

Inherently Delayed Germination of Elgon Teak (*Olea capensis*) Seeds in Kakamega Forest, Kenya

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ABSTRACT *Olea capensis* in Kakamega forest does not show any evidence of natural regeneration under the parent trees. This study sought to determine the factors that may be responsible for the lack of natural regeneration under the adult parent crowns in the Kakamega forest, Kenya. The study was carried out between November, 1999 and August, 2000 which marked the end of the fruiting period when one would expect to find a lot of germinated seeds on the forest floor. Contrary to this expectation, there was no observed germination. It was, therefore, envisaged that chemical inhibition by the parent tree may be responsible for the lack of seed germination and establishment under the parent crowns. Three principal bioassay methods were used to test whether chemical interaction indeed may be a possible mechanism inhibiting the germination and establishment of seeds and seedlings in the immediate vicinity of *Olea* adults inside the forest. To distinguish allelopathic interactions from others such as seed inviability, bioassays were applied on experimentally germinated seeds in the nearby forest tree nursery where sprouting of buds and shoots was monitored and measured. Controls were watered with distilled water. Live-shoot bioassay significantly inhibited seed germination. Both shoot and root leachates significantly ($p \leq 0.05$) reduced shoot and root budding in young experimental seedlings in the forest tree nursery. Shoot leachates appear to be the most effective inhibitors of seed germination and establishment of *Olea* seeds and seedlings inside the Kakamega forest. Shoot leachates also significantly ($p \leq 0.05$) retarded the growth of *Olea* seedlings. This has practical implications for the regeneration of *Olea* in the Kakamega forest and explains the clumped distribution of *Olea* adults in the forest. Inhibitory substances appear to delay germination of seeds for periods long enough for predation to occur. The implication is, only seeds that are dispersed distances away from *Olea* adults will germinate and establish, and the dispersal pattern determines the eventual spatial distribution.

INTRODUCTION

The idea that plants influence each other by chemical means (allelopathy), through competition for mineral nutrients (Muller 1969; Rice 1974; Harper 1977; Smith, 1984; Friedman and Walker 1985; Kumari and Kohli 1987; Herms and Mattson 1992; Berkowitz et al. 1995; Callaway and Walker 1997; Guarigata 2000; Bais et al. 2004; Kato-Noguchi 2004; Pablo et al. 2004), has been used to explain in part, patterns of spatial distribution and abundance in natural communities. Allelopathy, the production of biomolecules by one plant consisting mostly of secondary metabolites which can affect another plant adversely or beneficially has a number of community cited effects that include reduced seed germination and seedling growth (Rizvi et al. 1999; Kruse et al. 2000). Studies by Webb et al. (1967), on an Australian rainforest tree *Grevillea* sp. revealed that roots of the parent tree produced a water-soluble substance that inhibited the germination and establishment of adjacent seedlings

of the same species. Chemical inhibition, its common occurrence and significance in the field represents as very active area of chemical ecology (Harper 1977; Baldwin and Callahan 1993; Callaway 2002; Hierro and Callaway 2003).

An interesting feature of the Elgon teak (*Olea capensis*) in the Kakamega forest is that its seeds hardly germinate under its crown (Tsingalia 1988, 2009). One would expect at least some *Olea* seeds to germinate amid the factors that mitigate enhancement of germination on the forest floor below its crown (Tsingalia 1989). This rarely happens for *Olea* seeds even during peak fruiting and seeding season.

The objective of this study was to determine the factors that inhibit the germination of *Olea* seeds under the parent crowns. Chemical inhibition if proven, would delay germination for periods long enough for seeds (and perhaps fruits) to be attacked by either seed predators or by fungal pathogens. Such chemical inhibition would probably result from the parent adult *Olea* trees (Smith 1984; Strandberg and Strandberg 2000)

and this may have implications for the distribution of *Olea* adults in Kakamega forest (Tsingalia in press). *Olea capensis* is a canopy dominant in the Kakamega forest (Tsingalia 1988, 1989; Fashing et al. 2004) and the lack of germination and establishment of its seeds has generated a lot of interests in the possible allelopathic influences on the germination of the its seeds. Besides, this tree is a valued commercial timber species whose regeneration is of paramount importance to commercial forestry.

METHODS

Bioassays

Three principal bioassay methods were used to test allelopathic influences of *Olea* adults on their seeds and seedlings, and to determine whether chemical interactions indeed may be a possible mechanism inhibiting the germination and establishment of seeds and seedlings in the immediate vicinity of *Olea capensis* adult trees inside the Kakamega forest (Stowe 1979; Decker et al. 1983; Becerra et al. 2004; Kato-Noguchi 2004). The first bioassay method involved the use of live-root material. The objective was to test the possibility that phytotoxins exuded from living roots may in fact inhibit germination of seeds on the forest floor under the parent tree. Roots were taken from mature *Olea* trees in the forest. They were pounded to produce a fine mash which was soaked in distilled water to give a 5 per cent (by weight) solution. This solution was constantly stirred for two hours using a sterilized glass stirrer. The solution was filtered using a fine cloth. The extract was then used in the germination of *Olea* seeds. Fifty seeds in triplicates were used in this experiment. All the seeds were germinated on filter papers in Petri dishes under field conditions in a protected area within the forest tree nursery in order to emulate natural conditions under which seeds germinate.

