## Research -



# Fragment Size and Planting Depth Affect the Regenerative Capacity of Bushkiller (*Cayratia japonica*)

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Bushkiller (*Cayratia japonica*) is a herbaceous, perennial vine that reproduces from seed and vegetative root structures within its native range. However, this species is considered invasive in the United States due to prolific shoot production, which can overtop surrounding vegetation. Cultural control practices, such as mulching, have been observed to further the spread of this species through regeneration of root fragments. Research was conducted to determine the regenerative capacity of bushkiller root fragments (1 to 7 cm; 2.5 to 18 inches) buried at various depths (0 to 40 cm). Root length and planting depth affected leaf number, shoot number, plant height, and shoot biomass. Bushkiller leaf density, shoot density, plant height, and dry weight increased as root fragment length increased from 1 to 7 cm; conversely, these variables decreased as planting depth increased from 0 to 40 cm. Results indicate bushkiller regeneration capacity from root fragments is extremely high and control practices that fragment roots should be avoided to reduce further spread. **Nomenclature:** Bushkiller, *Cayratia japonica* (Thunb.) Gagnep.

Key words: Exotic plant, invasive species, vegetative reproduction.

Bushkiller [Cayratia japonica (Thunb.) Gagnep.] is a perennial herbaceous liana in the Vitaceae family with a twining, climbing growth habit. Bushkiller is native to temperate, subtropical, and tropical forests in Southeast Asia, Australia, India, Japan, Malaysia, and Taiwan where it reproduces through viable seed and vegetative root structures (Hsu and Kuoh 1999). Bushkiller was first reported in Texas in 1964 and has since been documented in Alabama, Louisiana, Mississippi, and North Carolina, where only vegetative reproduction has been observed (Brown 1992; Krings and Richardson 2006; Soule et al. 2008). Outside of its native range bushkiller is considered highly invasive due to its aggressive growth habit capable of overtopping surrounding vegetation, creating monocultures and limiting biodiversity (West et al. 2010). The invasive habit of bushkiller has been compared to that of kudzu [Pueraria montana (Lour.) Merr. var. lobata (Willd.) Maesen & S. Almeida], which is estimated to accrue \$500 million per year in forestry production loss (Forseth and Innis 2004).

\*Former Graduate Research Assistant, Graduate Research Assistant, and Associate Professor, respectively, North Carolina State University, Raleigh, NC 27695. Corresponding author's E-mail: rob\_richardson@ncsu.edu Recently, the North Carolina Department of Agriculture listed bushkiller as a Class B noxious weed (NCAC 2009).

To date, there is limited research on bushkiller control options. Bushkiller is difficult to control because shoots emerge prolifically from its extensive rooting network (Figure 1) (West et al. 2011). Bushkiller roots are creeping at the juvenile stage and develop elongated rhizomes as the plant matures (Ohwi et al. 1984). Because of the clonal growth habit of bushkiller, it is difficult to determine the spatial distribution and number of individual plants within a given area (Kakutani et al. 1989). Research has indicated selective control with herbicides may require multiple-season applications to reduce established bushkiller populations (West et al. 2011). Cultural control practices, including cultivation and tillage, have been observed to further the spread of bushkiller through regeneration from root fragments. However, past research examining the regenerative capacity of similar plant species from root fragments has been a useful predictor of its relative persistence and invasive potential, as well as elucidating potential control measures (Edwards and Oliver 2004; Hamdoun 1972; Raju et al 1964; Richardson 1975). Therefore, research was conducted to determine bushkiller regenerative capacity from various root fragment lengths when buried at increasing depths.

DOI: 10.1614/IPSM-D-12-00007.1

### **Management Implications**

Bushkiller is an invasive, perennial vine that grows rapidly and overtops surrounding vegetation. It has invaded several states in the Southern United States and several recently reported infestations have elevated concern about this species. Research was conducted to determine the regenerative capacity of bushkiller roots. Bushkiller roots just 2.8 inches in length produced over 1 shoot around 10 inches in length with approximately 13 leaves by just 34 days after planting. Even though smaller roots produced fewer and shorter shoots, vegetative reproduction was observed down to the smallest root fragment evaluated, 0.4 inches in length. Root fragments were also able to reproduce when planted as deep as 16 inches. Based upon this data, established bushkiller would be extremely tolerant of single disturbance events including cultivation, mowing, mechanical removal of shoots, grazing, and application of non-systemic herbicides. Further, disturbance that results in root fragmentation may increase stem density. Our results also suggest that fragmented roots would readily form new infestations when moved as soil contact is maintained.

