2.8 and 4.2 g pot\(^{-1}\)) were placed in a 13.5 cm diameter plastic pot. The levels of 0, 1.4, 2.8 and 4.2 g pot\(^{-1}\) were equivalent to 0, 0.1, 0.2 and 0.3 kg m\(^{-2}\), respectively. Tubers of CR were pre-sprouted before the experiments. The densities of 3, 6 and 9 sprouted tubers were planted for low-, medium- and high-density treatments, respectively. Experiment 1 was carried out in a greenhouse of Taitung DARES with two factors complete random design (CRD). Each combination (residue application rate × plant density) was repeated four times. All pots were watered as needed but no fertilizer was added during the experiment. After four weeks, plants were harvested, divided into shoot and root, and dried for 48 h at 80°C. The dry weight of shoot and root, as well as the numbers of tuber and tiller were determined.

4.4 Experiment 2: Interspecies Competition between B. pilosa var. radiata and C. rotundus

The method used in the interspecies competition was modified from the experimental protocols proposed by Snaydon [62]. A plastic pot (13.5 cm in diameter and 13 cm in depth) was equally subdivided by a plastic plate to serve as the belowground partition. The plastic plate was sealed with neutral silicone gel to the sides and base of the pot. Another plastic plate of 30 × 30 cm fixed vertically on the upper edge of the pot to serve as the aboveground partition. The relative arrangement of aboveground and belowground partition coupled with the disposition of BPr and CR were designed to compare various competitions, i.e., NO, SHOOT, ROOT and FULL competitions (Figure 7).

![Figure 7](image_url)

**Figure 7.** The design of the interspecies competition experiment. The relative arrangement of aboveground and belowground partition and the disposition of two plants were designed to give four modes of competition (i.e., NO, SHOOT, ROOT and FULL competitions).
For each competition, a 1.2 kg soil mixed thoroughly with (AC treatment) or without (N treatment) fine powdered activated carbon (pure grade) at the ratio of 50 to 1 was placed evenly in the two subdivisions of each pot. All pots were sprayed with R.O. water (50 mL per pot) per day and watered as needed but no fertilizer was added during the experiment. Experiment 2 was also conducted in the greenhouse of Taitung DARES with two factors complete random design (CRD). Each combination (competition × activated carbon addition) was repeated five times.

4.5 Experiment 3: The Tuber Sprouting of CR in the Field of Mature BPr Vegetation

To prevent the interference of the invaded CR, the field tuber sprouting experiment was carried out in a strip of three-year-old vegetation of BPr (24 m in length and 1.5 m in width) and its adjacent fallow field in Taitung DARES in January of 2020. The strip of BPr had been inspected before the experiment to assure no invasion of CR. Eight 30 cm × 30 cm quadrats were set randomly in the BPr strip. Half of the quadrats was selected to remove the aboveground plant shoots and litter to make the surface bare (VN treatment). Another half were left for shade (VS treatment). Four 1 m × 1 m plots were randomly selected in the adjacent bare ground (approximately 3 m far from the BPr strip). Weeds included tubers of CR in the plots were carefully removed by hand weeding. For each plot, one 30 cm × 30 cm quadrat was set in the center and mulched with an opaque plastic sheet (OP treatment). As a reference treatment with common weed management practice, the OP treatment was conducted to compare the tuber sprouting with VN and VS treatments. Twenty-five tubers (0.5–1.0 cm in diameter) of CR were sowed in each quadrat. Sprouted tubers, sprouts per quadrat and dry weight per sprout were explored two weeks after sowing.

