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RESEARCH ARTICLE

Impacts of *Ageratina riparia* (*Regel*) *R. M. King & H. Rob*. on natural regeneration of sub-montane forests at Knuckles Forest Reserve, Sri Lanka

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Abstract: Forest gaps and margins of sub-montane forests in the Knuckles Forest Reserve (KFR) are invaded by *Ageratina riparia.* It creates a dense cover and prevents the penetration of sunlight to the ground, which may affect the seedling establishment of indigenous species in invaded areas. Six forest gaps and four footpaths inside sub-montane forests were sampled for *A. riparia* cover, density of forest species, soil moisture, soil root density, and canopy openness. Soil seed bank experiments were conducted during wet and dry seasons. The percentage cover of *A. riparia* decreased significantly when moving from the center of gaps/footpaths into the forest interior. Mean density and species diversity of forest species decreased with the increase of percentage cover of *A. riparia*. Low root density of forest species was observed in areas with high density of this invasive species. Higher seedling emergence of *A. riparia* from soil seed bank was observed along footpaths (~1500 seedlings m^{-2}) than in forest gaps (~750 seedlings m^{-2}) during the wet season. *A. riparia* seedling emergence was higher during the dry season (~22%) than the wet season (7-11%). Lower number of forest seedlings emerged in locations with higher percentage of *A. riparia* seedlings. Availability of light affects the establishment of *A. riparia* inside forests. Native species, *Psychotria zeylanica* and *Symplocos cochinchinensis* can be used to restore forests invaded by *A. riparia*.

Keywords: *Ageratina riparia***,** forest gaps, footpaths, Invasive species, soil seed bank

INTRODUCTION

Although Sri Lanka is a small tropical island, a wide range of topographic and climatic variations in the country have led to evolution of many types of ecosystems, with high level of biodiversity per unit area, that is higher than most of other countries in the region (Bambaradeniya, 2002). When considering the forest cover of Sri Lanka, the richest biodiversity of the country is in the southwest of the country which include, 670 km^2 of montane forest, 740 km^2 of lowland forest, a total of 1,410 km^2 (roughly half being primary forest), or a mere 9% of original forest covering almost 16,000 km^2 (Myers, 1990). Sub-montane forests are located at 1,000-1,500 m elevation above the mean sea level and cover 1.1% of the total land area (Bastiaanssen and Chandrapala, 2003). These forests with high biodiveristy are important due to many ecosystem services provided by them including protecting of important watersheds and providing habitats for many endemic flora and fauna (Doumenge *et al*., 1995; Weerawardane, 2005).

The introduction of invasive species has become a major problem to biodiversity in many tropical countries of the world and some consider it as the second greatest global threat to biodiversity, after habitat destruction (Gould and Gorchov, 1999; Kairo *et al.*, 2003). Special characteristics unique to invasive species include the high dispersal capacity, physiological tolerance shown by the plant in its' immediate stresses in new habitats (environmental gradients such as temperature, photoperiod, the climate, resident species as competitors, predators, *etc*.) or the phenotypic plasticity (Maron *et al.*, 2004), production of small, short lived seeds and germination without permanent short juvenile periods (Goodwin *et al.*, 1999), high reproductive allocation, rapid vegetative growth rates, high potential for acclimation (McDowell, 2002) and production of allelopathic compounds (Orr *et al*., 2005). A large number of species extinctions were reported to have occurred due to the introduction of invasive species while others contribute to the degradation of catchment areas and irrigation systems causing severe economic losses (Mooney *et al.*, 1989; Marambe *et al.*, 2001; Vila and Weiner, 2004; Weerawardane, 2005). Invasive species that have escaped from the cultivations have infested lawns as pests, displaced native plant species, reduced wildlife habitats, clogged important water ways and have

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altered the processes in natural ecosystems (Marambe *et al*., 2001), thus it is important to prevent the introduction of new species to pristine environments.

Usually non-native invasive species colonize degraded habitats. Invasive species that are shade tolerant also can establish on disturbed forest habitats (Fine, 2002; Brown *et al.,* 2006). The disturbances in forest ecosystems influence soil conditions, propagule availability, species composition and community structure of the forest (Fine, 2002). Invasive species tend to dominate disturbed areas, as they are capable of tolerating harsh conditions. If invasive species dominate the initial stage of regeneration of a forest, it is most likely to limit the establishment of native species by competing aggressively with them for resources (Lichstein *et al.,* 2004). Many studies have documented reduced number of native species establishment in areas invaded by non-native species (El-Ghareeb, 1991; Pyšec and Pyšec, 1995; Dunbar and Facelli, 1999; Martin, 1999). In forest ecosystems, light is a critical factor affecting the growth of new seedlings of forest species and in disturbed areas invasive plants may suppress the native plant establishment by reducing the light availability (Wyckoff and Webb, 1999; Levine *et al.,* 2003; Lichstein *et al.,* 2003). Reduction of soil moisture, alteration of soil conditions, alteration of soil fauna and microbial communities are some of the other effects of these invasive species in an ecosystem, which lead to reduce the native species establishment (Melgoza *et al*., 1990; El-Ghareeb, 1991; Belnap and Philips, 2001; Ehrenfeld *et al*., 2001).

