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Seed germination behaviour of *Lantana camara* in response to smoke

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Abstract: *Lantana camara*, a native of the New World tropics, has invaded tropical and subtropical ecosystems across the world. Its growth is stimulated by disturbances such as mild fire, cutting, pruning, and grazing. We investigated the effects of smoke on seed germination of *L. camara*. Smoked seeds started germinating earlier than non-smoked seeds. The proportion of seeds that germinated, and values of the Germination Velocity Index (GVI) and Vigour Index (VI) were also higher in the smoke treatment compared to the control treatment. Further, seedling mortality was lower in the case of smoked seeds. Our experiment indicates that fire could enhance seed germination in *L. camara*. Besides mechanical and chemical control methods, burning is used as a method to control *L. camara*. This practice needs to be discouraged because it could promote - rather than check - the spread of *L. camara* in burnt areas.

Resumen: *Lantana camara*, especie nativa del trópico del Nuevo Mundo, ha invadido ecosistemas tropicales y subtropicales en todo el mundo. Su crecimiento es estimulado por disturbios como el fuego moderado, la tala, la poda y el pastoreo. Investigamos los efectos del humo sobre la germinación de semillas de *L. camara*. Las semillas ahumadas comenzaron a germinar antes que las no ahumadas. La proporción de semillas que germinaron y los valores del Índice de Velocidad de Germinación y del Índice de Vigor también fueron mayores en el tratamiento con humo que en el testigo. Además, la mortalidad de plántulas fue menor en el caso de las semillas ahumadas. Nuestro experimento indica que el fuego podría fomentar la germinación de las semillas de *L. camara*. Además de los métodos de control mecánicos y químicos, las quemas se usan para controlar a *L. camara*. Es necesario desfavorecer esta práctica porque podría estar promoviendo - en lugar de estar limitando - la expansión de *L. camara* en áreas quemadas.

Resumo: A *Lantana camara*, uma nativa dos Trópicos do Novo Mundo, invadiu os ecossistemas tropicais e subtropicais do mundo. O seu crescimento é estimulado pela ocorrência de distúrbios como fogos suaves, abates, desramações e pastagem. Investigámos os efeitos do fumo na germinação da semente de *L. camara*. As sementes fumadas iniciam a sua germinação mais cedo do que as não fumadas. A proporção de sementes que germinaram, e os valores do Índice de Velocidade de Germinação (GVI) e do Índice de Vigor (VI) foram também maiores nos tratamentos fumados do que no de controlo. Além disso, a mortalidade da semente foi também menor no caso das sementes fumadas. A nossa experiência indica que o fogo pode induzir a germinação da *L. camara*. Para além dos métodos mecânicos e químicos de controlo, a queima é usada como método de controlo da *L. camara*. Esta prática necessita ser desencorajada porque pode promover - em vez de conter - a expansão da *L. camara* nas áreas queimadas.

Key words: Fire, invasion, *L. camara*, management, recruitment, seed germination, smoke.

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Introduction

Fire is known to stimulate germination in a number of plants. For example, many species from the chaparral (southern California), kwongan (Australia), and fynbos (South Africa) ecosystems respond quickly to fire-induced germination signals (Sparg *et al.* 2005). Fire may lead to enhanced germination due to heat shock, or due to exposure to chemicals produced by fire or smoke (Keeley & Fotheringham 1998). Smoke exhibits stimulatory effects on seed germination in species from both fire-prone habitats and fire-free habitats (Pierce *et al.* 1995) and plays an important role in seedling recruitment during post-fire vegetation recovery (Weekley & Menges 2003).

Fire is also known to support invasive species proliferation by creating favourable conditions for their spread, either by their self-perpetuation and faster recolonization on burned areas (Brooks *et al.* 2004; D'Antonio 2000; D'Antonio & Vitousek 1992; Mack & D'Antonio 1998) or through enhanced seed germination (Hughes *et al.* 1991; Radford *et al.* 2001; Richardson & Bond 1991; Thomson & Leishman 2005). In this way a positive feedback between invasive species and fire occurs, which can result in major community- and ecosystem-level effects, changing local community dynamics, altering biodiversity, and generating long-lasting consequences on biogeochemical cycles (Grigulis *et al.* 2005; Keeley 2002; Mack & D'Antonio 1998).