The second bioassay method involved the use of decomposing material: decomposing material bioassay. The intention was to test whether decomposing plant material in contact with the seeds released phytotoxins that would inhibit germination. Ten quadrats of 25cmx25cm were established at random positions under the parent crowns of five randomly selected adult *Olea* trees. Soil sub-samples of upto 15cm deep were extracted from these quadrats. The sub-samples

were then thoroughly mixed. All live vegetation and solid decomposing materials were removed and the remaining litter bioassayed by soaking in distilled water for 24 hours. The mixture was then decanted and sieved using fine fabric. The filtrate was then applied to experimental seeds in Petri dishes.

The third bioassay method involved the use of live-shoots. This was based on the premise that rain can leach phytotoxins from live intact shoots and subsequently affect seed germination and establishment. Live shoots were collected from adult trees and sprayed with distilled water until about 300ml of leachate was collected. Two layers of filter paper were moistened with about 20ml of the leachate in five Petri dishes. Fifty dried seeds were then placed in each Petri dish. For controls, distilled water was used. Both experimental and control Petri-dishes were then incubated for 55 days at 22-25°C under normal laboratory conditions. At the end of 55 days, germinated seeds were tallied. The lengths of radicles were measured periodically during germination to provide information on the vigour of germination.

Testing for Allelopathic Effects

Tests on seedlings were also used to distinguish allelopathic from interactions like seed inviability resulting perhaps from seeds that fall before they mature (Fuerst and Putman 1983). The aim was to test the hypothesis that seedlings under parents may die in part from intraspecific chemical reactions.

Triplicates of five seedlings (a total of fifteen seedlings) per treatment were set up in the forest nursery. Three groups of seedlings were set up of which two groups were subjected to live-shoot and root bioassays as described above. The third group of seedlings was used as a control. Seedlings were watered daily with bioassay solutions for a period of 60 days. Sprouting or lack of sprouting of buds in seedlings was used as evidence of chemical inhibition or its absence. Data analysis was done using Seaby and Henderson (2006) QED Statistical package and Sokal and Rohlf (1995).

RESULTS

Results of the live-root bioassay did not show evidence of chemical inhibition by root leachates

on the ability of seeds to germinate. Of the fifty treated and control seeds, 46 and 47 seeds germinated respectively. There was no significant difference in mean percentage germination rates between experimental (92.0%) and control (94.0%) seeds ($t=-0.29$, $p=0.39$). Figure 1 shows the differences in germination rates between control and experimental seeds. There was no significant difference in the speed of germination of seeds between the two groups of seeds (Kolmogorov-Smirnov sample test, $p<0.01$).

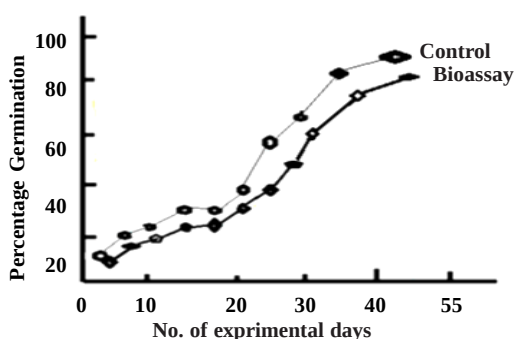


Fig. 1. Effects of live root bioassay on the germination of *Olea* seeds

Forty-eight of the fifty bioassayed and forty-six of the control seeds germinated in the decomposing material bioassay. There was no significant difference in mean germination rates between controls (96.0%) and experimental (92.0%; $p<0.05$). Figure 2 compares the difference in percent germination rates between the two groups. There was no difference in the rapidity of germination between experimental and control seeds. Decomposing material from the forest floor did not have any inhibitory effects on the germination of the *Olea* seeds.

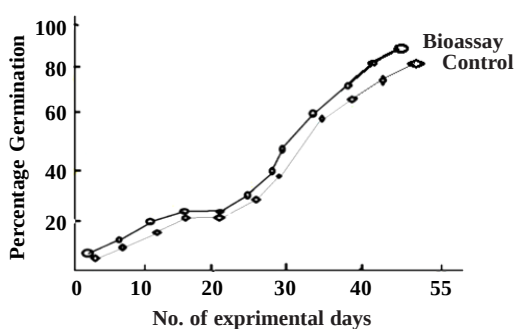


Fig. 2. Effects of decomposing material bioassay on the germination of *Olea* seeds

Figure 3 shows the differences in mean percent germination rates between experimental and control seeds. One control experiment was used for all the three experimental seed treatments. Germination rates were very similar towards the end of the experiment; perhaps as a result of continued use of distilled water to water the seedlings in order to prevent them from drying.