The mist flower or *A. riparia* (Regel) R. M. King & H. Rob. (Asteraceae), which is a native plant to Central America has been introduced to Hakgala Botanic Gardens of Sri Lanka in 1905 and in 1918 it was reported outside the botanic gardens (Weerawardena, 2005; Wijesundera, 1999). Since then, it has become a weed in the hill country of Sri Lanka (McFadyen, 2003). It is observed in the margins and interior of the disturbed montane and sub-montane forests of Sri Lanka. *A. riparia* is a moderately shade tolerant, shrubby perennial that grows very fast and attains a height of about 0.3 – 0.5 m at elevations between 1000-1500 m in Sri Lanka. Thus, seedlings of native plant species may get smothered by the growth of *A. riparia* due to lack of sunlight (Humphries *et al*., 1991). The most important character that makes this plant a serious threat to the native plant species is that many juvenile plants of *A. riparia* grow from a single primary stem, developing many branches and they intertwines with the adjacent plants giving a blanket effect (Zancola *et al.,* 2000). *A. riparia* is found along road sides, footpaths inside the forest and forest gaps in the Knuckles Mountain Range of Sri Lanka at elevation approximately 1,200-1,300 m a.s.l. Moreover, no or few seedlings or saplings of native forest species are observed in *A. riparia* invaded sites. In the areas where the ground layer of the forest had been cleared for cardamom cultivation, the invasion is greater than in the areas with a forest ground cover.

Investigation of threats caused by this invasive species on forest regeneration is crucial, because of the serious threats it may cause for the functioning of sub monatne forest ecosystems. No study has been conducted in Sri Lanka on the ecology of *A. riparia* to our knowledge. We hypothesize that dense stands of *A. riparia* have a negative impact on natural regeneration of forest species in these forests by preventing the establishment of seedlings of native plant species. The objectives of the study were to determine the distribution of *A. riparia* in sub-montane forests, the effect of *A. riparia* on regeneration of sub-montane forests species, the seasonal effect on the soil seed bank composition in sub-monatne forests infested by *A. riparia*, estimate the root density of native tree seedlings and *A. riparia*, and determine soil moisture content and canopy openness in areas infested by *A. riparia.*