Lantana camara L. (Family Verbenaceae) is an invasive shrub belonging to the tropical Americas. It has affected forests and grasslands of more than 60 countries across the world (Parsons & Cuthbertson 2001). *Lantana* disrupts succession, decreases biodiversity, and can reduce vigour of native plants due to allelopathy (Day *et al.* 2003; Holm *et al.* 1979). Disturbed open habitats, for example, along roads and railway tracks (Day *et al.* 2003), and canopy gaps provide favorable places for its invasion (Raizada *et al.* 2008). It can greatly alter fire regimes in natural systems (Day *et al.* 2003) and is reported to proliferate well after fires. It usually flowers early in the first growing season and is capable of flowering all year round if adequate moisture and light are available (Day *et al.* 2003; Duggin & Gentle 1998). Flowers are efficiently pollinated by butterflies and thrips, and sunbirds (in India), and humming birds (in Brazil) resulting in 85 % fruit set (Hilje 1985). Its seeds are capable of germinating any time of the year if sufficient soil moisture, light, and warmth are available (Duggin & Gentle 1998; Parsons & Cuthbertson 2001). Although seed germination rate in *L. camara* is low (4-45 %) due to seed dormancy, low seed viability, and meiotic instability (Duggin & Gentle 1998; Sahu & Panda 1998), low seedling mortality (Sahu & Panda 1998) and fast vegetative spread compensate for this deficit.

Although *L. camara* is known to proliferate and germinate well after disturbances such as grazing, cutting, and fire (Duggin & Gentle 1988; Gentle & Duggin 1997; Hiremath & Sundaram 2005), little is known about the germination requirements of *L. camara* seeds, especially in relation to fire and smoke. The objective of the present study, therefore, was to investigate the effects of smoke on *L. camara* germination.

Materials and methods

The present study was conducted in the polyhouse of the Botanical Garden of the Department of Botany, Banaras Hindu University, Varanasi. The University is located at 25° 18' N latitude and 80° 01′ E longitude with an elevation of 126 m above sea level. Varanasi has a tropical monsoonal climate with mean annual rainfall of 821 mm.

L. camara flowers year-round and its fruits are small, about 4-8 mm in diameter, hard and green when unripe, turning fleshy and purplish-black when ripe. The fruits have a single stony seed (Fig. 1) (Stone 1970). Germination of *L. camara* seeds is slow, and even under favorable conditions only up to 45 % germinate.

For this study, mature fruits of *L. camara* were collected from full grown flowering branches of approximately 50 plants from the local population. Fresh seeds (< 4 weeks old) were used for the experiments. *L. camara* seeds weigh nearly 12 to 14 mg, with moisture content varying from 0.006 % to 0.009 %. The seed has two embryos enclosed within a hard stony endocarp. Both embryos are viable and capable of germinating, but germination of both happens only rarely (Rao 1920). The viability of seeds was tested with 2,3,5-triphenyltetrazolium chloride (TTC) (ISTA 1993). Individual seeds were dissected to expose the embryo; embryos were defined as either fully formed or shriveled. Only 33 % of the total seeds dissected had embryos in them; 76 % of these seeds had single embryos and the remainder had two embryos.

Fig. 1. Unripe fruits (a), de-pulped seeds (b), sectioned seeds showing TTC-stained pink viable embryos (c), and double recruitment from a single seed (d) in *Lantana camara*.

Smoke treatment

De-pulped seeds were exposed to smoke by germinating them on smoke-fumigated filter papers. Seeds were de-pulped by rubbing seeds soaked overnight in water against a wire mesh. For the germination experiment the method described by Dixon *et al.* (1995) was followed, with seeds exposed to smoke using smoked filter paper, instead of direct exposure to smoke or fire. Three smoked filter papers were produced by hanging dry 9 cm Whatman seed testing papers (no. 182) in a fumigation tent and exposing them to smoke for 90 min. Smoke used for filter paper fumigation was generated by burning nearly 2-2.5 kg of mixed litter found in the dry tropical forest from where *L. camara* seeds were collected.

Seeds of *L. camara* were surface sterilised in 1 % sodium hypochlorite for 3-4 min, and washed in several changes of distilled water before transfer onto triplicate sets of plastic germination trays, with smoked or un-smoked (control) Whatman filter papers. A total of 100 seeds per tray were used in the experiment. The criterion for germination was radical emergence of at least 1 mm (Thomas *et al.* 2003).

Different germination traits like Germination Velocity Index (GVI), Vigor Index (VI) and Seedling Mortality (SM) were assessed to evaluate the impact of smoke on seed germination. The GVI indicates speed of germination, and was calculated using the following formula (Woodstock 1976):

$$GVI = \frac{N_1}{1} + \frac{N_2}{2} + \frac{N_3}{3} + \frac{N_4}{4} + \text{------- and so on.}$$

Here, N_1, N_2, N_3 and N_4, etc., are the number of new germinants on days 1, 2, 3, 4, etc., following the start of the germination test. Since the number of new germinants on a particular day is divided by the serial number of that day, the value of GVI is higher if more seeds germinate in the fewest number of days.

The VI indicates total proportion of a seed lot that can be used for the purpose of planting. It combines mean percent germination and average length of seedlings at a particular age, and is calculated using the formula:

VI = Mean percentage germination × Average length of seedlings at the age of ten days.

Seedling mortality (SM) is expressed as the proportion of all seedlings that die within ten days of germination, and is calculated using the formula:

$$SM = \frac{\text{No. of seedlings that died within 10 days of germination}}{\text{Total no. of seedlings emerging}} \times 100$$

During the course of the germination test, seedlings that died were counted before discarding. The effect of smoke on mean seed germination and germination traits was analyzed using t-tests in SPSS Version 10.0.

