Effects of Age, Length, and Pattern of Burial on Survival of *Mikania micrantha* Stem Sections

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Effects of Age, Length, and Pattern of Burial on Survival of *Mikania micrantha* Stem Sections[1]

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Abstract: For many landholders in the South Pacific, weed control of *Mikania micrantha* Kunth is conducted by manual or mechanical means, leaving fragments on or below the ground to reshoot and grow. Effects of age, length (number of nodes), and pattern of burial on the survival of stem sections of *M. micrantha* were examined in the field in Viti Levu, Fiji. The experiment was arranged in a randomized factorial design, with number of nodes, age of stem sections, and pattern (depth and orientation) of stem burial as factors. Stem sections with two or three nodes had significantly greater survival (30% and 25%, respectively) than those with one node (12%). Mature stem sections had a significantly greater survival rate (31%) than young stem sections (13%) when buried in either the horizontal or the vertical position. Vertical plantings had significantly greater survival (43%) than horizontal plantings (10%), and for both orientations survival decreased with depth of burial. Only 8% of stem sections survived when cut into smaller (3 to 5 cm) sections and buried at a depth of 10 cm. This study revealed that cutting the *M. micrantha* stems into smaller sections (<3 cm) and burying them at depths of 10 cm or greater would improve the overall management of *M. micrantha* in crop and noncrop systems.

Stoloniferous plants including perennial Asteraceae species such as Singapore daisy [*Sphagneticola trilobata* (L.) Pruski], Crofton weed [*Ageratina adenophora* (Spreng.) King & H. Rob.], and *Mikania micrantha* Kunth have the capacity to store important energy reserves, including proteins and carbohydrates, in their stolons (Corre et al. 1996, Alpert et al. 2003, He et al. 2011, Song et al. 2013). This capacity as a storage organ is very useful for those plant species that reproduce vegetatively (Stuefer and Huber 1999, Dong et al. 2010*b*) because such stored resources can either become available to the developing plantlet (ramet) or be retained in the internodes to facilitate survival and the production of new ramets (Suzuki and Stuefer 1999, Dong et al. 2012, Song et al. 2013). The capacity of a stoloniferous species to regenerate vegetatively increases when stem sections contain at least a single node (Stufer and Huber 1999, Dong et al. 2010*b*, Song et al. 2013). Many stoloniferous plants can readily produce roots and shoots at their nodes, with the survival rate of these stem sections being dependent upon their length (Dong et al. 2010*b*). For example, studies have demonstrated that stem sections with more than one node have a higher establishment and regeneration rate than single-nodal stem sections because they have a greater number of buds from which to shoot and can store more energy reserves (Truscott et al. 2006, Dong

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et al. 2010*b*, Dong et al. 2012, Lin et al. 2012, Song et al. 2013).

Physical or mechanical control methods of stoloniferous plants do not necessarily destroy all unwanted material, because tilling and/or slashing have the potential to slice and inadvertently spread and transplant vegetative material (Mann and Cavers 1979, Heap 1997, Johnson et al. 2003, Chicouene 2007). Residual stem sections of various lengths (ca. 2 to 30 cm) are often left on the surface or in the ground as green mulch (A.R.M. and M.D.D., pers. obs.), and many of these stem sections survive and reshoot to form new plants.

In addition, the various mechanical operations may affect stoloniferous weeds in different ways, thus influencing the weed's ability to reshoot. For example, a mold board plow may turn the soil over and in the process cut and bury some, but not all, plant material. A disk harrow may also slice the plants into smaller sections but bury the sections at different depths in the soil (Heap 1997, Johnson et al. 2003, Chicouene 2007).

Apart from the length of the stem section, there are other factors that may influence section survival after tilling and/or slashing. Mature stems have greater ability to store reserves than young stems. Young and mature stem sections of vegetatively propagated plants also perform differently depending on their orientation in the soil (Stuefer and Huber 1999). If sections are not in the normal upright orientation, the survival rate for some species decreases while for others it is enhanced (Mann and Cavers 1979).

Mikania micrantha is an aggressive perennial vine, native to tropical Central and South America (Holm et al. 1991, Ruas et al. 2000), that has invaded many ecosystems in Asia and the Pacific islands (Waterhouse and Norris 1987, Waterhouse 1994). *Mikania micrantha* can reproduce sexually, producing 94,500 viable seeds m^{-2} (Macanawai et al. 2011*a*), or through vegetative means (Waterhouse and Norris 1987).