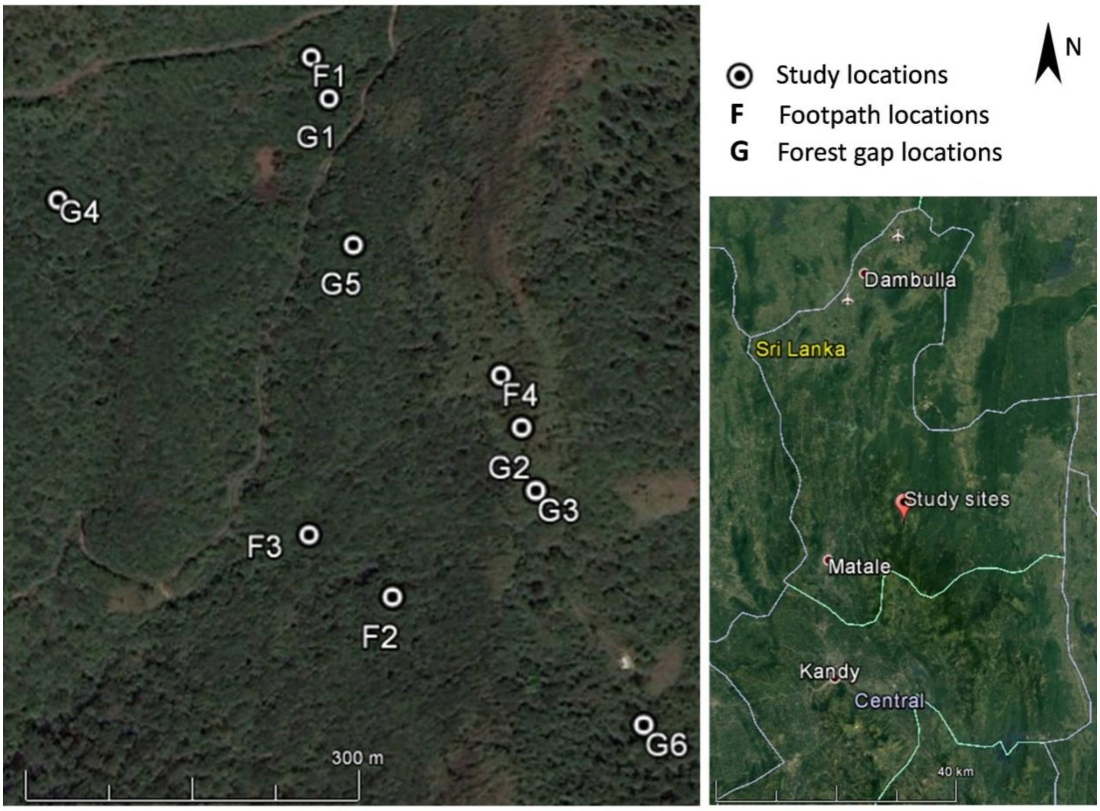
MATERIALS AND METHODS

Study Sites

The study was conducted in sub-montane forests at Riverston area from September 2011 to September 2012 at Knuckles Forest Reserve (KFR), Sri Lanka (7° 21'to 7° 24'N, 80° 45'to 80° 48.5' E). The KFR which is situated in Kandy and Matale administrative districts of central Sri Lanka covers an area of approximately 21,000 ha, and it spans to the upland and highland peneplains (Bambaradeniya and Ekanayake, 2003). The reserve which is high in biodiversity is an important watershed in the country, with several streams draining from the east of the reserve into the lower Mahaweli system (eg: Hasalaka oya and Heen ganga) from the south-west into the upper Mahaweli system (eg: Hulu ganga) (Bambaradeniya and Ekanayake, 2003). The KFR along with Peak Wilderness Protected Area and Horton Plains National Park was declared as part of Central Highlands World Heritage Site in 2010 by UNESCO as this region includes the largest and least disturbed remaining areas of the submontane and montane rainforests of Sri Lanka, with high endemism and biodiversity and they provide habitats for many threatened plant and animal species. (World Heritage Committee, 2012). However, the biodiversity of KFR is greatly threatened by cardamom cultivation, tea and paddy cultivation and introduction of invasive species (Bambaradeniya and Ekanayake 2003). The common invasive alien plants in the area include *Austroeupatorium inulifolium*, *Lantana camara*, *Clidermia hirta*, *Tithonia diversifolia* and *Eupatorium riparium* (*Ageratina riparia*) (Bambaradeniya and Ekanayake, 2003).

EXPERIMENTAL DESIGN

Six forest gaps and four footpaths infested with *A. riparia* were selected from the interior of the sub-montane forests at Riverstone (Figure 1). The angles of all the slopes were approximately 50°. The direction of the footpaths was recorded using a compass. Quadrats (1 m × 1 m) were established across each footpath at 0 m, 5 m and 10 m distances on either side of the footpath (in perpendicular to the footpath) towards the forest interior (Figure 2). Quadrats (1 m × 1 m) were established on the forest gaps at the center, edge and 5 m away from the edge into the forest on both sides, along the north-south direction.



Source: Google Earth

Figure 1: A map showing study sites at Knuckles Forest Reserve, Sri Lanka.

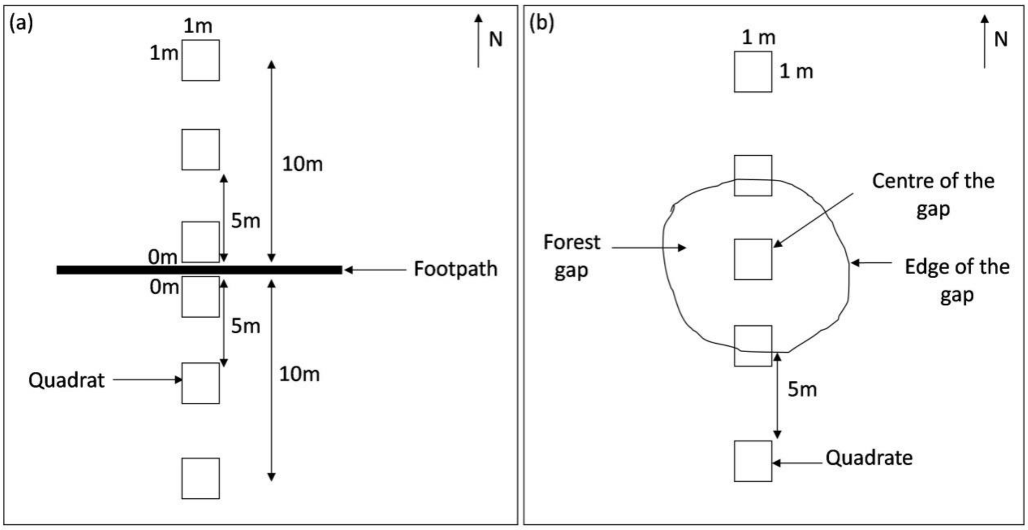


Figure 2: a) The layout of the quadrats along footpaths and b) in forest gaps.

