STUDIES ON GERMINATION AND SEEDLING ESTABLISHMENT OF *Napoleonaea vogelii* HOOK. & PLANCH. AND *Diospyros barteri* HIERN. UNDER DIFFERENT EXPERIMENTAL CONDITIONS

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DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, UNIVERSITY OF BENIN, BENIN CITY, IN PATRIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MASTERS OF SCIENCE IN PLANT BIOLOGY AND BIOTECHNOLOGY

SEPTEMBER 2019

CERTIFICATION

We certify that this project was carried out by Ejiroghene Okugbere with matriculation number **PG/LSC1209531** in the Department of Plant Biology and Biotechnology. Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

Prof. D.E Vwioko Date

Supervisor

Prof. (Mrs) F.I Okungbowa Date

Head of Department

DEDICATION

To God Almighty, who wishes for nothing but the very best for me.

ACKNOWLEDGEMENTS

I wish to say thanks to God whose love and mercies have kept me

My profound gratitude goes to my supervisor. Prof. D.E Vwioko who has been a teacher, a father and a model to me. I also wish to thank Prof Osawaru, who has been a source of encouragement and support. My profound gratitude also to the Head of Department, Plant Biology and Biotechnology, Prof.(Mrs.) F.I Okungbowa and all staff of the department.

To my beloved sister and her husband, Mr. and Mrs Saniyo who supported me financially through the program, I wish to say a very big thank you. May God continually bless you guy. Also, to the rest of my family- Niimo, Ena, Akp, Ruky, Ofe, Tsolaye and Maye, you have been wonderful.

To baba Uyi, for the role you have played as a friend and brother, I wish to say a big thank you and to my friends Osawaru, Uruemu, Solomon, Semi and Steve I couldn't ask for a more terrific family. Love you always.

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ABSTRACT

Hydropriming has proved to be the most potent form of seed germination enhancement in not just final germination but also in speed of germination and time taken to reach 50% of total germinated seeds of *Napoloenaea vogelii* Hook. and Planch. Priming seeds in solutions significantly affected seed germination ($P<0.05$), with smoke water treatments, hydro and halopriming having significantly higher germination means Maximum germination percentage was observed in hydropriming with 90% germination while Halopriming (NaCl 2, and 4g/l) had total germination percentages of 85% each. *Napoleonaea vogelii* seeds also responded positively to smoke water treatment for 10 and 20 minutes with 80% and 77.50% respectively. Effect of bush fire proved to be deleterious, showing the lowest germination percentage of 32.50%. Seeds collected from decaying fruits on trees has low mean germination percentage of 34.17%. The effect of various treatments was significant on the speed of germination. All forms of priming showed improved speed of germination with hydropriming being superior with a mean speed of 2.99 day^{-1}. Control showed the least speed with 0.39 day^{-1}. Plants in complete darkness showed the highest mean growth in height (32.42cm) while those in 24hrs light showed lowest growth in height (18.88cm). Hydropriming and Halopriming (2g/l) also showed significant growth in heights (24.12cm and 24.08cm respectively). The seeds of *Diospyros barteri* Hiern. did not show any germination under all treatments given to seeds.

CHAPTER ONE

INTRODUCTION

1.0 INTRODUCTION

There have been increased risks of species extinction as the rapid rates of forest loss and degradation across the tropics have continued to increase. In Nigeria as elsewhere, conservation of forest genetic resources is achieved through the protection of these resources in their natural habitat (*in situ*) or preservation of samples of the genetic diversity of endangered species away from their field habitats (*ex situ*) in facilities such as botanical gardens, seed gene banks, *in vitro* gene banks, and field gene banks (Owonubi and Otegbeye, 2004).

A seed represents the termination of a generation, as well as the beginning of a new generation in the life cycle of a plant. As the connection between generations, seeds pass along genetic information and maintain each species' characteristics. Ideally, seeds should germinate immediately after ripening to maximize reproduction (Fenner and Thompson, 2005). However, ideal germination conditions may not be common, since seed germination is influenced by various environmental factors, including light, moisture, and temperature, that may fluctuate in complex ways. To

preserve productivity in uncertain conditions, many species developed self-protecting mechanisms, such as seed dormancy (Fenner and Thompson, 2005).