Mikania micrantha was first reported in eastern Viti Levu, Fiji, in 1907, infesting sugarcane (*Saccharum officinarum* L.) and along roadsides (Knowles 1907). However, it is now widespread, affecting many other crops, including taro [*Colocasia esculenta* (L.) Schott] and cassava (*Manihot esculenta* Crantz) (Macanawai et al. 2010), two of the most economically important food crops in Fiji (Kumar et al. 1996, Lako and Nguyen 2001). The cost of controlling *M. micrantha* in taro and cassava production in Fiji was estimated to be US\$33 ha^{-1} or 16% of the total cost of controlling weeds (Macanawai et al. 2011*a*). In these crops, mainly physical or mechanical methods of weed management are practiced (Macanawai et al. 2011*b*). These methods may involve the use of a soil-tilling implement drawn by a tractor or an animal and/or hand cutting or slashing. However, such practices may also lead to an increased weed problem due to the ability of *M. micrantha* to shoot from stolons.

Because little is known of the effect of the placement or depth of stem section burial on *M. micrantha* reestablishment and regrowth in the field in Fiji, the aims of this study were to determine the survival rate and regrowth capacity of young and mature stem sections, with one to three nodes, and when placed in various burial orientations, mimicking the effects of precrop tilling and/or slashing. The purpose of this study is to provide insights into the vegetative propagation ability of *M. micrantha*, leading to a better understanding of its invasiveness and to identify better long-term management practices for this weed.

MATERIALS AND METHODS

Experimental Design

The experiment was arranged in a randomized three-way factorial design, with four young or four mature stem sections of *M. micrantha*, each having one (5 cm long), two (10 cm), or three (15 cm) nodes, respectively, as the first independent variable. The age of stem (mature or young) was considered as the second independent variable, and seven transplanting orientations were regarded as the third independent variable. Thus, stem sections were placed horizontally (H) at 0 (0H), 3 (3H), 6 (6H), or 10 (10H) cm deep; placed vertically upright with the whole stem section covered by soil (i.e., fully covered upright [FU]); placed vertically upright with only one node exposed (i.e., 5 cm above soil surface [VU]); or placed vertically but inverted, with only one node exposed (i.e., 5 cm above soil surface [VI]). The maximum depth tested to determine if *M. micrantha* could sprout was 10 cm. These treatments tested mimic those that might result from precrop tilling and/or plowing of fields. Therefore, this study had a three (number of nodes: one, two, or three) by two (age of stem: mature or young) by seven (pattern of stem burial: 0H, 3H, 6H, 10H, FU, VU, or VI) factorial design with three replications.

Stem Section Preparation

Healthy *M. micrantha* stem sections originating from the growing tips of stems (referred to as young), or from the base of stems (referred to as mature), were harvested from an area of ca. 20 m^2 infested with *M. micrantha* at the Koronivia Research Station (KRS), Nausori, Fiji. Ninety-eight stem sections, each with one node (5 cm), two nodes (10 cm), or three nodes (15 cm), for each of the young and mature stem sections were prepared, giving a total of 294 stem sections for each age group and 588 in total.

Of the 98 stem sections in each age and length class, 84 were used in the field experiments and 14 were used for an initial dry weight determination. Before experimentation, all leaves were removed from the mature stem sections, and all but the unopened leaves were removed from the young stem sections. The stem sections to be used in the field experiment were then wrapped in moist sterile tissue paper, placed in plastic bags and taken to the field, ca. 1 km away from the research station, for planting. The field experiment commenced on 19 May 2010 at the KRS farm, in a clay/loam soil (a typical soil used for crop production in Fiji) with a pH of 5.5. The 126 plots (two ages by three stem lengths by seven burial patterns by three replications) were prepared by removing the required volume of soil, depending on the depth of burial, from each plot, leaving a bed with a flat surface. The soil that was removed from each plot was then used to bury the stem sections. The study site was inspected daily and the plots watered to keep soil moist. The experiment was terminated after 40 days, which allowed sufficient time for the stem sections to sprout. At that time, each plot was exhumed to retrieve the buried stem sections. Immediately after exhumation (on 28 June 2010), the stem sections were examined and placed into one of three categories (modified from Tachibana et al. [2010]), where they were counted and recorded as being either sprouted and emerged, sprouted but not emerged, or decayed and/or desiccated. Survival was recorded if a stem section had sprouted, irrespective of the number of shoots produced.