VEGETATION SAMPLING

The percentage cover of *A. riparia* was estimated visually as a percentage from the total area of the quadrat (Klimes *et al.*, 2003). The number of individuals of seedlings (less than 1 m height) of different species was recorded. Unknown species were identified using the reference collections at the Department of Botany, Faculty of Science, University of Peradeniya and by comparing with the herbarium specimens at the National Herbarium at Royal Botanical Gardens, Peradeniya, Sri Lanka.

SOIL ROOT DENSITY

A total of 24 soil samples (15 × 15 × 10 cm^3) were collected from four footpaths from six quadrats in each location. Soil samples were collected into polythene bags and were sealed. Then the samples were mixed well and were sieved (< 2 mm) under the running water. The root samples were carefully separated (roots of forest species and *A. riparia*) and weighed (Lata *et al*., 2000). The root samples were dried in the oven at 75 ^{0}C until a constant weight was obtained (Sheng sand Hunt,1991).

SOIL MOISTURE CONTENT

Soil samples were taken using a soil corer (3.2 cm diameter) up to a depth of 6 cm. Two soil samples were taken from the center of the forest gaps and one soil sample each was collected from all other quadrats. Soil was collected in polythene bags, labeled and sealed tightly. All soil samples were oven dried at 105 $^{\circ}$C for 24 hours after taking their fresh weights. Samples were reweighed, and the dry weight of the soil samples were obtained. The soil moisture content was expressed as the ratio between the mass of water and the mass of wet soil (wet mass basis) (Angelis, 2007).

CANOPY OPENNESS

The canopy openness was determined by taking hemispherical photographs (CoolPix 5400 Nikon camera and a Fisheye lens). A single view of the canopy was taken at each location using a vertically oriented fisheye lens (center of the forest gaps and the footpaths) using a tripod. All the photographs were taken from 10.00 a.m. to 12.30 p.m. to reduce any errors due to reflection of light. Canopy openness was analyzed using the Hemiview software (Vincent, 2001).

SOIL SEED BANK

A soil sample was collected from each quadrat in footpaths and forest gaps except from quadrats placed in the center of gaps, where two samples were collected, using a soil corer (5 cm depth and 3.2 cm diameter) during the wet (February 2012) and dry (June 2012) seasons at KFR. The samples were laid separately on sterilized soil on trays in the plant house at the Department of Botany, University of Peradeniya, Sri Lanka. Each tray was separated into six sub divisions using Aluminum sheets and one division was kept as a control. The positions of the seed bank trays were changed weekly. Soil on each division was turned and mixed until no more seedlings emerged from samples. The seedling emergence was recorded for three months until no more seedlings emerged from the soil samples in both seasons. The procedure was repeated for both dry and wet seasons.

DATA ANALYSIS

The mean percentage cover of *A. riparia* was calculated for each distance for footpaths and forest gaps separately. The mean number of seedlings of the forest species at the footpaths and forest gaps were calculated for each distance. One-way ANOVA was carried out using MINITAB (2003) to compare the percentage cover of *A. riparia* and mean number of forest seedlings, for each distance for the two locations. One-way ANOVA was used to compare the soil moisture content, soil root density, root moisture content and the canopy openness.

RESULTS

The percentage cover of *A. riparia* decreased significantly ($p = 0.001$, $df = 2$, $F = 9.78$) when moving away from the center of forest gaps and footpaths into the forest interior (Figure 3). The lowest seedling density of the forest species was observed at the center of the forest gaps and 0 m distance of the footpaths, where the highest percentage cover of *A. riparia* was recorded [Figure 3 (b)]. However, the highest seedling density of forest species was observed at the edge of the forest gaps [Figure 3 (a)]. The number of forest species decreased with the increased cover of *A. riparia*. The shrub species, *Psychotria zeylanica* had the highest mean density of 0.75 ± 0.31 m^{-2} seedlings in the footpath at 0 m distance, and 1.2 ± 0.3 seedlings m^{-2} at the center of the forest gaps with the presence of the *A. riparia* (Figure 4). However, the regression values indicated that there was no relationship between the mean density of forest seedlings and the percentage cover of *A. riparia* (footpaths $R^2 = 0.114$, forest gaps $R^2 = 0.018$).