Seedling establishment is a critical stage in the life history of any plant species that relies on sexual reproduction for the persistence of its populations (Bu *et al.*, 2008). Variations in germination percentage are often interpreted as reflecting adaptations to specific ecological conditions (Grime *et al.*, 1981).

1.1 DEFINITION OF SEED GERMINATION

Germination begins with water uptake by the seed (imbibition) and ends with the emergence of the embryonic axis, usually the radicle, through the structures surrounding it. This latter event is sometimes referred to as "visible germination," at which point the seed has completed germination (Bewley *et al.*, 2013)

Various definitions of seed germination have been proposed, and it is important to understand their distinctions. Seed physiologist define germination as the emergence of the radicle through the seed coat. This says nothing about other essential structures such as the epicotyl or hypocotyls that become the above ground parts of a successful seedling (Pandey, 2015). To a seed analyst, germination is "the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favorable conditions." (Pandey, 2015). This definition focuses on the reproductive

ability of the seed, an essential objective in agriculture (Reddy *et al.*, 2001). Does it have the capacity to produce a normal plant? Others consider germination to be the resumption of active growth by the embryo resulting in the rupture of the seed coat and emergence of a young plant. This definition presumes that the seed has been in a state of quiescence, or rest, after its formation and development. During this period of rest, the seed is in a relatively inactive state and has a low rate of metabolism. It can remain in that state until environmental conditions trigger the resumption of active growth. Regardless of which definition is preferred, it should be emphasized that one cannot actually see the process of germination unfold. Therefore, all definitions include some measure of seedling development, even though this occurs subsequent to the germination event.

However, for brevity, the word germination is often used to indicate its completion, e.g., terms like "50% germination" indicate that 50% of a seed population has completed germination