#### **Materials and Methods**

Research was conducted on January 25, 2008, and February 29, 2009, at North Carolina State University in Raleigh, NC. Bushkiller root segments were collected from a residential population (Winston Salem, NC) and transplanted into commercial potting media (Metro MixH 200, Sun Gro Horticulture, Bellevue, WA) and grown until maturity. Mature plants were harvested for root fragments of 1-, 3-, 5-, and 7-cm (0.4, 1, 2, and 2.8 in) lengths (approximately 1 cm diam) and planted at depths of 0, 1, 3, 5, 7, 10, 15, 20, and 40 cm in 940-cm<sup>3</sup> (57 in<sup>3</sup>) pots. Supplemental lighting was provided to maintain a constant 12-h photoperiod. Pots were watered once daily and fertilized once weekly with a water-soluble fertilizer (Miracle-GroH water-soluble lawn food 36-6-6, The Scotts Company, Marysville, OH). Bushkiller shoot and leaf density (per 23.5 cm<sup>2</sup> surface area of pot) and plant height were recorded from 7 d after planting (DAP) to 34 DAP. Aboveground biomass was harvested 34 DAP and oven-dried at 50 C (122 F) for 72 h.



а 2.0 1.8 1.6 Shoot density (23.5 cm<sup>2</sup>) 1.4 Leaf density (23.5 cm<sup>2</sup>) 10 1.2 1.0 Shoot density P<0.01; r<sup>2</sup>= 0.98 Leafdensity 0.8 P<0.01: r= 0.98 0.6 0.4 0.2 0.0 2 3 6 Bushkiller root fragment length (cm) b 35 1.6 14 30 25 Plant height (cm) 1.0 6 20 Dry weight 0.8 Plant h eight P<0.01; r<sup>2</sup>= 0.99 15 • 0.6 Dry weight P<0.01; r<sup>2</sup>= 0.98 . 10 0.4 5 0.2 0 0.0 2 3 5 4 6 7 Bushkiller root fragment length (cm)

Figure 2. (a) Bushkiller shoot density and leaf density means vs. root fragment length 34 d after planting (DAP) plotted using the nonlinear equation  $f = a/(1 + exp(-(x-x_0)/b))$ . (b) Bushkiller plant height and dry weight means vs. root fragment length 34 DAP plotted using the nonlinear equation  $f = a/(1 + exp(-(x-x_0)/b))$ .

Experimental design was a randomized complete block with three replications in a factorial arrangement (four root fragment lengths by nine planting depths). ANOVA was conducted using mixed model methodology (SAS 2008). Root fragment length and planting depth were considered fixed variables in the model and experimental run, replication, and their interaction were considered random effects. Root fragment length by planting depth was evaluated to determine if there was an interaction. Means were subject to nonlinear regression analysis to determine the effect of root fragment length and planting depth on bushkiller leaf density, shoot density, plant height, and dry weight.

#### **Results and Discussion**

Figure 1. Bushkiller regeneration from a 5-cm length of root fragment.

Statistical analysis revealed no root fragment length by planting depth interaction (P > 0.05) for leaf number,



Figure 3. (a) Bushkiller shoot density and leaf density means vs. planting depth 34 d after planting (DAP) plotted using the nonlinear equation  $f = a/(1 + exp(-(x-x_0)/b))$ . (b) Bushkiller plant height and dry weight means vs. planting depth 34 DAP plotted using the nonlinear equation  $f = a/(1 + exp(-(x-x_0)/b))$ .

shoot number, plant height, or dry weight; therefore, the main effects (P < 0.05) will be discussed.