A total number of 45 different species of seedlings were identified from the footpaths and forest gaps invaded by *A. riparia* (Table 1, Appendix 1). The mean density and the composition of the forest species varied when moving away from the footpaths and forest gaps towards the forest interior. *Psychotria zeylanica* (Rubiaceae) had the highest abundance followed by *Symplocos cochinchinensis* (Lour.) S. Moore (Symplocaceae) (Table 1 and Figure 3). A higher root density was recorded for the seedlings of forest species than that of the *A. riparia* at all the distances in gaps and at footpaths. However, the root density of the seedlings of forest species is lower in the quadrats where the root density of *A. riparia* is high. The soil moisture content was not significantly different when moving away from the footpaths and the forest gaps into the forest. The canopy openness was higher in forest gaps than in footpaths.

Seedling emergence of *A. riparia* from soil seed bank was higher during the dry season (Figure 5: a and c) (~22%) than in the wet season (Figure 5: b and d) (9%). Lower seedlings density of forest species was observed from soil seed bank in locations with high seedlings density of *A. riparia*. However, there was no relationship between the seedling density of *A. riparia* and that of tree and shrub species that emerged from the soil seed banks. The *A. riparia* seedlings density at the footpaths (2021 seedlings m^{-2} at 0 m distance) were lower than that of forest gaps (2280 seedlings m^{-2} at center of the gap) during the dry season.

DISCUSSION

A. riparia is not capable of growing in undisturbed forests where the canopy is closed (Zancola *et al.*, 2000, Frohlich *et al*., 1999, Barton *et al*., 2003). In our study *A. riparia* was only recorded in disturbed areas such as forest gaps, footpaths and roadsides at margins or interior of sub-montane forests. According to Tripathi and Yadav (1987), forest leaf litter can inhibit the seed germination and seedling growth of *A. riparia* due to allelopathic compounds, which are released by the decay of the litter or may be due to the competition for the limited resources for the seedling growth (physical intervention) of *A. riparia* (Xiong and Nilsson, 1997). Zancola *et al.*, (2000) also reported that *A. riparia* volume index significantly negatively correlated with the forest leaf litter biomass. Moreover, branches of adjacent *A. riparia* intertwine with each other producing a blanket effect and produce a 100% ground cover (Zancola *et al.*, 2000). Due to this dense cover, seeds of forest species may not be able to reach the soil to initiate germination.

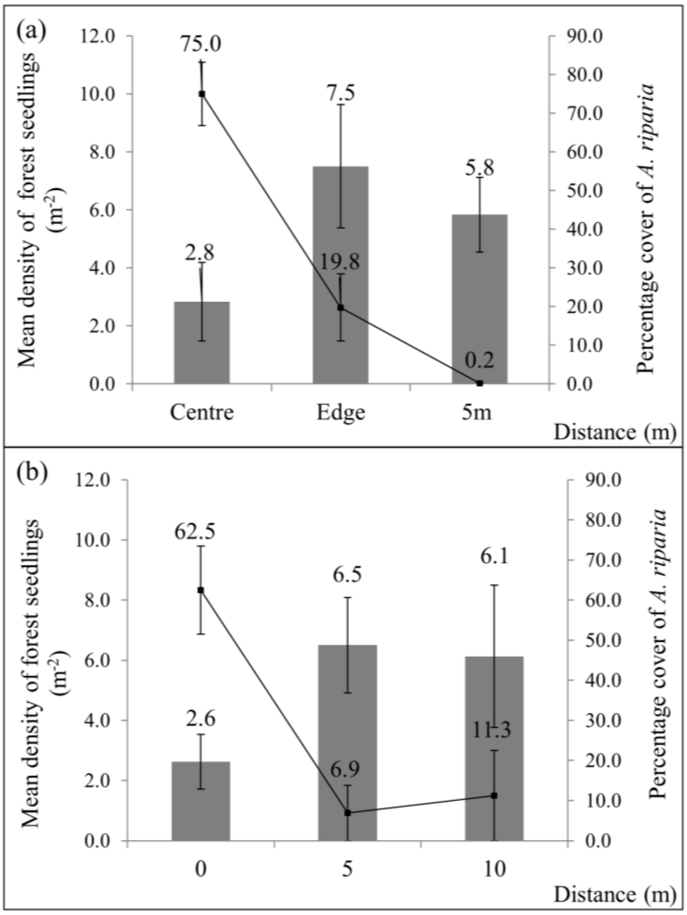


Figure 3: (a) The graph of the mean density of seedlings of forest species and the percentage cover of *A. riparia* vs the distance from the forest gaps (b) The graph of the mean density of seedlings of the forest species and the percentage cover of *A. riparia* vs the distance from the footpaths.

