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RESEARCH ARTICLE

Seed, cutting and air-layering reproductive inefficiency of noxious woody vine *Merremia biosiana* and its implications for management strategy

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Abstract *Merremia boisiana* (Gagnep) van Ooststr. is a noxious fast growing woody vine and is able to grow overtop other plants, causing the death of plants underneath and forming monospecies stands. To formulate management responses, we assessed its seed and vegetative reproduction efficacy through indoor and field experiments. The number of flowers counted from bagged infructescences in Guangzhou ranged from 25 to 172, with an average of 80.80. Counting the seeds of bagged infructescences had shown that there were only 1.58 hard testa seeds in each infructescence. Seed vitality tests using red ink indicated that only 68.6% of hard testa and filled seeds were viable. The emergence rate of scarified hard testa seeds in the sand bed was 31.96%. Under imitated natural conditions, 8% of hard testa seeds could germinate, and 9% still retained their germination potential in one year. Thus, seedlings should be monitored and removed in a timely fashion after any attempt of clearing. Moving soil or transplanting plant from infested patches should be strictly prohibited at least for several years. All considered, an infructescence contributed 0.3 seedlings. The investigation in the field found no seedlings either inside or at the perimeter of the patch, suggesting scant expansion by means of seed dispersal. Therefore, the elimination effort could be focused on a relatively restricted scale of patches. Of 630 cuttings of young shoots, old shoots and old lying shoots with or without growth regulators, there were only four (or 0.63%) established individuals. Air-layering shoots all died in two months. Poor cuttings and air-layering reproduction indicated that regeneration from fragments of removed stems or accidentally dropped ones was quite unlikely, and thus mechanical removal was safe.

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Keywords *Merremia boisiana*, seed vitality, germination rate, vegetative reproduction, management

1 Introduction

A fast growing perennial woody vine, *Merremia boisiana* (Gagnep) van Ooststr. (hereafter referred to as the vine), belonging to Convolvulaceae, is distributed in Indonesia, Malaysia, Laos, Vietnam and China. The vine is native to Hainan Island, China. Although the first specimen record in Guangdong was collected in 2005 (Chen et al., 2005), it had been seen in Boluo County (N 23°16′, E 114°03′), Guangdong Province as early as the 1960s (Wang et al., 2005b), and the vine in Guangzhou, Guangdong Province, had been detected not later than 1994 in the Longyandong Forest (about N 23°13′, E 113°25′) (Xu and Li, 1994) after the vine overgrew trees there. However, there has been no consensus on whether the vine is native (Wang et al., 2005b) or alien (Zeng et al., 2005; Sun et al., 2006; Lian et al., 2007; Wu et al., 2007) to Guangzhou.

The vine turned noxious after timber was harvested in Hainan Island, particularly in the central part of the Island (Wu et al., 2007). In Guangzhou, it has gradually expanded and damaged forestry areas (Xu and Li, 1994; Chen et al., 2005; Liu and Xu, 2005; Tong et al., 2005; Wang et al., 2005a, 2005b; Lian et al., 2007). The vine climbs on top of trees and shrubs (Fig. 1), rapidly forming a dense overtop cover and causing the deterioration of plants underneath (Wang et al., 2005a). The light saturated point and maximal net photosynthetic rate of the vine are even higher than those of invasive weed *Mikania micrantha* (Li et al., 2006), partially explaining the fast growing capability of the vine.

The detrimental effect of the vine is much more severe than ordinary lianas. It has long been known that trees with

Fig. 1 *Merremia boisiana* in Hainan Island, China. The vine climbs on top of trees and shrubs.

lianas suffer higher mortality rates than liana-free trees (Putz and Chai, 1987), while almost all the lianas could coexist with other trees. The vine, however, eventually forms a monospecific stand after plants underneath die and collapse (Fig. 2). The vine is thus termed “a forest killer” in the popular press.