PHASES OF GERMINATION

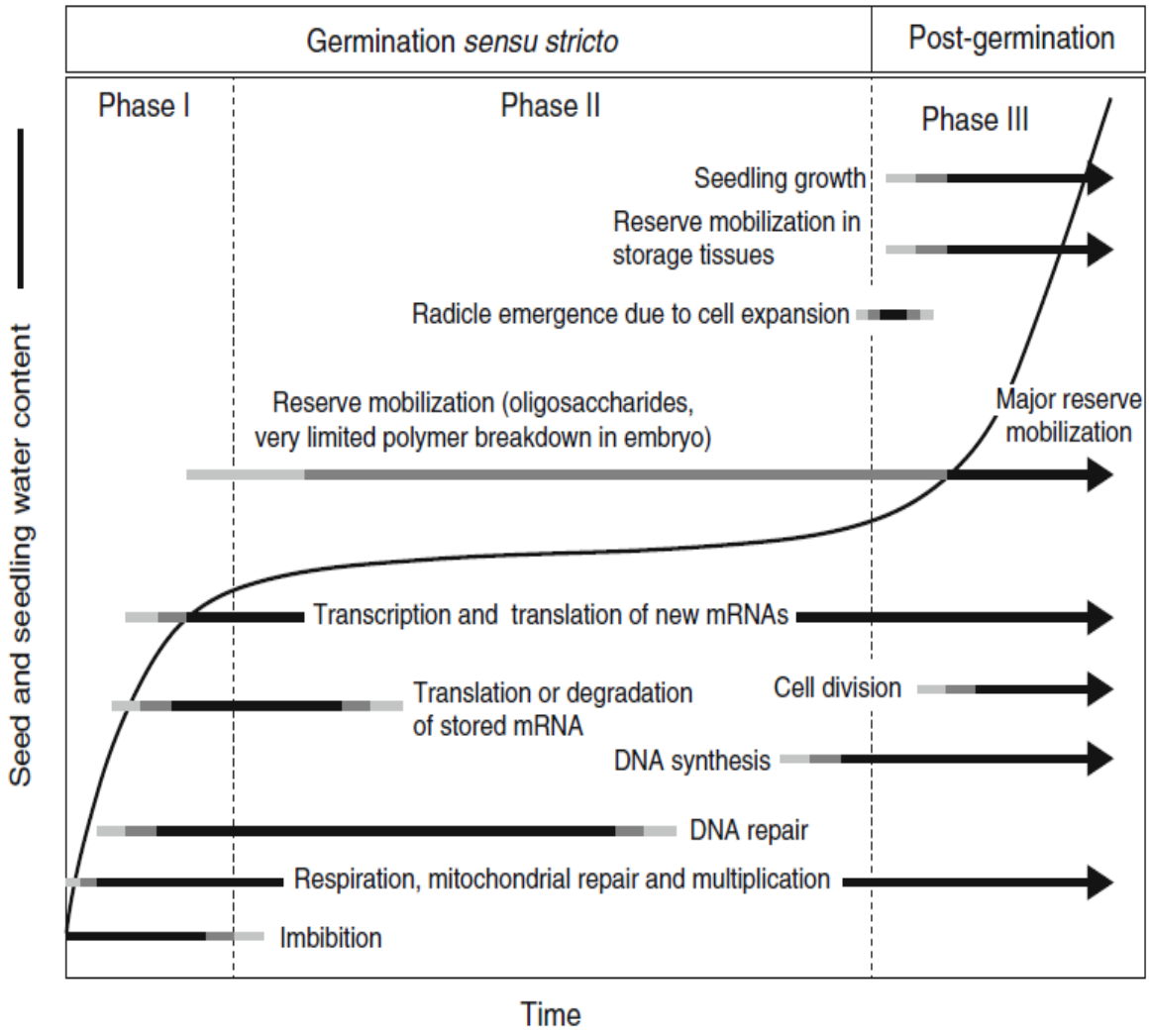

Fig. 1.1 Time course of water uptake and some important changes associated with germination and early seedling growth. Initial absorption of water, imbibition in Phase I, is primarily a physical process; physiological activities may commence within minutes of a cell becoming hydrated, well before all seed tissues become fully imbibed. During Phase II seed water content is fairly constant and metabolic activities increase with substantial transcription of new genes. Radicle emergence through the surrounding structures at the end of this Phase marks the completion of germination, and in Phase III there is further uptake of water as the young seedling becomes established, utilizing the major stored reserves. The curve is a stylized time course for water uptake. The time taken for these events to be completed varies among species and the germination conditions to which the seed is subjected. After Bewley *et al.* (2013).

1.2 SEED DORMANCY

In lots of naturally reproducing plant populations, there could be a delay between time of seed shed and subsequent germination. This could have a significant ecological advantage. Postponing emergence clearly allows extra time for dispersal to potentially greater distances. In any region with seasonal variations, it has the additional advantage that the germination of at least some seeds will coincide with the most favorable season for seedling growth and establishment (Gosling, 2007).

Natural selection has therefore favoured many species with seeds which do not germinate immediately after dispersal– even when the environmental conditions are ideal (Gosling, 2007).

When seeds are inactive because they are ‘immature’ or possess a layer of tissues that prevent water uptake or gaseous exchange, they are said to be ‘quiescent’. But when seeds can be shown to be fully hydrated and alive yet still remain outwardly inactive– they are said to be ‘dormant’(Gosling, 2007).

1.2.1 DEFINITION OF DORMANCY

Despite the importance of this subject, there is no clear and unique definition for seed dormancy. This lack of consensus may be due to dissimilar perspectives from different disciplines about this phenomenon. For example, what a seed physiologist

considers to be a dormant seed may be different from what an ecologist or seed technologist considers a dormant seed. The different types of seed dormancy mechanisms and treatments to overcome these dormancies further complicate a universal consensus on the definition.

Two common definitions for seed dormancy are

1. A mechanism that prevents germination of a seed at an inappropriate time
2. The absence of germination of an intact, viable seed under germination favoring conditions within a specific time lapse". (Hilhorst, 1995)

In these definitions, the description of "inappropriate time" or what "germination favoring conditions" is, are vague. If the germination favoring conditions for a particular species are optimal conditions used for the standard germination test for that species, this implies that after the standard germination test, any viable seed that fails to germinate would be considered dormant, and those that germinate (normal or abnormal) are non-dormant. In this case, a seed lot may have different levels of dormancy, from 0% if all the seeds germinate to 100%, if all the seeds are viable but do not germinate. Using this definition, however, each seed does not have a level of dormancy, it is simply dormant or non-dormant. In reality, this is not the case.

Seed dormancy has also been defined as: "*an innate seed property that defines the environmental conditions in which the seed is able to germinate*" (Finch-Savage and Leubner-Metzger, 2006). According to this definition, dormancy is not only associated with the absence of germination, but it is a seed characteristic that determines the conditions required for germination. This definition better fits the results of many studies in seed germination and dormancy. Many of these deal with seeds possessing different levels of non-deep dormancy and dormancy is evaluated according to the ability of the seed to germinate under different conditions, for instance: light or dark, different temperatures, different water potentials or different external ABA concentrations.

