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Do water buffalo facilitate dispersal of invasive alien tree species Acacia nilotica in Bekol Savanna Baluran National Park?

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DOES WATER BUFFALO (*Buballus*sp.)FACILITATE DISPERSAL OF INVASIVE ALIEN TREE SPECIES *Acacia nilotica*IN BEKOL SAVANNA, BALURAN NATIONAL PARK, EAST JAVA PROVINCE, INDONESIA?

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ABSTRACT

Invasion of *Acacia nilotica* in Baluran National Park, East Java Province, Indonesia, has caused significant loss to savanna cover which is the main natural feature of the park. This study aimed to describe whether water buffalo may play a role in the dispersal of *Acacia nilotica* seed in Bekol Savanna usinganalysis of buffalo stools/scats, observations and seed experiments. In total there were 30 plots set up around the Bekol Savanna to collect buffalo stools. In addition,*A. nilotica* pods matured from its trees were collected, as controls. Germination tests were conducted on seeds that were extracted from the collected stools and from pods (control). Some intact stools were left as they were to let the stored seeds germinate directly from the stools. Viability test on seeds was also conducted using tetrazolium solution. This study indicated that buffalo proved to be legitimate dispersers of *A. nilotica* seeds. The potential ofbuffalo acting as dispersal agent is apparent from the vast number of seeds found in their stools and the finding that although buffalo ingested the seeds, this caused no apparent damage to seeds. Result from tetrazolium test showed that both herbivore-grazed seeds and control seeds haveconsiderable good viability percentages of around 60-70%. This study led to conclusionsthat buffalo ingestion might not have direct scarification on the *A. nilotica* seeds which then leads to a higher number of germination as the percentage ofgermination was similar between control and grazed seed. However, buffalo stools mightact as an important microsite that benefits *A. nilotica* seeds and young seedlings. Buffalo stool might provide moist conditions that protect seeds from extreme air temperatures at Baluran Savanna.

Keywords: Endozoochory, invasion,protected areas,restoration, woody species

INTRODUCTION

Acacia (Mill.) is the main genus in the Leguminosae - Mimosoideae with roughly 1,200 species distributed mostly in tropical and subtropical regions (Mabberley 1997; Abari *et al*. 2012). One of woody species *Acacia* genus that is known to inhabit certain savanna ecosystems is *Acacia nilotica*. The *Acacia nilotica* tree is widespread in the northern savanna regions and its range extends from Mali to Sudan and Egypt. In 1850 *Acacia nilotica* was introduced to Java, by cultivation as fire break in teak forest. The species was spread also outside of Java Islands, such as Timor and Papua. *Acacia nilotica* is reported as dominant colonizer at Baluran National Park in East Java Province and Wasur National Park in Papua (Tjitrosoedirdjo 2008).

Acacia nilotica is known to be abundant in its native habitat in Africa (Brenan 1983), but has been scantly studied(Skowno*et al.*1999). In Australia, this species is wide spreading inwestern Queensland where it has been declared as a weed, and only a few are found in Western Australia, New South Wales, Adelaide and Northern Territory (Reynolds & Carter 1990). Adult trees of *A. nilotica* are fire tolerant and can have negative impact on savannas (Radford *et al*. 2001). Impact of *A. nilotica* on Mitchell grasslands in Australia was studied by Burrows *et al*. (1991) where it was observed that *A. nilotica* was invading the grasslands and forming thorny thicket formations. Although adult trees of A. *nilotica* are apparently fire tolerant, the effects of fire on juvenile plants of this species are unknown. In addition, fire is seen as a useful tool for the arrest of *Acacia* spp. and woody thicket formation and also for the maintenance of open savannas in many parts of Africa, however the effect of fire on *A. nilotica* are unclear and sometimes contradictory (Radford *et al*. 2001).

Similar phenomena can also be seen in Baluran Savanna in East Java Province, Indonesia. The same subspecies has been introduced into the Baluran Savanna that is overtaking Mitchell grassland in Australia, *Acacia nilotica* ssp. *indica*. Its first introduction was in the late 1960s, when its original purpose was to create fire breaks to prevent spread of fire from Baluran Savanna into the adjacent teak forests. However,nowadays, *A. nilotica* has spread rapidly and threatened the existence of Baluran Savanna as it has been observed to cause changes from open savannas to more closed canopy in some areas (Djufri 2004, Barata 2000). Over dominance of the woody species *A. nilotica* could shift the savanna into another ecosystem state, i.e. secondary/dry forest.