When measurements were taken 34 DAP, the main effect of root fragment length caused an increase in bushkiller shoot density, leaf density, plant height, and dry weight as the root fragment lengths increased. Shoot density was greatest for 7-cm root fragment (1.7 shoots) but decreased to 1.5 shoots for 5- and 3-cm root fragments and 0.7 shoots for 1-cm root fragments (Figure 2a). Leaf density followed a similar pattern, with 13.5 leaves from 7cm root fragments; 11 and 9.2 leaves from 5- and 3-cm root fragments, respectively; and 2.7 leaves from 1-cm root fragments (Figure 2a). Bushkiller plant height increased from 5.5 cm to 27.9 cm from 1- to 7-cm root fragments (Figure 2b). Dry weight mass was representative of the increasing plant growth from longer root fragments, ranging from 0.2 g (0.007 oz) from 1-cm fragments to 1.3 g from 7-cm fragments (Figure 2b).

The planting depth main effect resulted in a negative response to bushkiller leaf density, shoot density, plant height, and dry weight, which occurred as planting depth increased when measured 34 DAP (Figures 3a and 3b). Shoot density was greatest for planting depths of 0 to 20 cm, ranging from 1.3 to 1.5 shoots (Figure 3a). Leaf density decreased as planting depth increased, ranging from 11.8 leaves at the 0-cm planting depth to 2.8 leaves at the 40-cm planting depth (Figure 3a). Bushkiller plant height followed a similar pattern, with decreased plant height at greater planting depths, ranging from the greatest plant height of 29.6 cm at the 0-cm planting depth to 4.1 cm at the 40-cm planting depth (Figure 3b). Dry weight mass also decreased as planting depth increased, ranging from the greatest dry weight of 1.3 g at the 0-cm planting depth to the least dry weight of 0.1 g at the 40-cm planting depth (Figure 3b). Similarly, Edwards and Oliver (2004) determined that trumpetcreeper [Campsis radicans (L.) Seem. ex Bureau] was not significantly influenced by depth of planting.

When compared for the duration of the study, bushkiller root fragments of 5 and 7 cm produced greater plant heights than 1- and 3-cm root fragments 11 to 34 DAP (Figure 4a). Root fragments of 3 to 7 cm showed a greater growth response than 1-cm fragments. Edwards and Oliver (2004) reported similar results with *Campsis radicans*, in which biomass production was positively influenced by root fragment length. However, the response of bushkiller differs from Canada thistle [*Cirsium arvense* (L.) Scop.], which produced greater shoots per unit length of root fragment with smaller root fragments (Hamdoun 1972). These differing responses to root fragmentation length are likely due to the contrasting growth habits of bushkiller and *Cirsium arvense*.

Similarly, shallower bushkiller planting depths had greater plant heights when measured throughout the course of the study (Figure 4b). Planting depths of 0 to 20 cm had greater plant heights compared to the 40-cm planting depth 17 to 34 DAP. Although regeneration was less at greater planting depths, bushkiller was capable of emerging from the deepest planting depth (40 cm), indicating deep tillage or plant burial may not be a successful control option. Because 1-cm bushkiller root fragments were capable of plant regeneration and a 40-cm burial depth did not hinder emergence, control measures that cause root zone fragmentation and burial will likely not reduce bushkiller populations. Other research has indicated the invasive species leafy spurge (Euphorbia esula L.) was capable of regenerating from root fragments buried 2 to 8 m (Raju et al. 1964); therefore, future research should be conducted to determine the maximum planting depth at which bushkiller can no longer regenerate. Based on these results, bushkiller control measures should not include practices that may lead to root fragmentation and instead



Figure 4. (a) Bushkiller plant height means affected by root fragment length from 7 to 34 d after planting (DAP) plotted using the nonlinear equation  $f = y_0 + a \times x + b \times x^2$ . (b) Bushkiller plant height means affected by planting depth from 7 to 34 DAP plotted using the nonlinear equation  $f = y_0 + a \times x + b \times x^2$ .

should focus on carbohydrate depletion by nondisturbance techniques.

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Received January 13, 2012, and approved June 9, 2012.