Fig. 2 *Merremia boisiana* in Guangzhou, China. The vine forms monospecific stands after plants underneath die and collapse. The vine climbs over trees on the edge of a patch.

The monospecies stand is a ground cover of intercrossing shoots with broad leaves and woody stems. The stems and shoots roughly run horizontally and loosely pile up to 1–1.3 m high above the ground. At the perimeter of the stand, the vine grows vertically and climbs on adjacent trees or shrubs.

Analysis of experience in managing invasive plant species might provide insight into the control of the vine. For example, a research shows that *Cynara cardunculus*, an invasive perennial in the coastal California grasslands, USA, had almost all seedlings occurring within a few meters of their parents’ rosettes. Thus, management might be focused on adults (Marushia and Holt, 2008). *Carduus acanthoides*, an invasive weed in several North American ecosystems, relied on regular infusions of its seeds to the soil seed bank. Otherwise, its populations went to zero within two to four years since the soil seed bank was not long-lived. On the other hand, Australian *Acacia* sp., invasive in South Africa, produced a large number of seeds, and the extreme persistence of the seeds was above 50 years due to physical dormancy caused by the impermeability of their testas, which made it difficult to deal with once the massive seeds enter the seed bank (Richardson and Kluge, 2008).

Our experience during our endeavor in managing invasive weedy vine *Mikania micrantha* in China also enriches our thoughts for the control of the vine. Firstly, massive seed production of *Mi. micrantha* contributed to the majority of new establishments. The seed, however, persisted in the field for only less than eight months after fruiting from December to January of the following year (Li et al., 2002), thus spraying proper herbicides (e.g. sulfometuron-methyl) in autumn would effectively stop seed production of that year. Secondly, stems of *Mi. micrantha* left on the ground after manual removal re-grew easily (Zan et al. 2000), and the accidentally dropped stems of fragmented ones elsewhere became effective propagules to expand. Thirdly, reconstructing a damaged plant community to create an environment not suitable for *Mi. micrantha* to grow has been successful (Li and Zhou, 2004). As a result, *Mi. micrantha* became a minor important weed.

Effective managing measures to control the vine have not been available yet. Efforts to eradicate the vine by slashing stems, digging out roots or spraying herbicides were all unsatisfactory (Tong et al., 2005; Wang et al., 2005b; Sun et al., 2006). A trial on dripping benzoate aqueous solution from a hanging bottle to the vine shows that all target individuals were killed successfully (Lu et al., 2006), but it was quite difficult to perform on a large scale. Besides, plant community reconstruction has not been successful yet. However, plant eradication is not impossible given sufficient persistence (Simberloff, 2001).

Previously, we found that a considerable portion of seeds were soft and all soft ones were empty, indicating that the percentage of viable seeds was low. We also noticed that the vine only appeared in patches and no single individual outside patches was observed, suggesting that the seeds possibly contributed little to expansion. In addition, we did not find any successful re-sprouting from piles of slashed vines, suggesting that fragmented stems might not be an effective propagule, but different reports were seen (Xu and Li, 1994).

To establish management strategies, we should understand the properties of the seed and the stem fragment. In the present study, we address the following questions: How much do the seeds contribute to the proliferation of the vine? How long will the seed persist in the field? How high is the risk of regeneration of fragmented stems? Finally, what are their implications for management strategy?

2 Materials and methods

2.1 Proportion of hard testa seed per infructescence

Three hundred infructescences were bagged in the Longyandong Forest (N 23°12′38″, E 113°25′49″, altitude about 200 m asl) in a Guangzhou suburb in December, 2005. Each infructescence was put in a nylon mesh bag just after the flowers of inflorescences withered and fruits started to develop. The bag was 20 cm × 15 cm in size and its opening was tied with a string to retain all the structures inside. The infructescences were hand-harvested inside the bag when they turned dry in February, 2006, and then they were kept indoor at room temperature of about 20°C.