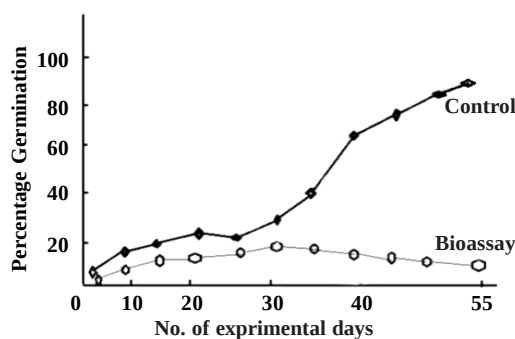


Fig. 3. Effects of shoot bioassay on percent germination of seeds

This appears to have significantly eliminated the inhibitory effects of the inhibitory chemicals involved through the "washing effect".

Shoot leachate significantly reduced the shoot growth in the experimental seedlings ($\chi^2=8.075$, $p=0.0176$). Root leachate also inhibited shoot growth. There was no statistical significance difference in the effects by shoot and root leachates on production of new shoots ($\chi^2=0.375$, $p=0.825$; Fig. 4).

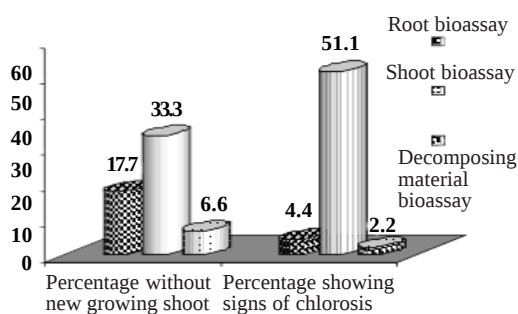


Fig. 4. Effects of root, shoot and decomposing material bioassays on percentage shoot sprouting and chlorosis in *Olea* seedlings

DISCUSSION

Of the three bioassays used in this study, only one produced significant inhibition of seed ger-

mination (Fig. 3). Leachates of shoots significantly inhibited germination than either root or decomposing materials from the forest floor. It has been suggested that growing shoots produce chemicals whose primary effects are protection from herbivory by phytophagous arthropods and other folivores (Swain and Lieberman 1987; Rosenthal and Janzen 1979; Waterman 1983). It might, therefore, appear that inhibition of germination of seeds may be a consequence rather than function of the phytochemicals (Francois 1993; Pidwirny 2006).

Shoot leachates also retarded the growth of seedlings of *Olea* suggesting that even if seeds were to germinate, establishment would be difficult in the forest. Since most seeds do not germinate, this stage of *Olea* regenerating appears to be of little practical significance in the field.

Results clearly show that both the live-root and decomposing material bioassays did not significantly affect percent germination of seeds (Figs. 1 and 2). Only the live-shoot bioassay significantly slowed down the germination. Shoots and root leachates also significantly inhibited the growth of shoots in experimental seedlings (Fig. 4).

The absence of inhibition of live-root bioassay contradicts a number of similar studies in which the root extracts have been shown to inhibit seed germination (Wilson and Rice 1968; Parent and Rice 1969; Newman and Rovira 1975; Kato-Noguchi 2004). This difference may have been due to the concentration of the bioassay used in this experiment. In earlier experiments, relatively larger doses of the bioassays were used while relatively smaller quantities of the bioassay were used in this experiment. In any case, inhibitory effects produced by high concentrations of inhibitory substances are, however, unlikely to be ecologically relevant.

Inhibition by phytotoxins in shoots has also been demonstrated by Stowe (1979) in an investigation in an old field in Illinois and in spruce germination (Francois 1999) in the United States of America. For germination of *Olea* seeds to be inhibited means that seeds must imbibe water soon after falling on the forest floor in readiness for germination. This is ensured by the fact that *Olea* seed fall at a time when there is ample rain to trigger germination. Such throughfall would necessarily be drops from shoots and leaves of the parent tree. Chemicals that inhibit germination and that may be dissolved in this water would

certainly be imbibed by the seeds. The result is delayed germination (Callaway and Waller 1997). This appears to confirm observation by forest nursery workers at the Kakamega forest that *Olea* seeds take on average 30 days or more to germinate. Most do not, however, germinate at all. This has caused great frustration to foresters in their efforts to grow *Olea* seedlings in forest tree nurseries around the Kakamega forest for reforestation.

The standard procedure for growing *Olea* seedlings in the forest tree nurseries is to collect seeds from the forest under the crown of fruiting *Olea* adults. Such seeds are then air-dried and placed in seed beds for germination. It appears that chemical inhibition may be at play at inhibiting the germination of *Olea* seeds inside the forest.

In summary, chemical inhibitors may delay germination of seeds and fruits for a period long enough for predation to occur. In some cases, however, these inhibitors, if imbibed in high concentrations during peak long rain season may inhibit germination completely as evidenced in seeds that fail to germinate completely in the forest tree nurseries.

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