To be released from dormancy, a seed must thus experience certain environmental factors for minimal lengths of time, the perception of which induces metabolic and structural changes within the seed that favor germination. Based on the above considerations, seed dormancy can now be defined as a temporary failure of a viable seed to complete germination under favorable conditions. (Bewley *et al.*, 2013)

Dormancy is a trait that has been acquired by many species during evolution by selection for the ability to survive in unfavorable environments, such as heat, cold and drought (Bewley *et al.*, 2013).

The origin of dormancy is possibly related to climatic changes that have occurred during the Earth's history. The number of plant species with seed dormancy tends to increase with the geographical distance from the equator, i.e., as seasonal variation in precipitation and temperature increases. (Bewley *et al.*, 2013)

Total germination is usually used to indicate dormancy change because germination is a measure of seed response to environmental conditions and germination requirements. Baskin and Baskin (2004), used germination percentage from a fixed duration germination test (4 weeks) under various environmental conditions to classify seeds into dormant (seeds fail to germinate in favourable conditions), non-dormant (seeds germinate to ≥80% in 4 weeks or less) and conditionally dormant (germinate to high percentages under some conditions, but not others). Seed dormancy promotes the persistence of seeds in the seed bank by prolonging their germination and emergence (Benech-Arnold *et al.*, 2000). The time at which a seed germinates is critical for seedling growth and subsequent survival (Bradford, 2002), because it may not be beneficial for a seed to germinate, even in favourable conditions. Seed dormancy may be desirable in some instances such as during seed development because it prevents premature seed germination, but it is generally undesirable where rapid germination and growth are desired.

1.2.2 CATEGORIES OF DORMANCY

Bewley *et al.* (2013), categorized dormancy into the following based on the cause of germination restraint.

In the case of *embryo dormancy* the properties of the embryo are of principal importance. In *coat-imposed dormancy* the properties of the covering tissues are determinative: these include mechanical, chemical and permeability features, all of which may interfere with or suppress the successful completion of germination by the embryo. Thus, in the case of coat-imposed dormancy, removal of the tissues surrounding the embryo (e.g., endosperm, pericarp, or extra floral organs) is sufficient for successful completion of germination. In the case of embryo dormancy, removal of the coat does not permit such embryos to germinate normally, and so the block to germination is, in a sense, more profound than in seeds with coat-imposed dormancy. Embryo dormancy is common in woody species, especially in the Rosaceae, but is sometimes found in herbaceous plants such as some grasses (e.g., wild oats). Very often both types of dormancy exist simultaneously or successively. In apple seeds, for example, embryo dormancy predominates, but a contribution is made by the endosperm and testa, and their removal reduces the amount of dormancy-breaking treatment (chilling) that is required.

They wrote that different types of seed dormancy also can be distinguished on the basis of the timing of the induction of dormancy rather than the location or mechanism of dormancy. Seeds that are shed from the parent plant in a dormant state display *primary dormancy.* Seeds in the soil may (gradually) acquire *secondary dormancy* if the conditions for germination are unfavorable or if seed germination is inhibited by other means, for example osmotic stress. Secondary dormancy imposed on imbibed light-requiring seeds maintained in the dark is termed *skotodormancy*, and that imposed by high temperatures is *thermodormancy* or *thermoinhibition* .

Seeds of several species display more complex patterns in which the parts of the embryonic axis differ in their depth of dormancy. In the so-called *epicotyl dormancy* (e.g., in *Paeonia* spp. and *Lilium* spp.), radicle emergence occurs readily but the epicotyl fails to grow. In *Trillium* spp. and *Caulophyllum thalictroides* , the radicle

has some dormancy but it is less deep than that of the epicotyl because the two organs differ in the duration of the chilling treatment needed to break dormancy; such cases are said to exhibit *double dormancy* .