The widespread occurrence of *A. nilotica* in the Baluran Savanna according to Tjitrosoedirdjo *et al*.(2013) may have been facilitated by large mammals such as the buffalo. According to them, at the end of the wet season and into the dry season, mature *A. nilotica* pods drop from the trees and are consumed by herbivores such as water buffalo (Fig.1). Tjitrosoedirdjo *et al*.(2013) suggested that the digestive system of the herbivores may scarify *A. nilotica* seeds, enhancing their germination, and that the herbivores would also facilitate the spread of *A. nilotica* in Baluran. Surprisingly, however, this assumption is lacking of empirical studies. This study aimed to describe whether water buffalo may play a role in the dispersal of *Acacia nilotica* in Bekol Savanna by stools/scats and seeds observation and experiment. The importance of the findings in preparing a restoration program at the national park as well as other important element in ecological restoration will also be discussed.

Figure 1. Buffalo (*Bubballus* sp.) in Baluran National Park

MATERIALS AND METHODS

Baluran is located at the north-end tip of East Java, plateaued in the rain shadow of mountain ranges. History of the area goes back to the early 1900, where in 1937 the Dutch Government stated this area as wildlife reserve (decree GB. No. 9 dated 25 September 1937 Stbl. 1937 No. 544) to conserve large mammals mainly *banteng* (*Bos sundaicus*) that had already inhabited the surrounding areas. This decree thenreinstated by the Indonesian Agriculture Minister in 1962 (decree No. SK/II/1962 dated 11 May 1962) and then it wasstated as national park in 1980 up untilnow. Baluran National Park covers vast area of 25,000 ha and it is located in Situbondo District, East Java Province. On its North part it is bordered with Madura Strait and on its Eastpartsit is bordered with Bali Strait (Fig.2)