Results and discussion

Since *L. camara* seeds are of medium size they tend to get buried deep in the litter layer. When there is a fire, seeds in deeper litter remain protected from fire and are exposed only to smoke while seeds that come into direct contact with fire are burnt and destroyed.

Smoke significantly enhanced seed germination ($F_{1,4}$ = 23.143, p = < 0.01) in *L. camara*. Out of 100 seeds, 16 ± 1.3 and 21 ± 1.2 seedlings were recruited in non-smoked and smoked conditions, respectively. In the case of smoked seeds, a third of the 21 seedlings were the result of recruitment of double seedlings from a single seed (Fig. 1).

Smoke exposure hastened seedling recruitment. Smoke-exposed seeds started germinating within a few weeks, while in non-smoked seeds germination only started after 2 months (Fig. 2).

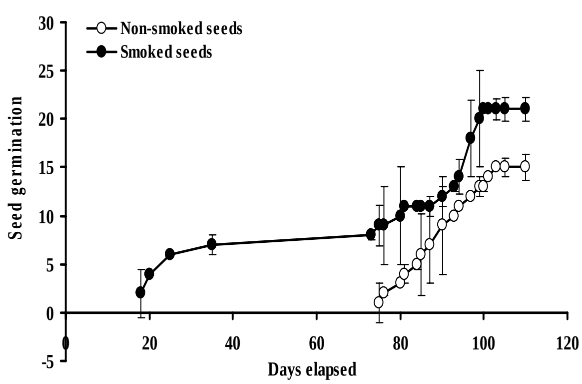

Fig. 2. Cumulative numbers of germinated *L. camara* seeds as a function of days after exposure to the experimental treatments.

There are two distinct phases of germination, one between 20 to 80 days and the other after 100 days for both smoked and non-smoked seeds. The treatments showed marked differences in the first phase of germination, while during the second phase germination was similar in the case of both treatments. It is likely that the effect induced by the smoke wore off after the first phase, so germination of seeds in the two treatments was similar thereafter.

The faster germination in smoked seeds led to a significantly higher value of the germination velocity index ($F_{1,4}$ = 18.263, p = < 0.05) compared to seeds in the control treatment. Further, the seedlings recruited from smoked seeds also had significantly higher values of the vigour index ($F_{1,4}$ = 57.627, p = < 0.01). The healthier seedlings emerging from the smoked seeds led to lower seedling mortality, as would be expected, although seedling mortality did not differ significant between treatments ($F_{1,4}$ = 1.730, p = 0.259) (Fig. 3).

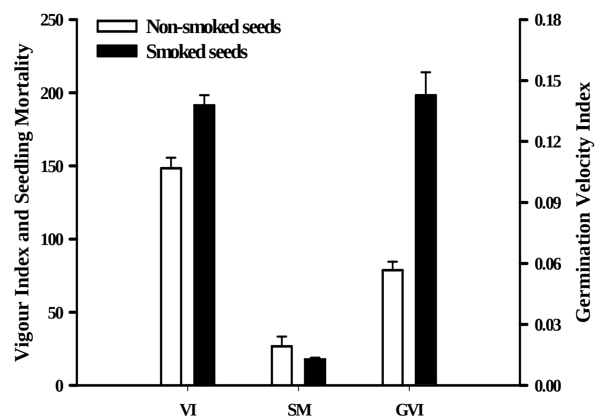

Fig. 3. Germination Vigour Index (GVI), Seedling Mortality (SM), and Vigour Index (VI) of smoked and non-smoked *L. camara* seeds.

Smoke has a combination of chemicals (Baldwin *et al.* 1994) and has been known to enhance germination in several species. Enhanced germination has been reported in both native and invasive species, but enhanced germination in invasive species is of greater concern. According to Keeley & Fotheringham (1997), smoke can enhance the permeability of sub-dermal cuticle in dormant seeds. Smoke is also found to be effective in enhancing germination in species with slow germination (Baskin & Baskin 1988; Flematti *et al.* 2004; van Staden *et al.* 2004). Nitrogen oxides (NOx) present in smoke are thought to be the reason for increased permeability of hard seeds, either directly due to oxidation effects, or after their hydration as acids.

Seed germination in *L. camara* is known to be stimulated by warm temperatures, light, and high soil moisture. It could be that chemicals present in smoke may help to enhance water absorption due to enhanced permeability and may lead to greater seed germination.

Observations from the present study show that smoke triggers faster germination and results in healthier seedlings in *L. camara* compared with un-smoked controls. It may be expected that greater germination would also confer greater invasiveness on *L. camara* and could play a pivotal role in its colonization of burnt ecosystems. Our study suggests that the use of fire as a management option to control *L. camara* should be discouraged, because fire may result in encouraging, rather than in checking, its spread.

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