4.6 Experiment 4: The Effects of Vegetation and Residue Mulch of BPr on the Reproduction of CR

In the winter of 2019, a field seriously invaded by CR was chosen for the present study and divided into 24 plots (3 m in length and 1 m in width). Each plot was separated by a 0.8 m wide ditch. On October 17, 2019, three soil cores (2 inches in diameter and 15 cm in depth) per plot were sampled to investigate the numbers and dry weight (dried for 48 hr at 80 °C) of CR tubers in the upper 15 cm of soil. All the plots were mowed and the seeds of BPr (8 g polt⁻¹) were sowed randomly in half of the plots on the following day (October 18, 2019). CR was allowed to grow in another twelve plots without sowing BPr. On December 26, 2019 (69 DAS), all the aboveground plant parts were mowed, the plant residues were weighted and left on the plot. After mowing, three soil cores per plot were sampled again for investigating the numbers and dry weight of CR tubers in the upper 15 cm of soil. Half plots of BPr (B-Py) and CR (C-Py) were mulched with an opaque plastic sheet while another half plots of BPr (B-Pn) and CR (C-Pn) were not mulched. The tiller numbers of CR of the aforementioned four treatments (B-Py, C-Py, B-Pn and C-Pn) were counted two weeks after mowing. Experiment 4 was conducted in two factors complete random design (CRD) with 6 replications for each combination (plant species × opaque plastic sheet mulch).

4.7 Statistical Analysis

Levene test was used to test for homogeneity of variance. The experimental data were subjected to analyses of variances (ANOVA) and Fisher’s LSD post-hoc test by the SAS software (SAS Enterprise Guide 7.1, SAS Institute Inc., Cary, NC, USA) except for experiment 3. The regression analyses were carried out with Sigmaplot software (Ver. 12.5, Systat Software Inc., San Jose, CA, USA). Prior to ANOVA, the percentage data were arcsine-square-root transformed; the data of tuber number and, tiller number and tuber density were square-root transformed; and the data of shoot to root ratio were log-transformed [63]. In experiment 3, the difference of tuber sprouting percentage, sprouts per quadrat and dry weight per sprout among quadrats were analyzed by Kruskal-Wallis non-parametric rank test and Dunn’s post-hoc comparison test (IBM SPSS Statistics V25, IBM Corp., Armonk, NY, USA).
5. Conclusions

In the present study, BPr exhibited less aggressive competitiveness than CR and did not affect the CR tuber proliferation in the pots. Different from the results of the pot experiment, both the BPr residues and soils exhibited phytotoxicity and reduced the CR reproduction in the field. The difference between the results of the pot and field experiments demonstrated that the pot volume might restrict the growth of BPr and reduce its effects of allelopathy and competitiveness. However, by combining the results of pot and field experiments, the present study revealed that BPr should have the potential for controlling CR through its allelopathy in the field.

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References

1. Peerzada, A.M. Biology, agricultural impact, and management of Cyperus rotundus L.: The world’s most tenacious weed. Acta Physiol. Plant. 2017, 39, 270.
2. Bendixen, L.E.; Nandihalli, U.B. Worldwide distribution of purple and yellow nutsedge (Cyperus rotundus and C. esculentus). Weed Technol. 1987, 1, 61–65.
3. Baloch, A.H.; Rehman, H.; Ibrahim, Z.; Buzdar, M.A.; Ahmad, S. The biology of Balochistani weed: Cyperus rotundus Linnaeus. A review. Pure Appl. Biol. 2015, 4, 171–180.
4. Nishimoto, R.K. Purple nutsedge tuber sprouting. Weed Biol. Manag. 2001, 1, 203–208.
5. Bangarwa, S.K.; Norsworthy, J.K.; Jha, P.; Malik, M. Purple nutsedge (Cyperus rotundus) management in an organic production system. Weed Sci. 2008, 56, 606–613.
6. William, R.D.; Warren, G.F. Competition between purple nutsedge and vegetables. Weed Sci. 1975, 23, 317–323.
7. Morales-Payan, J.P.; Santos, B.M.; Stall, W.M.; Bewick, T.A. Effects of purple nutsedge (Cyperus rotundus) on tomato (Lycopersicon esculentum) and bell pepper (Capsicum annuum) vegetative growth and fruit yield. Weed Technol. 1997, 11, 672–676.
8. Geretharan, T.; Sangakkara, U.R.; Arulnandhry, V. Competitive performance of purple nutsedge (Cyperus rotundus L.) and onion (Allium cepa L.) as affected by different sources of nitrogen. Trop. Agric. Res. 2012, 23, 290–299.
9. Cirujeda, A.; Anzalone, A.; Aibar, J.; Moreno, M.M.; Zaragoza, C. Purple nutsedge (Cyperus rotundus L.) control with paper mulch in processing tomato. Crop. Prot. 2012, 39, 66–71.
10. Inderjit; del Moral, R. Is separating resource competition from allelopathy realistic? Bot. Rev. 1997, 63, 221–230.
11. Duke, S.O. Proving allelopathy in crop–weed interactions. Weed Sci. 2015, 63, 121–132.
12. Singh, H.P.; Batish, D.R.; Kohli, R.K. Allelopathic interactions and allelochemicals: New possibilities for sustainable weed management. Crit. Rev. Plant Sci. 2003, 22, 259–311.
13. Scavo, A.; Abbate, C.; Mauromicale, G. Plant allelochemicals: Agronomic, nutritional and ecological relevance in the soil system. Plant Soil 2019, 442, 23–48.
14. Viji, N.; Chinnamuthu, C.R. Breaking dormancy and inducing germination of the world worst weed the Cyperus rotundus using nanoparticles. Ann. Plant Soil Res. 2015, 17, 361–363.
15. Viji, N.; Chinnamuthu, C.R. Nanoparticle effect on degradation of vanillic acid, a germination inhibiting dormancy factor present in Cyperus rotundus. Indian J. Weed Sci. 2019, 51, 98–100.
16. Babu, R.C.; Kandasamy, O.S. Allelopathic effect of Eucalyptus globulus Labill. on Cyperus rotundus L. and Cynodon dactylon L. Pers. J. Agron. Crop Sci. 1997, 179, 123–126.
17. Cheema, Z.A.; Khaliq, A.; Saeed, S. Weed control in maize (*Zea mays* L.) through sorghum allelopathy. *J. Sustain. Agric.* 2004, 23, 73–86.