Data Analysis

To test the effects of number of nodes, age of stems, and pattern of stem burial on survival rate, a three-way factorial design was conducted. Any interactions between the three factors were also determined. Data on the percentage of surviving stem sections were arcsine transformed to satisfy the analysis of variance requirements. The data were analyzed using STATISCA 12 (StatSoft, Inc., Tulsa, Oklahoma).

RESULTS

Effect of Number of Nodes on Stem Section Survival

The number of nodes on each stem section significantly affected the survival of those sections, irrespective of their age or pattern of burial ($F = 8.09$; df = 2,84; $P < .001$) (Figure 1). The survival rates of stem sections with two or three nodes were significantly greater than those with only one node (Figure 1). However, there was no significant difference in the survival rates between stem sections with two and three nodes (Figure 1). In addition, there was no significant interactions found between the number of nodes and the age of the stem sections ($F = 2.07$; df = 2,84; $P = .13$).

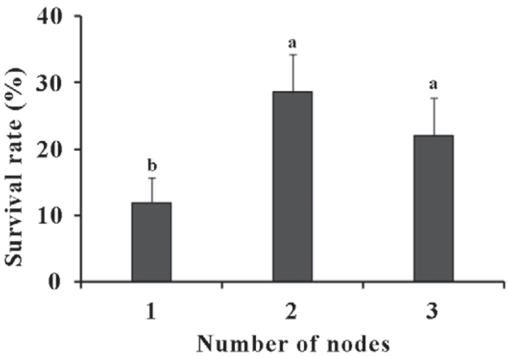

Figure 1. Effect of number of nodes on stem section survival (percentage) of *M. micrantha*. Interval bars with the same letters are not significantly different at $P < .001$, and error bars show two standard errors around the mean.

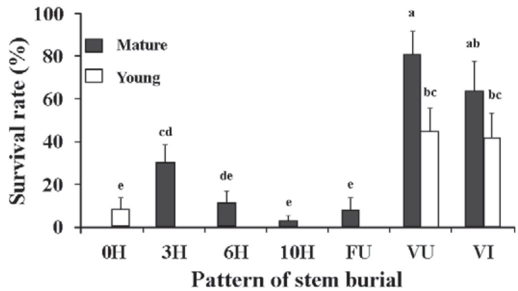

Figure 3. Effect of burial on survival rate of mature and young *M. micrantha* stem sections. Patterns of stem burial were as follows: placed horizontally (H) on the soil surface at 0 cm (0H), 3 cm (3H), 6 cm (6H), or 10 (10H) cm deep; placed vertically upright with all nodes covered (FU) by soil; placed vertically upright with only one node exposed (VU); and placed vertically but inverted, with only one node exposed (VI). Interval bars with the same letters are not significantly different at $P < .05$, and error bars show two standard errors around the mean.

Effect of Age on Stem Section Survival

Mature stem sections had a significantly greater survival rate than younger stem sections ($F = 18.05$; df = 1,84; $P < .001$), irrespective of length and orientation of burial (Figure 2). There was a significant interaction between age of the stem section and the pattern of burial ($F = 2.88$; df = 6,84; $P < .05$), indicating that the effect of the burial pattern on survival of *M. micrantha* stem section changes, depending upon the age of those stem sections.

The survival rate of mature stem sections was significantly greater ($F = 6.84$; df = 1,124; $P < .01$) than that of young stem sections when (1) buried horizontally at 3 (3H), 6 (6H), or 10 (10H) cm deep; (2) placed vertically upright with all nodes covered by soil (FU); (3) placed vertically upright with only one node exposed (VU); and (4) placed vertically but inverted, with only one node exposed (VI) for all treatments except for stems placed on the soil surface (Figure 3).