1.2.3 ECOLOGICAL ADVANTAGES FOR PLANTS WITH SEED DORMANCY.

The function of a seed is to establish a new plant but it can do this only once, because the completion of germination is essentially irreversible. Dormancy provides a

strategy for seeds to spread germination in time in order to reduce the risk of premature death in an unfavorable environment. According to Bewley *et al.* (2013), strategy occurs in three ways:

1. Seeds are dispersed from the same parent plant with different degrees of dormancy, a phenomenon known as polymorphism, heteromorphy, or heteroblasty. Frequently, the variation in dormancy is reflected in the appearance of the seeds or dispersal units-color, size, and thickness of coat. This may also be a reflection of different levels of maturity of the seeds, for at a given moment they may be at different stages of development on the parent plant and, hence, at different levels of dormancy since this is acquired during development. When there are polymorphic seeds, germination is spread temporally, with new seedlings emerging at irregular intervals, thus reducing competition and spreading environmental risks, increasing the likelihood that some individuals will survive. Such a temporal distribution clearly can have advantages with regard to the continuation and spread of the species.
2. Dormancy also results in the distribution of germination in time through the dependence of dormancy breakage on environmental factors which in turn have their own time distribution. For example, seeds are commonly released from dormancy by being chilled, sometimes for several weeks or months at 1-5°C. Since such temperatures occur only during the winter, seeds that rely on this

means of dormancy breakage must await the passage of this cold season before they can germinate. The advantage of this strategy is that the young seedling emerges in the spring and establishes itself over the favorable succeeding months; emergence before winter would entail the risk of succumbing to the severe conditions of that season.

3. Seed dormancy can also lead to a distribution of germination in space- another aspect of its biological importance. Dormant seeds may be dispersed over long distances by wind, water, and animals; these dispersal types are called anemochory, hydrochory, and zoochory, respectively.

Dormancy also helps to create a seed bank. This includes the seeds that are shed from the plant that do not germinate for years due to dormancy. A seed bank ensures that not all seeds of a species germinate in a single year. This is insurance against seedlings being exposed to catastrophic conditions (like drought or cold) that kill the entire next generation of a species. It also allows seedlings to grow during favorable years even if the mother plants failed to flower and make seeds.

1.2.4 BREAKING SEED DORMANCY

Dormancy in seeds can be broken in many ways (Bewley and Black, 1994), and breaking seed dormancy is an important step in improving seed germination (Bell *et al*., 1993). Some seeds lose their dormancy in the dry state (after-ripening) when

their rate of metabolism is low. However, imbibed, dormant seeds are metabolically active and can receive an external signal such as light, chilling, alternating temperatures, chemical, and/or hormonal treatment that can stimulate germination. Dormancy release involves the reception of germination stimulus by the embryo and the immediate signal transduction chain that leads other metabolic and hormonal changes (Bewley, 1997). Depending on the type of dormancy, stratification, light, scarification, growth regulators, alternating temperatures and/or exposure to chemicals, may break dormancy and promote germination (Bewley and Black, 1994). Smoke and its chemical components can also break dormancy (van Staden *et al.,* 2000). Smoke can stimulate germination in numerous species such as *Lactuca sativa,* but most studies have focused on species native to fire-prone ecosystems (Drewes *et al.,* 1995). The use of smoke, temperature, and light in dormancy breaking is discussed further in the following sections.

1.3 SEED GERMINATION AND CONSERVATION OF ENDANGERED SPECIES

The reinforcement of wild plant populations using individuals raised *ex situ* is considered a valid means of reducing the risk of extinction of threatened species or

populations (Bowes, 1999). Genetic diversity of local ecotypes is maximized when plants are multiplied from seed (Fay, 1992). However, each species has particular

requirements for seed germination as a result of adaptive radiation into patchy and changing environments (Schutz and Milberg, 1997).

Propagation from seed is inexpensive and usually very effective but germination requirements for native species are often unknown, particularly for rare and/or endemic species of which material is more difficult to obtain. Many species respond well to sterile *in vitro* conditions, including a nutrient rich medium and also phytohormone supply (Fay, 1992). However, for some species these techniques are not necessary for successful seed germination and would waste resources if used, and so appropriate techniques must be selected based on the requirements of each species (Benson *et al.*, 2000).

Germination of *Napoleonaea vogelii* and *Diospyros barteri* in non-sterile conditions and without phytohormones will allow widespread propagation by regional parks and botanic gardens without the use of specialist facilities. However, seed storage and germination are only the first steps in the reinforcement of populations of these species: studies to attain baseline data on *ex situ* plant development and establishment in the field following transplantation are now required. Seeds of both species are scarce, and should extensive population reinforcement be necessary, a good understanding of their seed biology and germination requirement will be useful.

