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Disturbance Facilitates Seedling Establishment of *Merremia boisiana* (Gagnep.) Ooststr in Natural Forests in Wuzhi Mountain, Hainan Island

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Abstract
Merremia boisiana (Gagnep.) Ooststr. (Convolvulaceae), a perennial evergreen woody vine in South Asia that is rapidly increasing its abundance and geographical range, has become a pest to forests in Hainan during the last two decades. *M. boisiana* typically invades secondary forests, shrub lands, and open woodlands, but has now expanded to natural forests in Wuzhi Mountain where there is tourism-associated disturbance. We did some survey and transplantation experiment to examine (1) where can seedlings of *M. boisiana* emerge, and (2) where do emerged seedlings have the potential to grow larger (e.g., reach the forest canopy). Surveys found that mature plants and emerged seedlings of *M. boisiana* were mainly distributed within 20 m from the tourist path, but a few reached the forest interior by more than 40 m. There were emerged seedlings in most of the plots where there were mature plants of *M. boisiana* in the forest canopy. Larger seedlings were nearer to the forest edge (within 6.19 m to the tourist path) compared with mature plants and small seedlings of *M. boisiana*. The transplant experiment further indicated that emerged seedlings could not survive in the forest interior, but a proportion of them could survive on the forest edge. These findings probably suggest that seedlings of *M. boisiana* cannot survive in the forest understory, but they may establish in disturbed sites and then expand to the forest interior through stolon elongation and climbing growth. Management of *M. boisiana* in natural forests in Wuzhi Mountain should focus on disturbed sites and canopy gaps near the tourist path to remove larger seedlings which have the potential to reach the forest canopy.

1. Introduction

Plant invasions significantly threaten native biodiversity and ecosystem functioning [1-3]. Determining which communities are more prone to invasion is a fundamental objective for invasion ecology [4, 5]. It is generally assumed that undisturbed closed-canopy forests are highly resistant to invasion [6-8], probably because of competition from native plants and attack by herbivores and diseases [9]. However, others found some evidence indicating that natural forests are not immune to invasion. For example, a five year field experiment found that seedlings of the Norway maple (*Acer platanoides* L.) could survive and establish in intact forests in New York [10]. Natural enemy release facilitates *Clidemia hirta* (L.) D. Don to invade tropical forests in Hawaii [11]. *Alliaria petiolata* (M. Bieb.) Cavara et Grande could invade natural forests and suppress native plant growth in North America [12]. In addition, some scholars argued that invasion ecology has traditionally focused on exotic plant species with early successional life-history traits (such as rapid growth and shade intolerance) while neglecting a very high percentage of the shade-tolerant exotics that could invade deeply shaded forests [13]. Discrepancies between these two lines of evidence raise the questions of if and why plants can or cannot invade natural forests.

In recent decades, probably because of global climate change and increasing human activities, some native species can also cause significant harm to biodiversity and ecosystem functioning [14, 15]. *Merremia boisiana* (Gagnep.) Oostr. (Convolvulaceae), a perennial evergreen woody vine native to South Asia, has become a pest to forests in Hainan province and Guangzhou city of China during the last two decades [16, 17]. The first discovery of *M. boisiana* in Hainan was in the Chengmai county in 1882 and in the Danzhou city in 1922 [18]. *M. boisiana* was also discovered in Indochina Peninsula and Kalimantan Island [19]. This species was published as a new one in 1915, using specimens collected from Indochina Peninsula [20]. There are no records documenting any introductions or dispersal of *M. boisiana* from one distributional region to another, so in this paper we consider it as a native species in Hainan that is increasing its abundance and geographical range to the detriment of native species. After its first discovery in Hainan, in the 1940's it was recorded in more counties such as Yazhou, Lingshui, Wanning, Ledong and Baisha, and it has been widely distributed in the island after 1990 [18]. It establishes from seeds in a new place, and a single plant may form a monotypic stand after several years through stolon elongation and ramification [16, 21]. Once established, *M. boisiana* overtops other plants nearby. In several mountains in Hainan, it has formed a continuous canopy reaching 10 hectares [16].