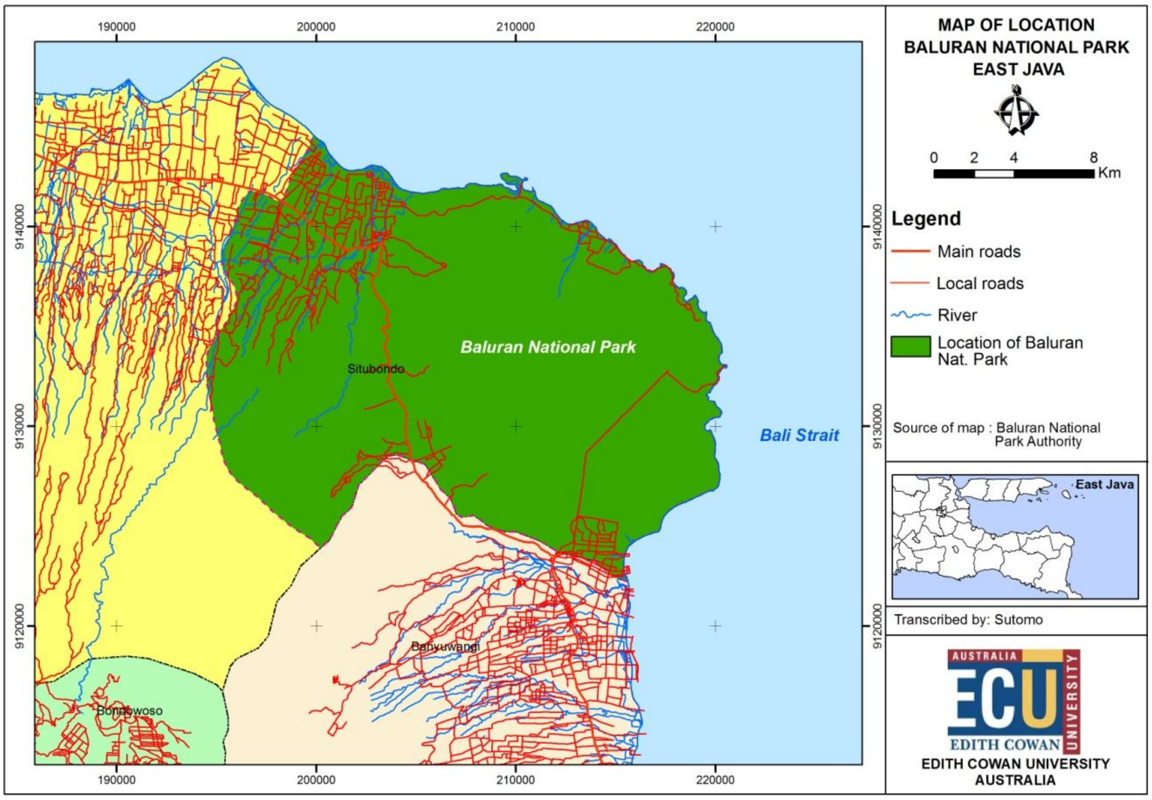

Figure 2. Location of Baluran National Park

Mount Baluran is a small volcano mountain which is currently non-active. The geographical set up produces a peculiar dry area, leading to the establishment of a savanna ecosystem. The area receives only a meager rain as the water vapors carried by wet southern wind has been deposited as rain at the other side of the mountain. The park located in between 7°29'10" and 7°55'55" South Latitude and 114°29'20" and 114°39'10" East Longitude, characterized by a single cone of Baluran Mountain having a summit at 1,247 m (Tjitrosoedirdjo*et al.* 2013). According to Schmidt and Fergusson climate classification, the Baluran is classified as type-F climate (dry climate) with temperature ranging from 27-31°Cand relative humidityof 77%. Rainy season starts from November up to April, whereas dry season occurs from April to October. The highest rainfall usually occurs in December up to January. Baluran Savanna has alluvial soil type. In dry season or drought, the soil will crack and thedepth of the crack could reach up to 80 cm (Sabarno 2002).

Plots were laid out in Bekol Savanna whenever a stool was found. The stool was then collected, put in a plastic bag and labeled. Most of stoolswere not in intact condition, some already broken to pieces, exposing the *A. nilotica* seeds (Fig.3). Plant species composition and abundancewere also recorded in the 2 x 2 m plot if it is on the savanna, but if it is in the *A. nilotica* stand the record was made on the plants inside a 5 x 5 m plot. In total there were 30 plots (20 plots in the savanna and 10 plots in the *A. nilotica* stand) set up around the Bekol Savanna. In addition *A. nilotica* pods matured from its trees were also collected, for control.

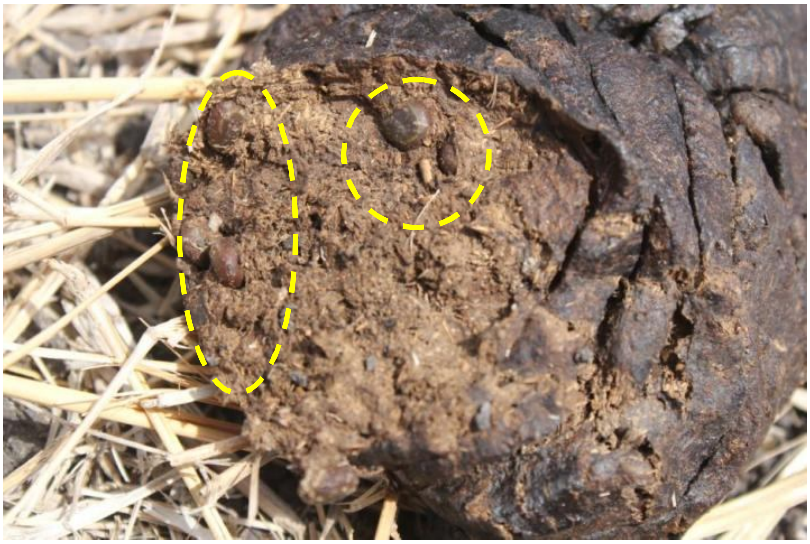

Figure 3. Buffalostools with seeds of *Acacia nilotica* in it (yellow dash) which were found inBekol Savanna in Baluran National Park