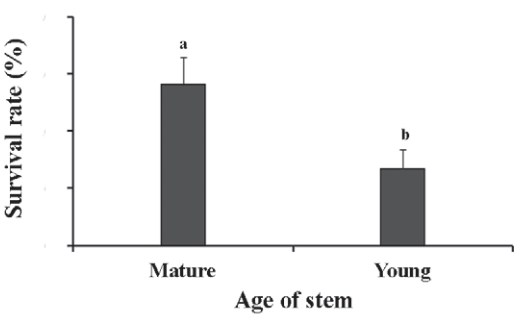

Figure 2. Effect of age of the stem section on survival rate of *M. micrantha* stem sections. Interval bars with the same letters are not significantly different at $P < .001$, and error bars show two standard errors around the mean.

Effect of Stem Burial Pattern on Stem Section Survival

The pattern of stem burial significantly affected stem section survival ($F = 28.92$; df = 6,84; $P < .001$). Both vertical planting orientations (upright and inverted) with one node exposed (VU and VI) had significantly greater survival rates than the other five patterns of stem burial (Figure 4). There was no significant interaction between length of the stem sections (number of nodes) and pattern of burial ($F = 1.43$; df = 12,84; $P = .17$). Survival rate of stem sections with two or three nodes was significantly greater than survival rate of stem sections with one node when buried with 6H, FU, and VI orientations (Figure 5).

Although a proportion of all stem sections was able to survive irrespective of pattern of

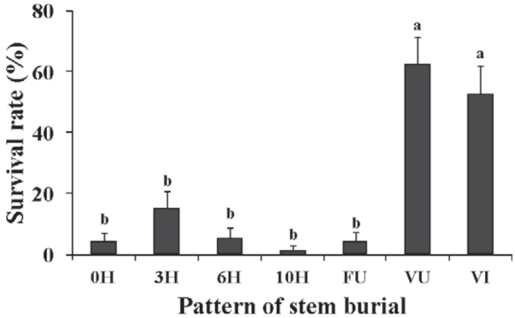

Figure 4. Effect of burial on survival rate of *M. micrantha* stem sections. Patterns of stem burial were as follows: placed horizontally (H) on the soil surface at 0 cm (0H), 3 cm (3H), 6 cm (6H), or 10 (10H) cm deep; placed vertically upright with all nodes covered (FU) by soil; placed vertically upright with only one node exposed (VU); and placed vertically but inverted, with only one node exposed (VI). Interval bars with the same letters are not significantly different at $P < .05$, and error bars show two standard errors around the mean.

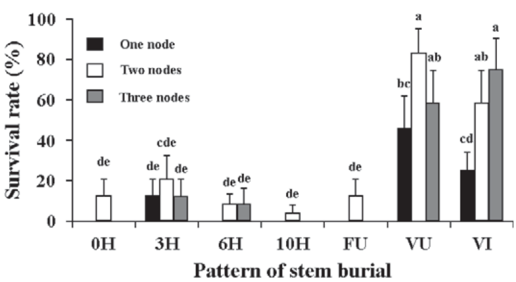

Figure 5. Effect of burial on survival rate of *M. micrantha* stem sections with one, two, or three nodes. Patterns of stem burial were as follows: placed horizontally (H) on the soil surface at 0 cm (0H), 3 cm (3H), 6 cm (6H), or 10 (10H) cm deep; placed vertically upright with all nodes covered (FU) by soil; placed vertically upright with only one node exposed (VU); and placed vertically but inverted, with only one node exposed (VI). Error bars show two standard errors around the mean.

burial, a greater percentage survived when buried in a vertical and upright orientation with one node exposed, compared with those buried completely or in the inverted orientation (Figures 4 and 5). Stem sections buried in an upright orientation with one node exposed sprouted mainly on the exposed node. However sprouting was also observed on buried nodes of some stem sections.

DISCUSSION

The ability of freshly cut stem sections without roots to survive when buried may suggest that they contain sufficient reserves to allow them to form roots at a time when conditions are more conducive for their growth. One of the functions of the stem internode is to store energy reserves that may then be translocated to other parts of the stem section for regrowth (Baur-Höch et al. 1990, Corre et al. 1996, Volenec et al. 1996, Stuefer and Huber 1999, Lin et al. 2012).