M. boisiana typically invades disturbed forests, shrublands and open woodlands, but has expanded to natural forests in Wuzhi Mountain [22]. Natural forests in Wuzhi Mountain have been disturbed to some extent because of tourism. Tourism may facilitate the invasion of *M. boisiana* by accidentally dispersing seeds to natural forests and creating disturbed sites (e.g., the parking area and the tourist path) for its establishment. We observed that some plants of *M. boisiana* has climbed and covered the forest canopy, killing the trees and plants below by preventing them from absorbing sunlight, and reducing biodiversity. The species is mostly distributed within 100 meters to the forest edge (i.e., the tourist path). Stolons of mature plants (i.e., plants that have reached the forest canopy) can be covered by soil to a length of more than ten meters, and it is difficult to locate the exact points where they germinated. As a vine, *M. boisiana* may germinate and establish in the forest understory, or germinate and establish in open and disturbed sites and then expand to the forest interior through stolon elongation and climbing growth. To explore which is likely the case, we aim to answer the following questions: (1) where can seedlings of *M. boisiana* emerge, and (2) where do emerged seedlings of *M. boisiana* have the potential to grow larger (e.g., reach the forest canopy)? We answer these questions by surveying plots where *M. boisiana* plants of various sizes could be found, and by transplanting its seedlings and examining the seedling survival in plots in the forest interior and on the forest edge.

2. Materials and Methods

2.1. Study Site

Wuzhi Mountain is located in the center of Hainan island of China with a total area of 13,435 ha (18°49'～18°59'N, 109°40'～109°48'E). Its elevation reaches 1,867 meters and is the highest mountain in Hainan. It has a high biodiversity with rich genetic resources [23]. Average annual precipitation ranges from 2,350.7 mm to 2,488.3 mm with a distinct seasonal pattern: 80% of precipitation occurs between May and October [23]. Average annual temperature is 22.5°C, with the hottest being 25.7°C in July and coldest being 18°C in January [23]. Soils are usually alfisols that are formed after weathering of granite [23].

Vegetation in Wuzhi Mountain is usually evergreen broad-leaved forests. In sites with lower elevation (< 800 m), vegetation is often disturbed secondary forests or human plantations with open canopies, and some of the trees have been entirely overgrown and shaded by *M. boisiana*. When elevation is above 800 m, vegetation is usually closed-canopy natural forests, and plant communities remain undisturbed [23]. In most of these forests, *M. boisiana* is not found. However, some natural forests have been disturbed to some extent because of tourism. In particular, areas near the administrative office of Wuzhi Mountain, the parking area, and the tourist path (about one to two meters wide) have been disturbed and invaded by it extensively. The elevation of these areas ranges from 750 m to 850 m. *M. boisiana* has overtopped and partly or completely covered some trees and can reach the forest interior by about 100 m. We did our research in these areas (18°54'N, 109°41'E). Common species in the natural forests nearby include *Ficus hirta* Vahl, *Macaranga tanarius* (L.) Muell. Arg., *Ficus altissima* Bl., *Cratoxylon ligustrinum* (Spach.) Bl., and *Camptotheca acuminata* Decne.

2.2. Emergence Pattern of *M. boisiana* Seedlings and Seedling Survival in the Forest Understory

M. boisiana produces seeds from May to July in Hainan, and seeds usually begin to germinate in the spring (April) when temperature begins to rise. In May 2012 and May 2013, we examined if there were seedlings of *M. boisiana* in the forest understory where there were mature plants in the canopy. Mature plants in the canopy might indicate that there were seeds in the soil underneath, although there were also many seedlings in areas where there were no mature plants in the canopy. In the study area, we tried to locate as many plots where there were mature plants of *M. boisiana* in the canopy as possible, except where we were difficult to get to. We went into the forest interior by 100 to 150 meters far from the tourist path, because we occasionally went into the forest interior and found that there were no mature plants further than this distance in the forest interior. In each plot, matures plants of *M. boisiana* were in the canopy, and the nearest distance from their vertical projection on the ground to the edge of the plot was within 3 meters. The tourist path was not included in any plot. The plots were rectangular. Each plot was at least 2 m away from other plots. In May 2012, we were able to locate 41 plots. However in May 2013 we were only able to locate 26 plots because many mature plants of *M. boisiana* were cut by forest managers between the two surveys. In May 2013, we also measured the nearest distance from a plot to the tourist path. In May 2012, we marked all of the emerged seedlings found in the 41 plots, and in December 2012, we re-surveyed these plots to examine the survival of these emerged seedlings in the forest understory, because we expected that most mortality would occur during this time period.