1.4 REQUIREMENTS FOR GERMINATION

1. WATER. Water is a basic requirement for germination. It is essential for enzyme activation, breakdown, translocation, and use of reserve storage material. In their resting state, seeds are characteristically low in moisture and relatively inactive metabolically. That is, they are in a state of quiescence. Thus, quiescent seeds are able to maintain a minimum level of metabolic activity that assures their long-term survival in the soil and during storage. Moisture availability is described in various ways. Field capacity moisture is about optimum for germination in soil; however, germination varies among species and may occur at soil moistures near the permanent wilting point. Most seeds have a critical moisture content for germination to occur. For example, this value in corn is 30%, wheat 40% and soybeans 50%. Once that critical seed moisture content is attained in the seed, sufficient water is present to initiate germination and the seed is committed to that event and cannot turn back. If the internal moisture content decreases below the critical moisture content, seeds will essentially decay in the soil.

2. GASES. Air is composed of about 20% oxygen, 0.03% carbon dioxide, and about 80% nitrogen gas. If one provides different proportions of each of these gases under experimental conditions, it soon becomes clear that oxygen is required for

germination of most species. Carbon dioxide concentrations higher than 0.03% retard germination, while nitrogen gas has no influence on germination.

3. TEMPERATURE. Seed germination is a complex process involving many individual reactions and phases, each of which is affected by temperature. The effect on germination can be expressed in terms of cardinal temperature: that is *minimum, optimum,* and *maximum* temperatures at which germination will occur. The minimum temperature is sometimes difficult to define since germination may actually be proceeding but at such a slow rate that determination of germination is often made before actual germination is completed. The optimum temperature may be defined as the temperature giving the greatest percentage of germination in the shortest time. The maximum temperature is governed by the temperature at which denaturation of proteins essential for germination occurs. The optimum temperature for most seeds is between 15 and 30^{o}C. The maximum temperature for most species is between 30 and 40^{o}C. Not only does germination have cardinal temperatures, but each stage has its own cardinal temperature; therefore, the temperature response may change throughout the germination period because of the complexity of the germination process. The response to temperature depends on a number of factors, including the species, variety, growing region, quality of the seed, and duration of time from harvest. As a general rule, temperate-regions seeds require lower temperatures than do tropical region seeds, and wild species have lower temperature

requirements than do domesticated plants. High-quality seeds are able to germinate under wider temperature ranges than low-quality seeds.

1.5 *Napoleonaea vogelii* Hook. and Planch.

1.5.1 CLASSIFICATION/TAXONOMY

Division: Magnoliophyta

Class: Magnoliopsida

Order: Ericales

Family: Lecythidaceae

Genus: *Napoloenaea*

Species: *vogelii*

Binomial Name: *Napoloenaea vogelii* Hook. and Planch.

1.5.2 DISTRIBUTION OF PLANT SPECIES. *N. vogelii* (Family: Lecythidaceae) is tropical flora that is exclusively African and widely distributed in the coastal regions of West African countries including Nigeria, Ghana, Liberia, Guinea and Côte *d'Ivoire*. In Nigeria, it is found predominantly in the rainforest regions. It has been reported in Osun, Ondo, Ekiti, Oyo, Edo, Delta, Akwa-Ibom, Cross-River and Imo State.

Popularly called "Ukpagberagi" by the people of Benin and "Udarutobo or Nkpodo" among the Igbo speaking people. It is one of the most potent species of Lecythidaceae that is richly endowed with pigments.

1.5.3 HABIT AND ECOLOGY. *Napoleonaea vogelii* Hook. and Planch. is an evergreen shrub or a low-branching tree (treelet) with a dense crown growing from 2 to 6 meters tall. The fruits are green when unripe, and reddish-orange when ripe.

1.5.4 FLOWERING. Flowering in the dry season and fruiting in the dry season and early rainy season, the tree bears attractive white to pale reddish flowers. It flowers sometimes on the old wood.