18. Iqbal, J.; Cheema, Z.A.; An, M. Intercropping of field crops in cotton for the management of purple nutseed (*Cyperus rotundus*) (*L*.). *Plant Soil* 2007, 300, 163–171.

19. Campbell, G.; Lambert, J.D.H.; Arnason, T.; Towers, G.H.N. Allelopathic properties of α-terthienyl and phenylethanthiophene, naturally occurring compounds from species of Asteraceae. *J. Chem. Ecol.* 1982, 8, 961–972.

20. Deba, F.; Xuan, T.D.; Yasuda, M.; Tawata, S. Herbicidal and fungicidal activities and identification of potential phytotoxins from *Bidens pilosa* var. *radiata* Scherr. *Weed Biol. Manag.* 2007, 7, 77–83.

21. Hsu, H.M.; Kao, W.Y. Contrasting effects of aqueous tissue extracts from an invasive plant, *Bidens pilosa* var. *radiata*, on the performance of its sympatric plant species. *Taiwania* 2009, 54, 255–260.

22. Zhang, K.; Shen, Y.; Fang, Y.M.; Liu, Y. Changes in gametophyte physiology of *Pteris multifida* induced by the leaf leachate treatment of the invasive *Bidens pilosa*. *Environ. Sci. Pollut. Res.* 2016, 23, 3578–3585.

23. Xuan, T.D.; Khanh, T.D. Chemistry and pharmacology of *Bidens pilosa*: An overview. *J. Pharm. Investig.* 2016, 46, 91–132.

24. Poonaiboonpipat, T.; Poolkum, S. Utilization of *Bidens pilosa* var. *radiata* (Sch. Bip.) Scherr integrated with water irrigation for paddy weed control and rice yield production. *Weed Biol. Manag.* 2019, 19, 31–38.

25. Galon, L.; Concenço, G.; Perin, G.F.; da Silva, A.F.; Forte, C.T.; David, F.A.; Radúz, L.L.; Radunz, A.L.; Andres, A.; Tironi, S.P. Comparison of experimental methods to assess the competitive ability of weed species. *Am. J. Plant Sci.* 2015, 6, 2185–2196.

26. Wang, R.; Feng, Z.; Liang, X.; Xu, W.; Su, Y.; Song, Y.; Zeng, R. Comparative allelopathic and competitive abilities of 3-native forage legumes and the invasive weed *Bidens pilosa* L. *Allelopathy* J. 2012, 29, 297–306.

27. Weidenhamer, J.D. Distinguishing allelopathy from resource competition: The role of density. In *Allelopathy: A Physiological Process with Ecological Implications*; Reigosa, M.J., Pedrol, N., Gonzalez, L. Eds.; Springer: Dordrecht, The Netherlands, 2006; pp. 85–103.