Fifty infructescences were randomly sampled. Flowers were identified and counted based on the number of persistent pedicles with bract. Fruits were peeled off to collect seeds. There were two kinds of seeds: hard testa ones and soft testa ones. The proportions of hard testa seeds over the total number of seeds were then calculated.

2.2 Seeds used in the following experiments

For all experiments involving the seeds described below, mature fruits of the vine were collected from Wanning (N 18°47′55″, E 110°23′17″), Hainan Island in July 2006, which is the mass fruiting period on the Island. In total, we obtained about 8000 hard and soft testa seeds by peeling off the fruits.

2.3 Seed vitality test

Seed red-dye staining was used for the vitality test. One hundred and fifty full grain hard testa seeds were selected and merged in water for 24 h. Swollen and disintegrated seeds were discarded. The remaining seeds were scarified by removing a small spot of testa with a razor blade to expose the embryo. A seed with developed embryo was termed filled seed, while a seed without noticeable embryo was termed empty seed. While empty seeds were discarded, scarified filled seeds were merged in water for another 24 h to soften the hard testas. Then, the testas were carefully removed by hand and the embryos were collected. Each embryo was cut into two pieces of cotyledons. The two pieces were placed into two batches, respectively. One batch was placed in a Petri dish and the other batch was put into boiling water for five minutes to kill their cells before placed in another Petri dish. After that, both batches were completely immersed in red ink for 15 minutes followed by tap water rinsing until no red color could be seen in water. The red stained boiled batch was used as control. The cotyledon of the unboiled batch was examined singly to differentiate the viable from the dead based on the color: nonviable cotyledons were red due to increased membrane permeability in dead cells, whereas colorless or slightly stained ones were considered viable.

2.4 Scarified seed emergence in sand bed

Scarified filled seeds were obtained as described in the seed vitality test, except for loosening the strict standard to only hard testa and not necessarily requiring full grain in appearance. They were then soaked in water at room temperature for imbibing. Twenty four hours after the radicles were emerged, they were transplanted onto a sand bed and covered with 1 cm thick of sand and put in the laboratory. Emergence count was based on the emergence of the cotyledon growing out from the sand.

2.5 One year hard testa seed germination potential under imitated natural conditions

To estimate the viability of seeds under natural condition but without interference of herbivores, the emergence experiment was performed in pots. One hundred and fifty hard testa seeds were randomly divided into three batches of 50 seeds on 19 July, 2006. Each batch was sown in a pot (26 cm in diameter and 22 cm in height) covered by 1 cm thick of sand. Water was supplied until some water came out from the bottom of the pot and then each batch was placed outdoor and watered as necessary.

The experiment lasted for almost one year. The seeds from two pots were recovered from medium particles through sieving on 7 June, 2007, and then they were scarified for the germination test.

2.6 Seedling investigation in the field

Two transects of 10 m × 1 m were established in early 2005 to investigate seedling emergence and the investigation lasted for about 12 months until the end of 2005. One transect was set up about 30 m from the edge of a vine's patch. The other one was placed at the perimeter of a patch. Both transects were visited at two month intervals.

In June, 2006, another survey along the perimeter of patches and nearby area was performed to find seedlings of the vine.

2.7 Vegetative cutting propagation

Cuttings of two categories of shoots and one category of stems were obtained from the Longyandong Forest in October, 2005. The two categories of shoots included young ones less than one month old and old ones more than two months old. Both of them were green in color. The third category was old stems which had no green color or leaves, and were lying on the ground with some adventitious roots.

The shoots were excised into cuttings of about 15 cm long with two nodes having leaves. The lower nodal leaf and half of the upper one were removed to decrease evaporation. The old stems were cut into about 25 cm long cuttings.

The three categories of cuttings were divided into two batches, respectively. One batch was put in a beaker and about 5 cm of the bottom end was merged in 800×10^{-6} indole-3-butyric acid (IBA) for 10 min. The other batch was merged in tap water as the control. After treatment the cuttings were immediately explanted into plastic trays filled with the medium of three portions of sand and one portion of soil. The medium had been fumigated by spraying 0.15% $KMnO_4$. Cuttings were inserted 5 cm vertically into the medium. After that, the trays were placed in the shadow and watered as needed.