Stools (30 stools) were then transported to Bali Botanical Garden. Some stools (25) were crushed and *A. nilotica* seeds were extracted from it. The rest of the stools were also crushed and mixed together and left as they were on a plastic tray. Seeds extracted from the crushed stools were then separated and only well looked seeds were selected for the germination test. As many as 519 seeds of *Acacia nilotica* were extracted from the stools. However, only 310 of them were well looked and counted. For the purpose of simulating germination, 20 of very well looked seeds were selected. Germination bedswere prepared under a shade of paranet at the nursery of Bali Botanical Garden. The planting media composed of soil, humus and sand with equal size comparison 1:1:1. Then 20 seeds from the stools were planted in two rows on the beds (two repetitions), this was called as herbivore treatment. Next, the other 20 seeds were extracted from the *A. nilotica* pods which were also prepared and planted in the same waywiththe herbivore treatment, this acted as control or no treatment. The stools that were still intact were left asthey were, under the paranet shade for observation of any emerging seeds coming out from the stools.

In order to test viability using Tetrazolium solution, another 10 seeds from buffalo extracted stools repository were taken, as well as 10 seeds from another *A. nilotica* extracted pods. To do this test, seeds were soaked in water for one night, cut to facilitate stain intake and incubated in a 2% solution of 2,3,5-triphenol-tetrazolium chloride in phosphate buffer for 24 hours in the dark (Peters 2000, Elias & Garay 2004, Calviño-Cancela *et al.* 2006). Germination and viability data were then analyzed and graphed using Microsoft Excel and then statistical test wasperformed using SPSS.

RESULTS AND DISCUSSION

The germination test showed that germination started to occur in the 25th day of observation (Fig.4), on that 25th day, herbivore treatment and control germinated only one seedling each whereas stools germinate 2 seedlings. There was no additional germination in the herbivore treatment throughout the 55 observation days. At the 30th day of observation, stools seeds started to germinate again and the total number was double the number of the previous germination (4). Meanwhile, two seeds started to germinate on the 35th day of observation of control. The control seeds retained the same total number of seeds that had germinated (2) until observation ended, while the stools seeds continued to germinate up until 50th day with total number of 38 seedlings (Fig.4).

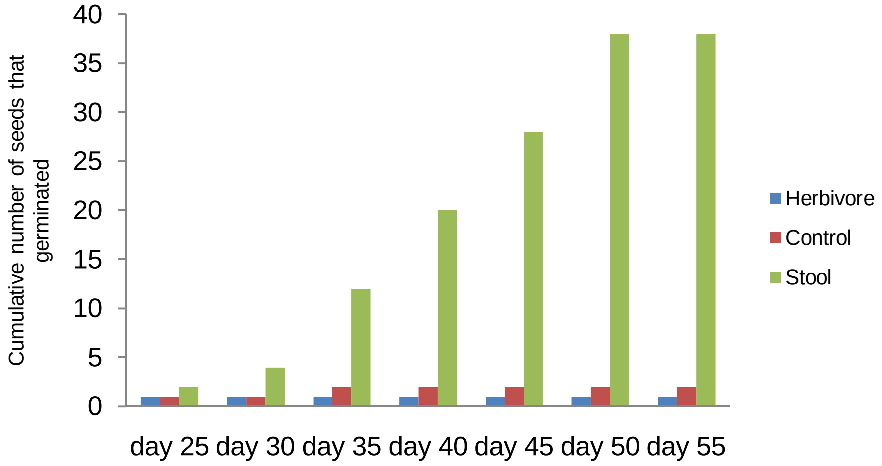

Figure 4. Cumulative number of seeds that germinate from herbivore treatment, control and seeds that germinate directly from Buffalo stools during the observation days.