1.5.5 ECONOMIC IMPORTANCE. *Napoleonaea vogelii* is a sweet and edible fruit found in holly- a tree with hard prickly leaves all through the year and the ripe fruits in the dry seasons. The trees are grown wildly on a prickly rocky bush in Edo, Delta and most parts of Ebonyi State of Nigeria. The fruits are widely eaten by bush monkeys hence the local name "udarutobo", meaning monkey apple. Farmers and rural dwellers also eat the flesh of *Napoleonaea vogelii* fruits. The colorful skins also attract the attention of other animals, which may eat the fruits and disperse the seeds. Many researchers such as Muñoz-Espada *et al.* (2004), Hosseinian and Beta, (2007), Wada and Ou, (2002) and Siriwoharn *et al.* (2004), have noted that the red coloration of the fruits may camouflage them from herbivores blind to red wavelengths, or signal un-palatability, since anthocyanin often coincides with unpalatable phenolic compounds. *Napoleonaea vogelii* also showed a relatively high

crude protein when compared to other browsed plants by cattles (Ahamefale *et al.*, 2006).

Preparation from the bark of *N. vogelii* has been used in the management of cancer in the western part of Nigeria (Soladoye *et al*., 2010) Topical preparation from the stem bark has been used and found beneficial in the treatment of dermatosis, ingested to treat sexual asthenia, and extract from the leaves has been used in the treatment of external wounds (Akah *et al*., 2007).

Pushparani *et al*., (2018) reported that it is used in the treatment of ulcers, stomachaches and diarrhoea. The extract from this plant has antimicrobial activity against bacterial agents. The bark of *N. vogelii* has been used in the management of cancer. Topical preparation from the stem bark has been used and found beneficial in the treatment of dermatosis, ingested to treat sexual asthenia, and extract from the leaves has been used in the treatment of external wounds. They also reported that the methanol extract of *N. vogelii* leaves demonstrated wound healing activity and caused a significantly reduction in the wound area. Comparatively, the extract relatively accelerated wound healing significantly in the treated animals than the natural healing process.

Land use intensification through shifting agriculture was found to impact negatively on tree abundance of *N. vogelii* in an agrarian community within the Niger Delta region of Nigeria (Chima and Omoemu, 2012)

Plate 1.1- Matured fruit of *Napoleonaea vogelii* (magnification x 0.75)

Plate 1.2- The flower of *Napoleonaea vogelii* on the stem of the plant (magnification x 0.7)

Plate 1.3- Matured seed of *Napoleonaea vogelii* (magnification x 0.67)

1.6 *Diospyros barteri* Hiern.

1.6.1 CLASSIFICATION/TAXONOMY

Division: Magnoliophyta

Class: Magnoliopsida

Order: Ebenales

Family: Ebenaceae

Genus: *Diospyros*

Species: *barteri*

Binomial Name *Diospyros barteri* Hiern.

1.6.2 DISTRIBUTION OF PLANT SPECIES. The family Ebenaceae, is widely spread in tropics and subtropics. Hiern (1873), reported that it consists of 5 genera namely *Diospyros, Euclea, Maba, Royena and Tetraclis.* However, Duangjai *et al.*, (2006) proposed a new infrafamilial classification based on a phylogenetic approach,

consisting of two subfamilies, *Lissocarpoideae* and *Ebenoideae*, and four genera, *Lissocarpa* Benth., *Euclea, Royena, and Diospyros*.

The genus *Diospyros* is found in the tropics and subtropics areas. It is the only genus of *Ebenaceae* represented in West Tropical Africa, including Nigeria (Omokafe *et al.*, 2016). It is known as the ebony family with more than 350 species. There are thirty-nine species in West Tropical Africa, out of which twenty-five and additional two imperfectly known species are found in Nigeria (Hutchinson and Dalziel, 1963)

Diospyros barteri have alternate leaves which are simple and have entire margins with no stipules. The fruit is a berry, surrounded at the base by the persistent and often enlarged calyx. *Diospyros barteri* is a forest shrub or a small tree 3 to 5 feet high. It is sometimes scrambling, young plants are covered with ferrugineous hairs. The leaves are glaucous beneath. Fruits are orange and sometimes pale yellow.