In March 2006, the above experiments were repeated but without the growth promoter IBA.

2.8 Air-layering propagation

The air-layering propagation experiment was performed to evaluate the capability of vegetative propagation. In March 2006, 60 young shoots and 60 old ones (as described above) were girdled by removing one third of the circle of epidermis by 2 cm long with a sharp knife. The girdled shoots were treated with 500×10^{-6} IBA solution. Another 30 young shoots and 30 old ones were treated as above but without growth regulators. Shoots were wrapped at the girdled point with moist soil and held in place with clear plastic wrapping. They were examined in three weeks and two months, respectively.

2.9 Data analysis

Data analysis was performed using SPSS version 12.0 (SPSS, Chicago).

3 Results

3.1 Proportion of hard testa seeds per infructescence

The number of flowers, fruits and seeds per infructescence from Guangzhou varied markedly among the 50 infructescences sampled randomly. There were a total of 4040 flowers; 15 (or 30%) of infructescences had no fruits; 21 (or 42%) of infructescences had no seeds. There were 79 (or 37%) hard testa seeds out of the total 210 seeds (Table 1).

The average number of fruits and seeds per infructescence was only 2.48 and 4.20, respectively. Seeds with hard testas accounted for 37.6% of the total number of seeds. All considered, there were only 1.58 hard testa seeds per infructescence on average (Table 2).

3.2 Seed vitality test

Of the 150 full grain hard testa seeds used, 48 (or 32.0%) seeds did not develop embryos and were removed. Only the remaining 102 (or 68.0%) seeds were used in vitality testing using 5% red ink dye. Seventy filled seeds were found viable and 32 were dead. Thus, of all filled seeds, 68.6% were viable and 31.4% dead. That was to say, of all full grain hard testa seeds, 46.7% was filled and viable, and the remaining 53.3% non-viable seeds was composed of 32.0% empty ones and 21.3% filled but dead ones.

3.3 Scarified seed emergence in sand bed

Of 1588 hard testa seeds scarified, there were 716 (or 45.1%) filled seeds. Scarified filled seeds were soaked in water and noticeably imbibed, but only a total of 508 (or 70.9%) seeds germinated, while the others disintegrated. Explanted seeds in the sand bed emerged within one week. The total emergence was 504 (or 70.39%) of filled seeds and no more seeds emerged after a week. Considering all 1588 hard testa seeds, the emergence rate was 31.96%.

3.4 One year hard testa seed germination potential under imitated natural conditions

Of 150 hard testa seeds sown in three pots of 50 seeds each in June, 2006, 11 emerged within 30 d, and another one emerged in five months. No more seedlings emerged thereafter. Thus, the overall emergence rate was 8%. Twelve months after sowing, *i.e.* until 7 June 2007, out of two pots with a total of 100 sown seeds, 14 intact seeds were recovered. Four seeds were found empty during scarification. Nine out of the remaining ten filled seeds germinated. Thus, 9% of the hard testa seeds retained their germination potential after a one year period in pots placed outdoor. Supposing all these ten germinated seeds could also successfully germinate with no termination by scarifying, and taking into account those that had been germinated before, the possible maximum germination rate of hard testa seeds in this experimental condition would be 17%, and the percentage of hard testa seeds that vanished during the one year course would be 78%.

3.5 Seedling investigation in the field

Either inside or at the perimeter of the patch, no seedling was seen in transects totaling 20 m^2 in the year of 2005, although flowering was abundant at the end of 2004. The survey along the perimeters and nearby patches in June, 2006 found no seedlings at all.