2.3. Survey of Plots Where Larger Seedlings of *M. boisiana* Could Be Found

In July 2012 and May 2013, we surveyed in the study area where larger seedlings of *M. boisiana* could be found, including the plots where there were mature plants in the canopy. This may indicate where seedlings of *M. boisiana* could potentially establish and grow to the canopy. We define larger seedlings as those with at least three true leaves but have not reached the forest canopy. In July 2012, we recorded the nearest distance from a site where we found larger seedlings to the tourist path or the parking area. In May 2013, we measured the distance from a larger seedling to the tourist path.

2.4. Seedling Transplant Experiment

To further examine where emerged seedlings of *M. boisiana* could survive in the forest, we conducted a seedling transplant experiment. In May 2013, we selected two plots for the transplant experiment: one in the forest interior and the other on the forest edge. There were no mature plants or seedlings of *M. boisiana* within a radius of 5 m around the plots. The plot on the forest edge was adjacent to an abandoned tourist path and about 3.5 m wide and 4 m long. Probably because the distance from the plot to the administrative office of Wuzhi Mountain was short (72 m to the nearest house), seeds of herbaceous species could reach this plot. In most of the cases where we found larger seedlings of *M. boisiana*, there was little herbaceous vegetation on the ground. We thus cleared all vegetation in the ground to mimic conditions found for larger seedlings. On May 13, 2013, we transplanted 80 seedlings of *M. boisiana* into the plot. Another plot with the same size was 60 m away from the tourist path. Such a distance would keep the plot away from human intervention. This plot was typical of the natural forest and there were no mature plants of *M. boisiana* in the canopy or seedlings in the understory. We also transplanted 80 seedlings into the plot. In the two plots, each seedling transplanted was at least 20 cm away from other seedlings. The 160 seedlings for transplantation were with cotyledons only and were gathered the same day from the forest (outside of the 26 plots surveyed). Each seedling was randomly assigned to a plot. During the next four days, we watered these seedlings to make sure they survived the transplantation. One year later on May 20, 2014, we recorded their survival and the longest stem length of the surviving plants.

2.5. Statistical Analysis

For the 26 plots surveyed in May 2013, we analyzed the relationship between distance to the tourist path and number of *M. boisiana* seedlings found in a plot using exponential decay regression. We also compared the average distances from the tourist path between larger seedlings found in May 2013 and the plots where we found at least one emerged seedling. Data were ln-transformed to achieve normality. Student's t-test for unequal variances was performed for this analysis.

3. Results

3.1. Emergence Pattern of *M. boisiana* Seedlings and Seedling Survival in the Forest Understory

There were a total of 213 and 222 emerged seedlings found in the 41 plots surveyed in May 2012 and in the 26 plots surveyed in May 2013, respectively. The area of the plots was 33.6 ± 3.09 m^2 (mean ± SE) in 2013, ranging from 15.5 to 69 m^2. Most of the seedlings were with cotyledons only and without true leaves, and only four (all in the May 2013 survey) can be termed as larger seedlings. Most of the plots (87.8% and 88.5% in 2012 and 2013, respectively) with mature plants of M. boisiana in the canopy contained at least one emerged seedling. For the survey in May 2013, number of seedlings found in a plot decreased exponentially with distance to the tourist path ($P = 0.009$), and most of the plots (and seedlings within them) were restricted within 20 m from the tourist path (Figure 1). In December 2012, all of the emerged seedlings found in the 41 plots in May 2012 died.

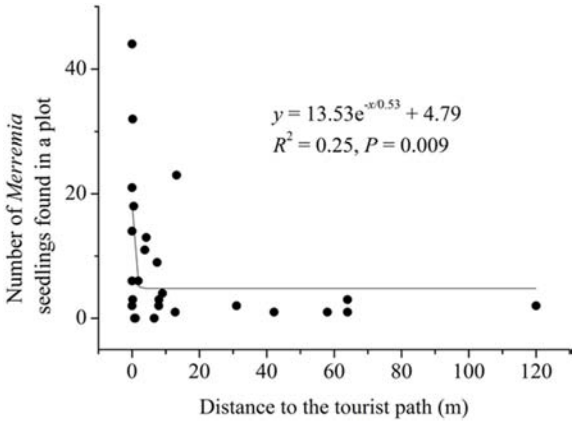

Figure 1. *Distance to the tourist path of and number of emerged Merremia boisiana seedlings found in the 26 plots surveyed in May 2013.*