Although there was no relationship between the mean density of forest species and the percentage cover of *A. riparia*, lower number of forest species and lower mean densities were observed in quadrats with higher percentage of *A. riparia*. The high shade inside the forest can suppress the growth of the seedlings of native species and the invasive species. Moreover, cardamom cultivation may influence the seedling establishment of forest species in the forest interior. Although cardamom cultivation is legally prohibited inside the KFR, advanced regeneration of sub-montane forests is frequently cleared by locals to enhance the growth of cardamom plants. Thus, management practices used during cardamom cultivation and spread of *A. riparia* play a crucial role on the establishment of sub montane forest species in the KFR.

The native species *P. zeylanica* has shown the capability to survive in the presence of *A. riparia*. Root systems of certain species can affect the other species by certain mechanisms such as allelochems or alteration of microbial populations which are associated with the neighboring plants (Christie *et al.*, 1978). Lower root density of forest species was observed with higher percentage cover of *A. riparia*. Hence, *A. riparia* seem to exert high competition for below ground resources such as water and nutrients thereby limiting the growth of forest seedlings.

The center of the forest gaps is wetter than the forest interior and the soil moisture content can vary based on the distance from the gap and gap orientation (Ziemer, 1964; Gray *et al.*, 2002). Gálhidy *et al.*, (2006) reported that the soil moisture content in the center of the gap always has the maximum value, regardless of the size of the gap and is usually higher than the forest interior. However, our results indicate that the center of the forest gaps had similar moisture content to the forest interior probably due to small size of the gaps in our study. The results suggest that establishment of *A. riparia* has reduced the soil moisture content in invaded areas.

Table 1: The species recorded at the study locations.

			Number of seedlings	
key	**Species**	**Family**	**Footpaths**	**Forest gaps**
Sc	*Symplocos cochinchinensis*	Symplocaceae	11	19
Ta	*Toddalia asiatica*	Rutaceae	0	5
Sp.4	U1	-	0	3
Sp.5	U2	-	0	2
Ps	*Psychotria zeylanica*	Rubiaceae	43	76
Sp.7	U3	-	0	5
Sw	*Semecarpus walkeri*	Anacardaceae	10	3
Lw	*Lasianthus walkerinus*	Rubiaceae	2	4
Sp.11	U4	-	0	2
Sp.12	*U5*	Rubiaceae	0	9
Ga	*Glycosmis angustifolia*	Rutaceae	0	2
Sp.14	U6	-	0	3
Sf	*Syzygium fergusoni*	Myrtaceae	4	4
Sp.16	U7	-	0	4
Sp.18	U8	-	0	1
Rd	*Rauvolfia densiflora*	Apocynaceae	0	5
Sp.21	U9	-	1	1
Sch	*Sacandra chloranthoides*	Chloranthaceae	6	3
Sp.25	U10	-	3	0
El	*Elaeagnus* sp.	Elaeagnaceae	0	0
Sp.29	U11	-	1	0
Sp.30	U12	-	1	0
Sp.32	U13	-	0	2
Ca	*Calophyllum acidus*	Clusiaceae	6	2
Sp.34	U14	-	0	0
Me	*Mallotus ariocarpus*	Euphorbiaceae	1	1
Sp.36	U15	-	0	3
Stc	*Strobilanthes calycina*	Acanthaceae	3	0
Sp.39	U16	-	3	0
Sp.40	U17	-	1	0
Sp.45	U18	-	3	0
Sp50	U19	-	1	1
Pc	*Procris crenata*	Urticaceae	0	2
Sp.55	U20	-	0	1
Fi	*Flacourtia indica*	Flacourtiaceae	1	0
Sp.56	U21	-	1	0
Sp.57	U22	-	0	1
Sp.58	U23	-	1	0
Lm	*Lasianthus mooni*	Rubiaceae	2	0

key	Species	Family	Number of seedlings	
			Footpaths	**Forest gaps**
Sp.60	U24	-	0	0
Ap	*Acronychia pedunculata*	Rutaceae	0	3
Aa	*Aglaia apiocarpa*	Meliaceae	0	2
Ct	*Cissus trilobata*	Vitaceae	0	3
Sp.64	U25	-	0	3
Sp.65	U26	-	1	0