3.6 Vegetative cutting propagation

Of 630 cuttings from three categories with or without growth regulator treatment, only eight developed

Table 1 Number of flowers, fruits, total number of seeds and hard testa seeds per infructescence of *Merremia boisina* in Guangzhou

infructescence order number	number of flowers	number of fruits	number of seeds	number of hard testa seeds
1	25	0	0	0
2	28	0	0	0
3	33	0	0	0
4	35	0	0	0
5	36	0	0	0
6	37	0	0	0
7	38	0	0	0
8	45	0	0	0
9	45	0	0	0
10	46	0	0	0
11	56	0	0	0
12	57	0	0	0
13	64	0	0	0
14	64	0	0	0
15	67	0	0	0
16	70	1	0	0
17	71	1	0	0
18	72	1	0	0
19	73	1	0	0
20	74	1	0	0
21	74	1	0	0
22	76	1	1	0
23	79	1	1	0
24	80	2	2	0
25	81	2	2	0
26	83	2	3	1
27	83	2	3	1
28	84	2	3	1
29	84	2	3	2
30	88	2	3	2
31	89	3	3	2
32	89	3	3	2
33	91	3	4	2
34	91	3	4	2
35	91	3	5	3
36	92	4	6	3
37	95	4	6	3
38	99	4	6	3
39	99	4	7	3
40	99	4	8	3
41	101	5	8	3
42	101	5	9	4
43	105	5	9	4
44	109	5	9	4
45	110	6	11	4
46	130	6	15	4
47	141	7	15	4

(*Continued*)

infructescence order number	number of flowers	number of fruits	number of seeds	number of hard testa seeds
48	143	7	16	4
49	145	9	18	6
50	172	12	27	9

Table 2 Average number of flowers, fruits, total number of seeds and hard testa seeds per inflorescence/infructescence of *Merremia boisina* in Guangzhou

flowers	fruits	seeds	
		total (hard and soft testa)	hard testa
80.80±31.78	2.48±2.67	4.20±5.81	1.58±1.99

$n = 50$; data are mean±SD.

adventitious roots, and four eventually established themselves (Table 3). Thus, with all pooled, the established cuttings represented 0.63% of the total. IBA did promote the development of adventitious roots and establishment of cuttings.

3.7 Air-layering propagation experiment

Two weeks after air-layering, ten wrappings were opened. No adventitious roots developed. Two months after treatment, all shoots were found dead starting at the girdled point. Thus, none of the 180 air-layerings was successful.

4 Discussion

4.1 Percentage of viable seeds

Two experiments were done for seed viability test. When filled seeds were concerned, the seed viability of the two experiments was consistent: 68.6% from seed vitality test and 70.39% from scarified seed emergence in the sand bed. However, full grain appearance did have a higher percentage of developed embryo, *i.e.*, 68.0% in the seed vitality test, while only 45.1% in the scarified seed emergence experiment due to the non-exclusive use of full grain seeds in seed selection. The difference between the two indicated that seeds with full grain appearance represented better quality.

4.2 Flowers produced a small number of viable seeds

A large portion of flowers failed to develop fruit and some fruits did not contain any seed. Some seeds produced contained soft testa ones, which were not viable. Furthermore, even among hard testa seeds, still a proportion of them were not viable.

Our seed counting from 50 infructescences resulted in 4040 flowers with 79 hard testa seeds, *i.e.*, 1.58 hard testa seeds per infructescence. Taking into account the emergence rate of scarified hard testa seeds being 31.96%, roughly 0.5 seed per inflorescence (1.58×31.96%) was potentially able to germinate. If the seeds were placed under natural condition for one year, the eventual seed germination would be much lower. The results of maximum germination potential under imitated natural condition in one year was 17%, and the estimated number of germination rate would be only about 0.3 (1.58×17%). It could be even lower if the seeds were not put in a pot but

Table 3 Results of cuttings propagation of *Merremia boisiana*

items	cuttings without growth regulator			cuttings treated with 800×10^{-6} IBA		
	young shoot (a)|(b)	old shoot (a)|(b)	old lying stem (a)|(b)	young shoot (a)	old shoot (a)	old lying stem (a)
number of cuttings	120|120	60|60	30|30	120	60	30
number of cuttings developing adventitious roots	0|0	0|0	0|1	7	0	0
number of established cuttings	0|0	0|0	0|1	3	0	0

(a) experiments started on 19 Oct 2005; (b) experiments started on 19 March 2006. IBA: indole-3-butyric acid.

were directly sown in the field because there would be no preventive barrier from frugivores.