28. Weidenhamer, J.D.; Hartnett, D.C.; Romeo, J.T. Density-dependent phytotoxicity: Distinguishing resource competition and allelopathic interference in plants. *J. Appl. Ecol.* 1989, 26, 613–624.

29. Blum, D.J.W.; Suffet, I.H.; Duguet, J.P. Estimating the activated carbon adsorption of organic chemicals in water. *Crit. Rev. Environ. Sci. Technol.* 1993, 23, 121–136.

30. Nilsson, M.C. Separation of allelopathy and resource competition by the boreal dwarf shrub *Empetrum hermaphroditum* Hagerup. *Oecologia* 1994, 98, 1–7.

31. Mahall, B.E.; Callaway, R.M. Root communication mechanisms and intracommunity distributions of two Mojave Desert shrubs. *Ecology* 1992, 73, 2145–2151.

32. Khaliq, A.; Matloob, A.; Irshad, M.S.; Tanveer, A.; Zamir, M.S.I. Organic weed management in maize (*Zea mays* L.) through integration of allelopathic crop residues. *Pak. J. Weed Sci. Res.* 2010, 16, 409–420.

33. Matloob, A.; Khaliq, A.; Farooq, M.; Cheema, Z.A. Quantification of allelopathic potential of different crop residues for the purple nutseed suppression. *Pak. J. Weed Sci. Res.* 2010, 16, 1–12.

34. Fuerst, E.P.; Putnam, A.R. Separating the competitive and allelopathic components of interference. *J. Chem. Ecol.* 1983, 9, 937–944.

35. Batish, D.R.; Lavanya, K.; Singh, H.P.; Kohli, R.K. Phenolic allelochemicals released by *Chenopodium murale* affect the growth, nodulation and macromolecule content in chickpea and pea. *Plant Growth Regul.* 2007, 51, 119–128.

36. Weiner, J. Asymmetric competition in plant populations. *Trends Ecol. Evol.* 1990, 5, 360–364.

37. Tuor, F.A.; Froud-Williams, R.J. Influence of nitrogen on competition between purple nutseed, maize and soybean. *Int. J. Pest Manag.* 2002, 48, 73–79.

38. Horowitz, M. Competitive effects of three perennial weeds, *Cynodon dactylon* (L.) Pers., *Cyperus rotundus* L. and *Sorghum halepense* (L.) Pers., on young citrus. *J. Hortic. Sci.* 1973, 48, 135–147.

39. Kobe, R.K.; Iyer, M.; Walters, M.B. Optimal partitioning theory revisited: Nonstructural carbohydrates dominate root mass responses to nitrogen. *Ecology* 2010, 91, 166–179.

40. Williams, R.D.; Quinby, P.C.; Frick, K.E. Intraspecific competition of purple nutseed (*Cyperus rotundus*) under greenhouse conditions. *Weed Sci.* 1977, 25, 477–481.

41. Bergmark, C.L.; Jackson, W.A.; Volk, R.J.; Blum, U. Differential inhibition by ferulic acid of nitrate and ammonium uptake in *Zea mays* L. *Plant Physiol.* 1992, 98, 639–645.