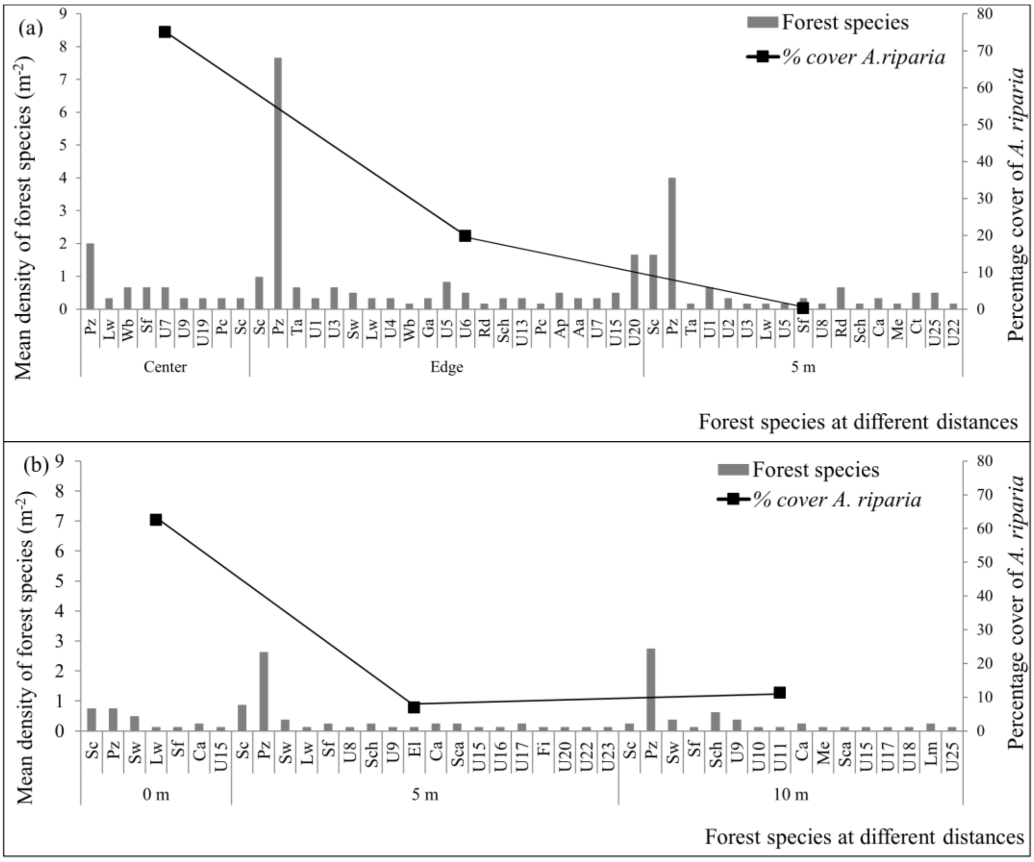


Figure 4: (a) The graph of the mean density of forest species and the percentage cover of *A. riparia* vs the distance from the forest gaps (b) The graph of the mean density of forest species and the percentage cover of *A. ripria* vs the distance from the footpaths.