3.2. Survey of Plots Where Larger Seedlings of *M. boisiana* Could Be Found

In July 2012, we found larger seedlings of *M. boisiana* in three sites. The first site (area: 8.8 m^2) was 0 m away from (on the edge of) the parking area. There were some small herbs and grasses, and six *M. boisiana* seedlings in the place. Seedlings ranged from 14 cm to 94 cm long. The second site was near a rest place along the tourist path, and a *M. boisiana* plant with 250 cm long was found. The plant was 0.8 m away from the tourist path. The third site was 0 m away from the tourist path. The length and width of the site were 12 m and 0.5 m, respectively. Soils in the site were dug up for the placement of a water pipe, and thus there were very little leaf litter on the ground. There were many seedlings emerged in the place with seven having no less than four true leaves.

In May 2013, we found a total of 30 larger seedlings. The number of true leaves ranged from 3 to 16. The distance to the tourist path of these 30 seedlings ranged from 0 to 6.3 m, and the distance to the tourist path of the 23 plots (another 3 plots surveyed in May 2013 had no emerged seedlings) with at least one emerged seedling of *M. boisiana* in the understory ranged from 0 m to 120 m. The average distance to the tourist path of these larger seedlings was shorter than that of the 23 plots with at least one emerged seedling in the understory ($t = 3.081$, $P = 0.005$; Figure 2).

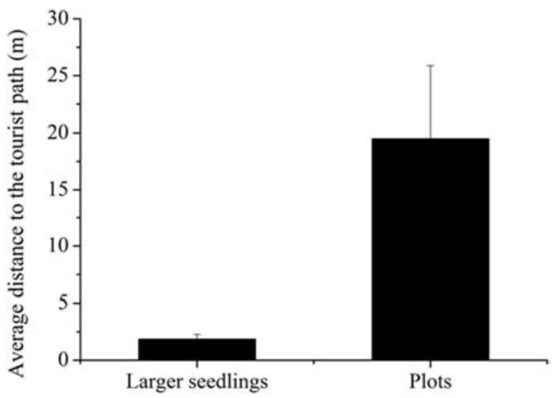

Figure 2. *Average distance (mean ± SE) to the tourist path between the 30 larger seedlings and the 23 plots (with mature plants of Merremia boisiana in the canopy and at least one emerged seedling of Merremia boisiana in the forest understory) surveyed in May 2013.*

3.3. Seedling Transplant Experiment

After transplantation of one year, seedlings in the plot on the forest edge had a higher survival rate (22.5%) than those in the plot in the forest interior (0%). Actually, there were no seedlings survived in the plot in the forest interior. The longest stem length of the surviving seedlings on the forest edge was 67.88 ± 12.51 cm (mean ± SE), ranging from 5 to 170 cm.

4. Discussion

Our finding suggests that *M. boisiana* was mainly distributed near the tourist path, but larger seedlings were nearer to the tourist path compared with mature plants and emerged seedlings. Seedlings of *M. boisiana*could emerge but could not survive in the forest interior, and have the potential to survive on the forest edge. These results together indicate that *M. boisiana* was a shade-intolerant plant with a high light saturation point [24]. *M. boisiana* seedlings may establish in disturbed sites and then expand to the forest interior through stolon elongation and climbing growth.

M. boisiana was mainly distributed near the tourist path, indicating that the species is a ruderal living in environments with low stress and high intensity of disturbance. Others also found that distance to a road is a significant predictor of invasive species presence [25, 26]. Seedlings of *M. boisiana* could emerge in most of the plots where there were mature plants in the forest canopy, although there might be differences in seed germination rate among plots. Other studies also found that natural forests could reduce but not completely inhibit the seed germination or seedling emergence of invasive plants [8, 10]. However, emerged seedlings could not survive in the forest understory. This suggests that *M. boisiana* is able to survive and grow, but not reproduce inside the forest.