42. Schenk, H.J. Root competition: Beyond resource depletion. *J. Ecol.* 2006, 94, 725–739.
43. El-Rokiek, K.G.; El-Masry, R.R.; Messiha, N.K.; Ahmed, S.A. The allelopathic effect of mango leaves on the growth and propagative capacity of purple nutsedge (*Cyperus rotundus* L.). *J. Am. Sci.* 2010, 6, 151–159.
44. Einhellig, F.A. Mechanism of action of allelochemicals in allelopathy. In *Allelopathy, Organisms, Processes and Applications*, Inderjit, Dakshini, K.M.M., Einhellig, F.A. Eds.; American chemical society: ACS Symposium Series, VOLUME 520, Washington, DC, USA, 1995, pp. 96–116.
45. Abenavoli, M.R.; Lupini, A.; Oliva, S.; Sorgonà, A. Allelochemical effects on net nitrate uptake and plasma membrane H^+ -ATPase activity in maize seedlings. *Biol. Plant.* 2010, 54, 149–153.
46. Blum, U. Effects of microbial utilization of phenolic acids and their phenolic acid breakdown products on allelopathic interactions. *J. Chem. Ecol.* 1998, 24, 685–708.
47. Blum, U. Allelopathy: A soil system perspective. In *Allelopathy: A Physiological Process with Ecological Implications*; Reigosa, M.J., Pedrol, N., Gonzalez, L., Eds.; Springer: Dordrecht, The Netherlands, 2006; pp. 299–340.
48. Schmidt, S.; Ley, R.E. Microbial competition and soil structure limit the expression of allelochemicals in nature. In *Principles and Practices in Plant Ecology: Allelochemical Interactions*; Inderjit, Dakshini, K.M.M., Chester, L.F., Eds.; CRC Press: Boca Raton, FL, USA. 1994; pp. 339–351.
49. Mahmood, A.R.I.F.; Cheema, Z.A. Influence of sorghum mulch on purple nutsedge (*Cyperus rotundus* L.). *Int. J. Agric. Biol.* 2004, 6, 86–88.
50. Boz, O. Allelopathic effects of wheat and rye straw on some weeds and crops. *Asian J. Plant Sci.* 2003, 2, 772–778.
51. Patterson, D.T. Suppression of purple nutsedge (*Cyperus rotundus*) with polyethylene film mulch. *Weed Technol.* 1998, 12, 275–280.
52. Wang, G.; McGiffen Jr, M.E.; Ogbuchiekw, E.J. Crop rotation effects on *Cyperus rotundus* and *C. esculentus* population dynamics in southern California vegetable production. *Weed Res.* 2008, 48, 420–428.
53. Politycka, B.; Adamska, D. Release of phenolic compounds from apple residues decomposing in soil and the influence of temperature on their degradation. *Pol. J. Environ. Stud.* 2003, 12, 95–98.
54. Stevens, K.L., Polyacetylenes as allelochemicals. In *The Science of Allelopathy*; Putnam, A.R., Tang, C.S., Eds.; John Wiley & Sons: New York, NY, USA, 1986; pp. 219–228.
55. Xuan, T.D.; Anh, L.H.; Khang, D.T.; Tuyen, P.T.; Minh, T.N.; Khanh, T.D.; Trung, K.H. Weed allelochemicals and possibility for pest management. *Int. Lett. Nat. Sci.* 2016, 56, 25–39.
56. Ng, C.C.; Wu, S.J.; Wang, C.Y.; Tzeng, W.S.; Shyu, Y.T. Emergence and growth of beggarticks (*Bidens pilosa* var. *radiata*) in different plant communities under experimental field conditions. *J. Agric. Technol.* 2011, 1, 950–962.
57. Hong, N.H.; Xuan, T.D.; Eiji, T.; Khanh, T.D. Paddy weed control by higher plants from Southeast Asia. *Crop Prot.* 2004, 23, 255–261.
58. Krumski, R.; Suwunnamek, U.; Homhaul, W.; Laosinwattana, C.; Poonpaiboonpipattana, T. Allelopathic effects of *Bidens pilosa* var. *radiata* and its preliminary utilization to control weeds in rice. *J. Agric. Technol.* 2015, 11, 1875–1886.
59. Scavo, A.; Restuccia, A.; Abbate, C.; Mauromicale, G. Seeming field allelopathic activity of *Cynara cardunculus* L. reduces the soil weed seed bank. *Agron. Sustain. Dev.* 2019, 39, 41.
60. Hsiao, C.L. Safe production techniques of *Bidens pilosa* for high content of functional compounds. *Stock-Farming Ten Days* 2017, 1908, 43–44.
61. Tembo, Y.; Mkindi, A.G.; Mkenda, P.A.; Mpumi, N.; Mwanauta, R.; Stevenson, P.C.; Ndakidemi, P.A.; Belmain, S.R. Pesticidal plant extracts improve yield and reduce insect pests on legume crops without harming beneficial arthropods. *Front. Plant Sci.* 2018, 9, 1425.
62. Snaydon, R.W. An analysis of competition between plants of *Trifolium repens* L. populations collected from contrasting soils. *J. Appl. Ecol.* 1971, 8, 687–697.
63. Shen, M.L. *Experimental Designs*; Jeou Chou Book Co, Ltd.: Taipei, Taiwan, 2010.

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