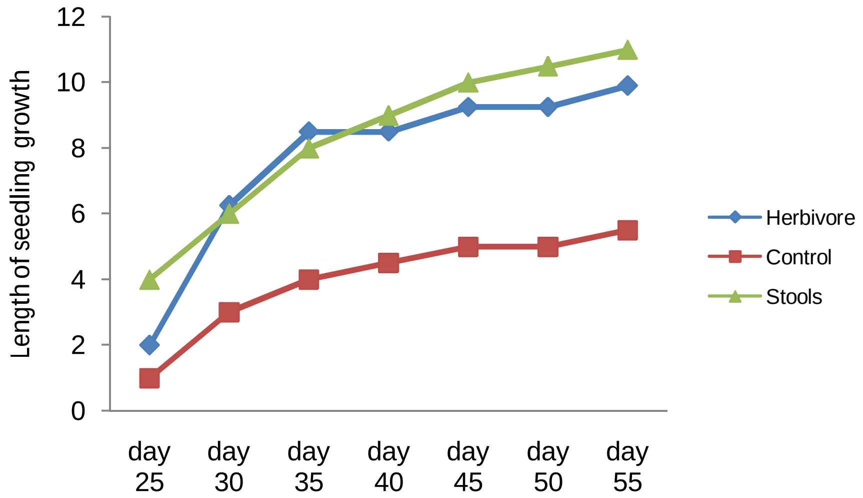

Figure 5. Comparison oflength (in centimeter) of seedlings growth between Buffalo grazed treatment, control and stools on seeds of *Acacia nilotica*

Measurement of seedling length on the treated and control seeds revealed that seeds from herbivore treatment had longer size of seedling compared to seedling germinated from pods or control (Fig. 5). Day 25 to day 35 was the time when the growths of seedlings were at the fastest rate and this applied for all treatments (herbivore, control and intact stools). Number of seedlings emerged directly from buffalo's stools were reaching up to 38 seedlings for the 50 days observation period (Fig. 4). The seedlings that grew from buffalo's stools had relatively similar length with the seedlings that germinated from planting media.

Tetrazolium (fully known as 2,3,5 –Triphenyl tetrazolium Chloride) is a colorless solution having a reddish-pink color that stains living tissue, such as embryos. Test of tetrazolium showed no significant difference ($\alpha = 0.05$) on seed viability between the herbivore grazed seeds and the control seeds. The test result also showed that the herbivore grazed seeds and the control seeds had high percentage of viability (around 60-70%).

For biological invasion to begin, dispersal capability is significantly required. Many weeds possess well-adapted accessory to aid in long-distance movement of their seed (Radosevich*et al*. 2007). To be considered as a dispersal agent, animal has to supply some benefits to dispersed seeds. Perhaps animal movement is one of the advantages so that the concerned seeds could have long range of dispersal (Comins *et al.* 1980). Endozoochory (carry seeds internally, by ingesting them) is most common among vertebrates. Birds and mammals are therefore, the most common animal seed dispersal agent in many plant communities (Calvino-Cancela & Rubido-Bará 2012). In Serengeti and in tropical savanna generally, according to Anderson *et al*.(2013) ecological processes including seed dispersal is dominated by large herbivores.

In this study in a savanna ecosystem of Baluran National Park, buffalo proved to be legitimate dispersers of *A. nilotica* seeds, based on several reasons, i.e. (1) Thepotential of acting as disperser agent is apparent from the vast number of seeds found in their stools. Number of seeds that was found inside buffalo stools was predicted to be lower in the wet season when other options of food sources were available; (2) Buffalo ingested the seeds, but it causing no apparent damage to the seeds. Surprisingly, when sown on a media, buffalo ingested seeds did not show higher germination success compared to the seeds from the control treatment. However, the seedlings that emerged directly from buffalo's stools showed a high number of seedlings germinating. Mean length of seedling growth also showed different results. Seedling emerged from buffalo stools had longer seedling length compared to seedling originated from pods that were sown in a planting media. (3) Tetrazolium test showed that both herbivore grazed seeds and control seeds showed high percentage of viability test of around 60-70%. This implies the buffalo's ingested seeds were viable according to the tetrazolium test.