Stem sections with two or three nodes had a greater survival rate (29% and 22%, respectively) (Figure 1) than those with one node (12%) (Figure 1), and this trend was true for both young and mature stem sections. This finding concurs with that of a similar study on *M. micrantha* in China, which demonstrated the regeneration rate from stem sections with several nodes to be ca. 40% greater than that from stem sections with one node (Song et al. 2013). This suggests that longer stem sections with more than one node may contain more stored reserves than shorter ones (Suzuki and Stuefer 1999, Dong et al. 2010*a*, Dong et al. 2010*b*, Song et al. 2013). The higher survival rate of *M. micrantha* stem sections with more than one node in our study is in general agreement with other studies undertaken on other vegetatively reproductive species such as *S. trilobata*, *A. adenophora*, dayflower (*Commelina communis* L.), pennywort [*Centella asiatica* (L.) Urb.], wandering Jew [*Tradescantia pallida* (Rose) D. R. Hunt], and paragrass [*Brachiaria mutica* (Forssk.) T. Q. Nguyen] (Song et al. 2013), common silverweed (*Potentilla anserina* L.) (Stuefer and Huber 1999), peak downs curse (*Polymeria longifolia* Lindl.) (Johnson and Sindel 2005), and alligator weed [*Alternanthera philoxeroides* (Mart.) Griseb.] (Dong et al. 2010*a*, Dong et al. 2010*b*, Song et al. 2013).

Studies on *Portulaca oleracea* L. (Miyanishi and Cavers 1981), *P. longifolia* (Johnson and Sindel 2005), and 39 stoloniferous species (Song et al. 2013) have shown that new shoots can form from the smallest of fragments, suggesting that even small fragments may contain adequate stored energy reserves to enable them to produce new shoots. Indeed, in our study, a small percentage of stems with one node also survived.

Mature *M. micrantha* stem sections with more than one node had a greater percentage survival than young sections, regardless of orientation. These results are consistent with those of Stuefer and Huber (1999), who also reported that fragmentation is more harmful to young stem sections than it is to mature sections. Mature stem sections are likely to contain more parenchyma cells, which can store more energy reserves in their internodes, than younger stem sections (Stuefer and Huber 1999).

In our study, all young stem sections with one, two, or three nodes failed to survive when buried horizontally at 3, 6, or 10 cm depth or when completely buried vertically. However, a few young stem sections with two nodes did survive on the soil surface, and those placed vertically, with a single node exposed, survived very well.

The failure of the majority of *M. micrantha* stem sections to survive when placed horizontally on the soil surface may be attributed to a higher desiccation rate caused by sun exposure, which can slow carbohydrate mobilization, which, in turn, can reduce new root production (Stuefer and Huber 1999). However, the survival of some young stem sections with more than one node when placed on the soil surface suggests that under favorable conditions, freshly cut *M. micrantha* stem sections could still survive and establish on the soil surface.

Several mature stem sections that were buried horizontally at depths of 6 or 10 cm survived. However, none of these stem sections produced shoots that could emerge from the soil after 40 days. Thus, stem sections may survive at greater depths but may also deplete all stored reserves before the new shoots reach the soil surface.

Overall, the results demonstrated that *M. micrantha* stem sections may survive after tilling and/or slashing, with survival rates decreasing as the stem sections become smaller and more deeply buried. The study reported here examined burial to depths of only 10 cm, but many plows and tillers can bury sections to 30 cm, which is likely to reduce survival even further, even for the longer mature stem sections. Slashing or cutting mature stem sections into smaller pieces (i.e., one node length) and burying them deeper than 10 cm would further reduce the ability of stem sections to reshoot, thus improving the overall management of *M. micrantha*. Therefore, effective mechanical control may require plowing/tilling the ground more than once to increase the chance of stem sections being cut smaller and buried.

Our results are particularly important with regard to manual weed control, which often involves slashing *M. micrantha* or hand pulling plants, leaving large stem sections lying on the soil surface. Although the exposed sections may dry out, sections below would be partially protected from the sun and may be able to reshoot. To improve weed management, manual control efforts may need to implement other techniques because cutting all sections into one-node lengths and burying would be unfeasible. One option would be to pile and burn slashed stem sections, although the effectiveness of this needs to be tested.

The ability of *M. micrantha* stem sections to survive under various conditions suggests that weed management practices need to be modified to reduce plants reshooting and reinfesting control areas. By knowing the attributes and reshooting capabilities of various weeds, such as *M. micrantha*, management techniques can be structured to optimize better control.

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