Larger seedlings of *M. boisiana* found in May 2013 were distributed not more than 6.19 m from the tourist path. We suggest that this result was conservative because six larger seedlings distributed more than 6 m from the tourist path were all in a plot with an open canopy. The canopy of the plot was formally dominated by mature plants of *M. boisiana* and they were cut by the time of survey. This might indicate that human manual control of mature plants of *M. boisiana* may act as a disturbance to facilitate its seedling growth. The distance to the tourist path of the remaining 24 larger seedlings was 0.766 ± 0.182 m (mean ± SE), ranging from 0 to 3.75 m. The survey from the two years all indicated that larger seedlings were distributed in sites adjacent to the tourist path or the parking area. These sites were usually disturbed with a lower coverage of leaf litter on the ground, and our observation indicated that continuously dropping leaf litter is likely a significant contributor to the death of emerged *M. boisiana* seedlings in the forest understory. This has two main reason: toxic substances released in the decomposition of leaves inhibit the germination of other individuals (allelopathy) or a thick layer of litter prevents the radiation from reaching the seed, making it impossible to emerge. Also, light level and temperature in these sites might be higher than that in the forest interior, and there was evidence that high temperature could promote the seed germination and growth of *M. boisiana* [27, 28]. The low light under natural forest understory has also been highlighted in other studies as the source of resistance to invasion [29, 30]. One may argue that soils may also have a significant effect on the growth of invasive plants [8, 10]. However, our study has found that seedlings of *M. boisiana* could grow as well on soils from the natural forest understory as on soils from disturbed sites, indicating that soil may not be a constraint on the invasion of this species into natural forests [22].

Most emerged seedlings cannot survive in the forest understory. It should be noted that the distance of seedlings found in a plot to the tourist path was farther than the distance of a plot to the tourist path, because the latter was measured as the nearest distance from a plot to the tourist path. Although we did not examine the survival of the seedlings found in the 26 plots surveyed in May 2013, we found in December 2012 that the 213 seedlings found in the 41 plots surveyed in May 2012 all dead. This might be because the canopy of the plots we surveyed, including those near the tourist path, was closed (i.e., like those deep in the natural forest interior), and factors such as low light levels and continuously dropping leaf litter might cause the death of emerged *M. boisiana* seedlings. Many studies found that most invasive plants do not spread into intact forests even when found in adjacent disturbed forests [6, 30, 31], probably also because seedlings of invasive plants cannot establish in the understory of intact forests.

One may argue that larger seedlings of *M. boisiana* were not found in the forest interior because there were fewer emerged seedlings in the forest interior (Figure 2), and that there might be other larger seedlings in the forest interior that were not found by our surveys. However, we think that these were unlikely the case because of several reasons. First, there should be many seedlings that emerged before the time of our surveys or were in sites outside of the plots we surveyed, but we did not find other larger seedlings by the time of our surveys. Second, aside from the plots we surveyed, we also frequently walked into the forest interior to find mature plants and larger seedlings of *M. boisiana*. Therefore, although we did not completely survey the studied area, our surveying sites were representative. Finally, the transplant experiment further corroborated the results that emerged seedlings of *M. boisiana* could not survive in the forest interior but could survive on the forest edge. Although there was only one pair of plots for the transplant experiment, the result can be considered as some weak and supplementary evidence that seedlings of *M. boisiana* cannot survive in the forest interior but have the potential to establish on the forest edge. We also stress that many larger seedlings found in our study were still small and might not eventually reach the forest canopy, and these seedlings may survive, grow, but not reproduce under these adverse conditions, but it is probable that a part of these larger seedlings could eventually reach the canopy.

Overall, our results together suggest that *M. boisiana* seedlings probably establish in disturbed sites and then expand to the forest interior through stolon elongation and climbing growth. Our results partly support the idea that disturbance facilitates invasion [32, 33]. More importantly, we present a novel pathway through which shade-intolerant species could invade natural forests in the adult form (i.e., stolon), but hardly by germination. To our knowledge, this is the first study to report the invasion of a shade-intolerant species into natural forest. Such an invasion process by lianas species should be paid with more attention, especially considering that lianas regenerate aggressively in disturbed forests or on forest edges in recent decades [34, 35]. If forest managers want to limit the regeneration of *M. boisiana* in natural forests in Wuzhi Mountain, they may need to focus on disturbed sites and canopy gaps near the tourist path to remove larger seedlings of *M. boisiana* which have the potential to reach the forest canopy.

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