These results further confirmed the important roles of seed dispersal through animal guts or Endozoochory. By droppings/stools sampling of emu (*Dromaius novaehollandiae*) in three different locations in Western Australia, Calviño-Cancela*et al*. (2006) considered emu to be an important non-standard dispersal agent for long-distance plant dispersal. In other studies, Calvino-Cancela & Rubido-Bará (2012) found that seeds having been through the guts of slugs escaped from physical damage and the ingestion helped seed scarification and sped up its germination. In this study, seeds that were extracted from buffalo stools mainly also escaped damage, however, these didnot mean that their germination was also better compared to the seeds that were not from buffalo stools (=seeds from pod/control). This result suggested that buffalo grazing and ingestion might not have scarification effect to the seeds that would speed up the germination. In another ecosystem, a volcanic terrain in Mount Koma in Japan, Nomura and Tsuyuzaki (2015) reported that hares was the agent of dispersal and also aided the establishment of seedling following volcanic eruption. Seedling started to emerge in a few weeks after being sowed.Crushed stools retained the highest germination rate according to their findings. Similar to the findings in this study, seeds of *A. nilotica* also started to germinate after 3 weeks of sowing. Also, seedling that emerged directly from buffalo stools grew more gregariously compared to the seeds that extracted from the stools and planted in other media.

Based on the results of this study, it is concluded that buffalo ingestion might not have direct scarification on the *A. nilotica* seeds which then lead to a higher number of germination. However, buffalo stools might act as an important microsite that benefited *A. nilotica* seeds. Buffalo stool might have distinct microclimate which provided humid condition, a shelter that protected seeds from extreme air temperature at Baluran Savanna that could reach 40°C during dry season. Water properties that these stools retained, played important role in seeds germination. The importance of microsite had been recognized as an important element in plant invasion and succession, especially in primary succession in volcanic environment (Gomez-Romero *et al.* 2006, Mori *et al*. 2008, Titus & del Moral 1998). In this case, microsite provided microclimate and substrate amelioration. In a study on plant colonization following eruption of Mt. St. Helen, Titus and del Moral (1998) found that colonization patterns of plants in microclimates were changing over the years which highlighted the dynamic nature of the landscape and significance of climate, substrate amelioration and seed rain to plant establishment. Availability of micro sites for overcoming seed dormancy are essential for increasing the likelihood of seed germination and seedling establishment, particularly in dry environments (Abari*et al*. 2012).

Understanding the seed biology and characters of invasive alien species (IAS) in native community is important as to drafting management plans of the ecosystem (Booth *et al*. 2003) especially when preparing for a restoration program. There have been many restoration efforts at Baluran savanna conducted by various institutions and with various methods (Sutomo, 2014).However in more than three decades these efforts showed no significant results yet. The inability of the invaded savanna ecosystem to return to its original state is now recognized in ecology as multiple stable state (Lindig-Cisneros, 2009). Starting in 2011, an eradication effort was led by the Forest Research and Development Agency (FORDA). Basically the method wasto apply strong herbicide (triclopyr) solution that mixed with diesel and then applied it to the bark of *A. nilotica* (stem brushing). In one year the leaves began to falls and the bark wasdecaying and it would break and tree would fall. Soon after the *A. nilotica* trees were burnt and grasses planting was begun. However, although the above method seemed quite promising, its effectiveness still neededto be assessed. First of all, how can we be sure that there are no seed stored in the soils around the restoration site? Seeds can disperse by mammals everywhere including in the restoration site as there are no fences. Seeds can also come from pods of the mature seeds when it was still alive. These seeds were potentially stored in the soil as seed bank. Secondly, burning method that wasused perhaps wouldaccelerate the germination of these soil seedsin the restoration site. Therefore, soil seeds bank study studiesare also needed to be conducted as one of the key variable to measure the success of the trial above. In near future, eradication program need to be based on the concept of restoration. Restoration would include ecological theory such as succession, biological invasion and various relevant researches.

CONCLUSIONS

The importance of studies on seed ecology and dispersal through animal's guts is inurgent need, especially when discussing Baluran savanna and off course elsewhere, where savanna is the main feature biome. Therefore, result from this study may also be relevant in other savannas. In this study, Buffalo is shown to be an important dispersal agent of exotic – invasive plant *Acacia nilotica*. In order to prepare restoration program of the savannas in Baluran National Park, dispersal agent such as the Buffalo, the distribution and population of *A. nilotica*, its seed ecology and seed bank are of vital component that should be taken into account.

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