4.3 Seeds contributed little to the expansion of the vine

Were seedlings abundant outside a patch, the seeds must have an effective dispersal mechanism. But this was not the case of the vine. Investigation in the Guangzhou area found no seedlings or scattered individuals outside the boundary of a patch. The absence strongly suggested that expansion by means of seed dispersal was scant.

Unlike the tiny seed of noxious herbal vine *Mikania micrantha*, which has pappus that facilitates wind dispersal in air or on the ground, seeds of the vine which are round and have relatively heavy weight (15 g per 1000 seeds, unpublished data) are not suitable and have no device for wind dispersal. Under natural conditions, the seed would fall to its immediate surrounding ground. Furthermore, no seedling outside a patch also indicates that the mechanism for the efflux or influx of a seed is unlikely to exist.

4.4 Vegetative fragment was ineffective propagule

Of 630 cuttings of shoots and old lying stems with or without growth regulator treatments, only eight developed adventitious roots and eventually only three cuttings of shoots and one cutting of old lying stems were established. None of the air-layerings could produce adventitious roots. However, all those air-layerings died in two months. These show that vegetative reproduction through fragments was ineffective, but different results were also reported (Xu and Li, 1994).

The result implies that mechanical removal was safe. Regeneration from the pile of removed stems or accidentally dropped stems of fragments was quite unlikely.

4.5 Re-infestation through seeds could not be estimated

Eradication of a target plant is the elimination of every potentially reproducing individual plant and seed from an area (Myers et al., 1998). To eliminate new individuals established from the seed, monitoring and timely removal for several consecutive years is required. In the field, we never saw a seedling under dense cover of the vine, but a number of seedlings emerged and grew after the aboveground layers were removed, indicating some seeds were in the seed bank. Our experiment indicated that about 10% hard testa seeds kept in the imitated field conditions were still viable within one year. The results from the germination experiment in the pot might overestimate the proportion of viable seeds and their longevity because seeds in the pot were protected against many detrimental natural processes in the field. Nonetheless, it indicates that not all mature seeds were ready to germinate in the field, and it is reasonable to believe that a portion of seeds remained dormant for at least one year.

4.6 Implications for management strategy

The vine has been expanding slowly but continuously over the past 14 years and had shown no signs of stopping. Previous manual removal attempts were not successful and people turned to herbicides but failed. It called for new management responses to terminate the expansion trend and to rectify the damage it had caused. Such attempts require large amounts of resources. Just like in any large scale eradication effort, a relatively long-term financial and institutional commitment would be required (Panetta and Timmins, 2004; Regan et al., 2006). Hence, to make sure that many resources would not be spent in vain, evidence-based responses should be established.

Compared to the massive and easy dispersal of the seed of invasive weed *Mikania micrantha*, the vine expansion through seeds is of minor importance. Additionally, no obvious influx or efflux of seeds from a patch occurred. Therefore, it is reasonable that the elimination effort should be focused on a relatively restricted scale of patches.

Vegetative cuttings and even layerings were both poor propagules. Thus, unlike a fragment of *Mi. micrantha*, which was an effective propagule, the slashed or dug stems of the vine would not regenerate and mechanical removal was safe.

The seed of the vine has hard testa and scarifying breaks seed dormancy. In the field, the seeds could persist for at least one year. Thus, the seed increases the chances of resurgence. It should be monitored and seedlings should be eradicated in a timely fashion. On the other hand, moving soil or transplanting plant from infested patches should be strictly prohibited for at least several years.

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