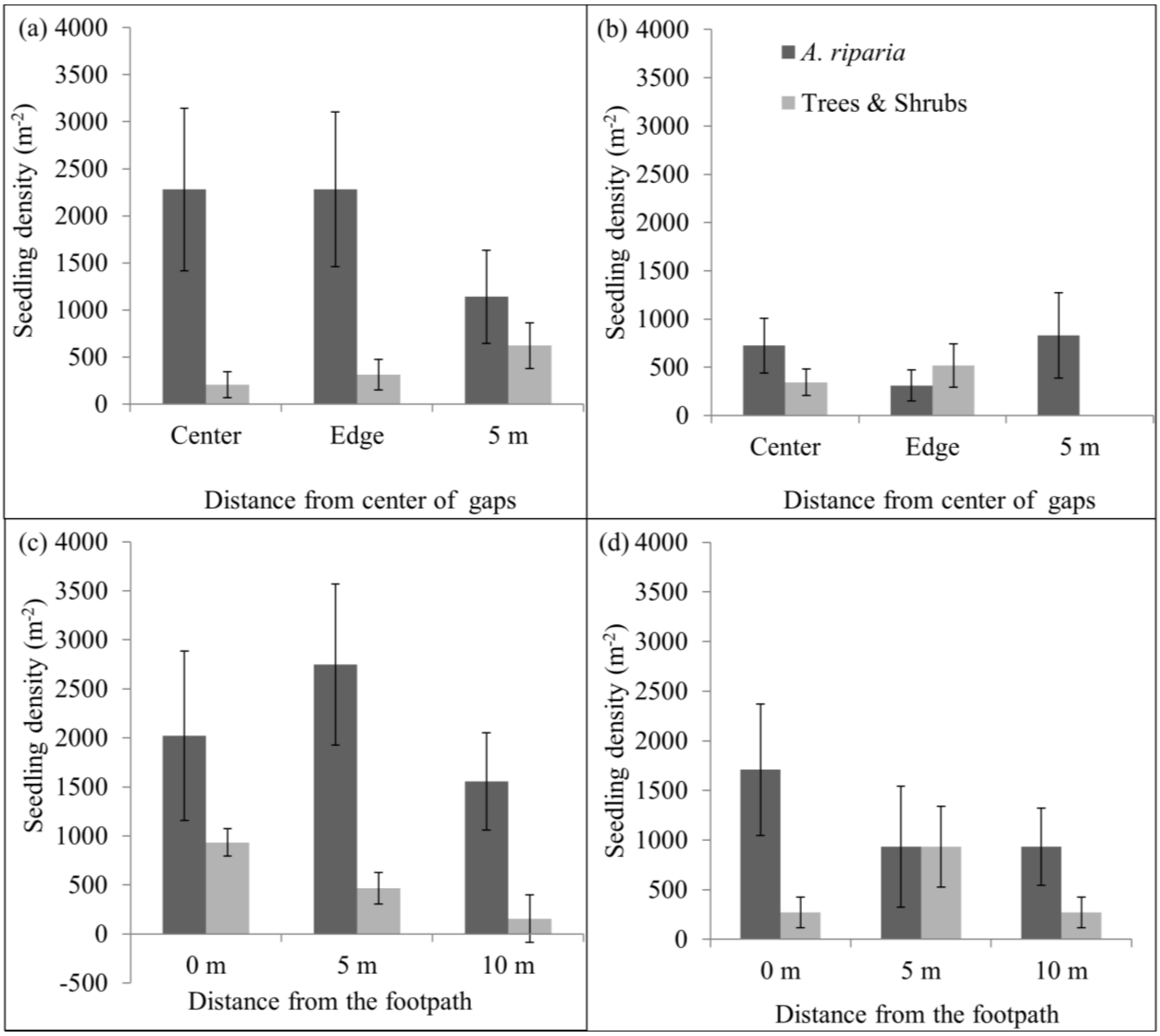


Figure 5: (a) The graph of seedling density vs. the distance from the forest gaps during the dry season (b) The graph of the seedling density vs. the distance from the forest gaps during the wet season (c) The graph of the seedling density vs. the distance from the footpaths during the dry season (d) The graph of the seedling density vs. the distance from the footpaths during the wet season.

A. riparia tends to prefer areas with partial shade but not open areas, such as areas adjacent to grasslands. According to Zancola *et al.* (2000), the availability of sunlight had a significant positive relationship with the volume index of *A. riparia* plant biomass. Similarly, higher availability of light in forest gaps than footpaths may have resulted in higher cover of *A. riparia* in the forest gaps than the footpaths.

The invasion of *A. riparia* in the forest interior may be related to the type and the frequency of disturbance. Footpaths are disturbed frequently compared to forest gaps. Such frequent disturbances may have a negative effect on the growth of this invasive species. *A. riparia* prefers the edges of footpaths probably due to low light intensities than that of the center of the footpath and also due to less disturbances. Another possible reason is that undisturbed forest could be observed from one meter or less away from the edge of the footpath. Along most footpaths, *A. riparia* is grown only as a thin strip (a width of approximately 0.5 m) parallel to the footpath.

Understanding the disturbance regimes and regeneration patterns in forests is essential to determine the health of the forests and to recommend restoration strategies to conserve biodiversity and ecosystem services. The natural regeneration in tropical sub-monatne forests at KFR is affected by cardamom cultivation as well as the spread of *A. riparia*. The invasive, *A. riparia* has suppressed the growth of seedlings of forest species by altering the microhabitat conditions and resource availability along footpaths and in canopy gaps. Since *A. riparia* threatens the regeneration of the forest species in sub-montane forests, their spread must be controlled. Based on the results of the current study, native forest species including *Psychotria zeylanica* and *Symplocos cochinchinensis* can be used to restore these affected sub-montane forest patches.

Prevention of the spread of *A. riparia* could be carried out by several methods, including the use of chemicals (herbicides), mechanical control and the biological control. The aerial application of the herbicides before the production of mature seeds by the plant could be effective in restricting further spread of the invasive species (Land protection, 2006). The mechanical control could be done by physically uprooting small plants and disposing them either by burning or putting into black plastic bags to rot down. Cultivation, grubbing, hoeing and burning along with replanting of competitive pastures or replacement of *A. riparia* by native flora will successfully control *A. riparia* (Land protection, 2006). Biological control could also be done using agents such as gall fly, *Procecidochares alani* Steyskal; the plume moth or defoliator, *Oidaematophoru*s sp., and the leaf spot fungus, *Cercosporella ageratinae* (Nakao *et al.*, 1981) and *Entyloma ageratinae* sp. nov. (Barreto *et al.*, 1988). Use of chemicals can pollute the water sources of local communities and biological control needs thorough testing prior to introduction of new species to areas with high endemism like the KFR. Thus, in order to have the minimal impact on the functions of the natural ecosystem, we recommend mechanical control of *A. riparia*.

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Appendix 1: Seedling reference collection of the identified species.