1.6.3 ECOLOGY/HABITAT- In Nigeria, members of the genus *Diospyros* consist of trees and shrubs which are characterized by simple, exstipulate, entire-margin leaves, without latex; the heartwood is usually black, yielding commercial ebony. The inflorescence is cymose, solitary; the flowers are unisexual with 3–7 united sepals, 3–7 united petals and stamens, which vary from two to more than 100. The fruit is a berry, which is often surrounded at the base by a persistent calyx

(Hutchinson and Dalziel, 1963 and Keay, 1989). *Diospyros barteri* occurs naturally in comparatively dry mixed evergreen forests as an understorey tree

1.6.4 ECONOMIC IMPORTANCE

Diospyros have considerable economic importance with a lot of the species having edible fruits, ebony and valuable timbers (Sinha *et al.,* 2009). *Diospyros* species have long been known for their medicinal uses. Almost all the parts of these plants have been used as medicine e.g., the leaves are good for lumbago, fruits are carminative, astringent and cure biliousness and vata in Ayurveda, seeds are sedative, whereas bark is bitter, astringent and febrifuge (Sinha *et al.,* 2009). Many species of *Diospyros are* known to accumulate substantial amounts of naphthoquinones which are possibly responsible for the observed anti bacterial activities found in the genus (Alireza *et al.,* 2011).

Diospyros barteri locally known as Elugbe and ivin-oha has commonly been used by traditional medical practitioners as anti-infectious agent. Its twig is chewed and used for cleaning teeth among the Esan tribe of Edo State (Idu *et al.,* 2009). The methanol extract of *Diospyros barteri* was found to be effective in the control of cowpea Aphid-Borne Mosaic virus (CABMVa-onne strain) and other viruses (Moody *et al.,* 2002)

Plate 1.4- Matured fruit of *Diospyros barteri*

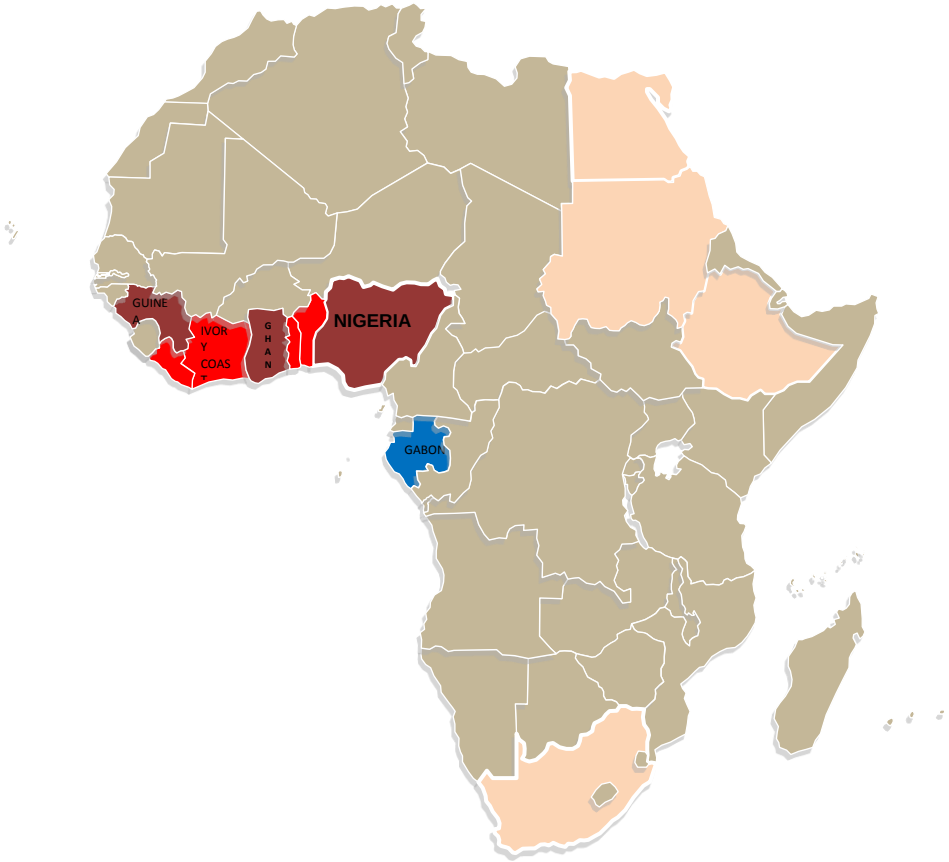

Figure 1.2: Map of Africa showing countries where *Napoleonaea vogelii* and *Diospyros barteri* have been reported

- Areas marked red indicates countries where *Napoleonaea vogelii* have been reported
- Areas marked blue indicates countries where *Diospyros barteri* have been reported
- Areas marked brown indicates countries where both plants have been reported

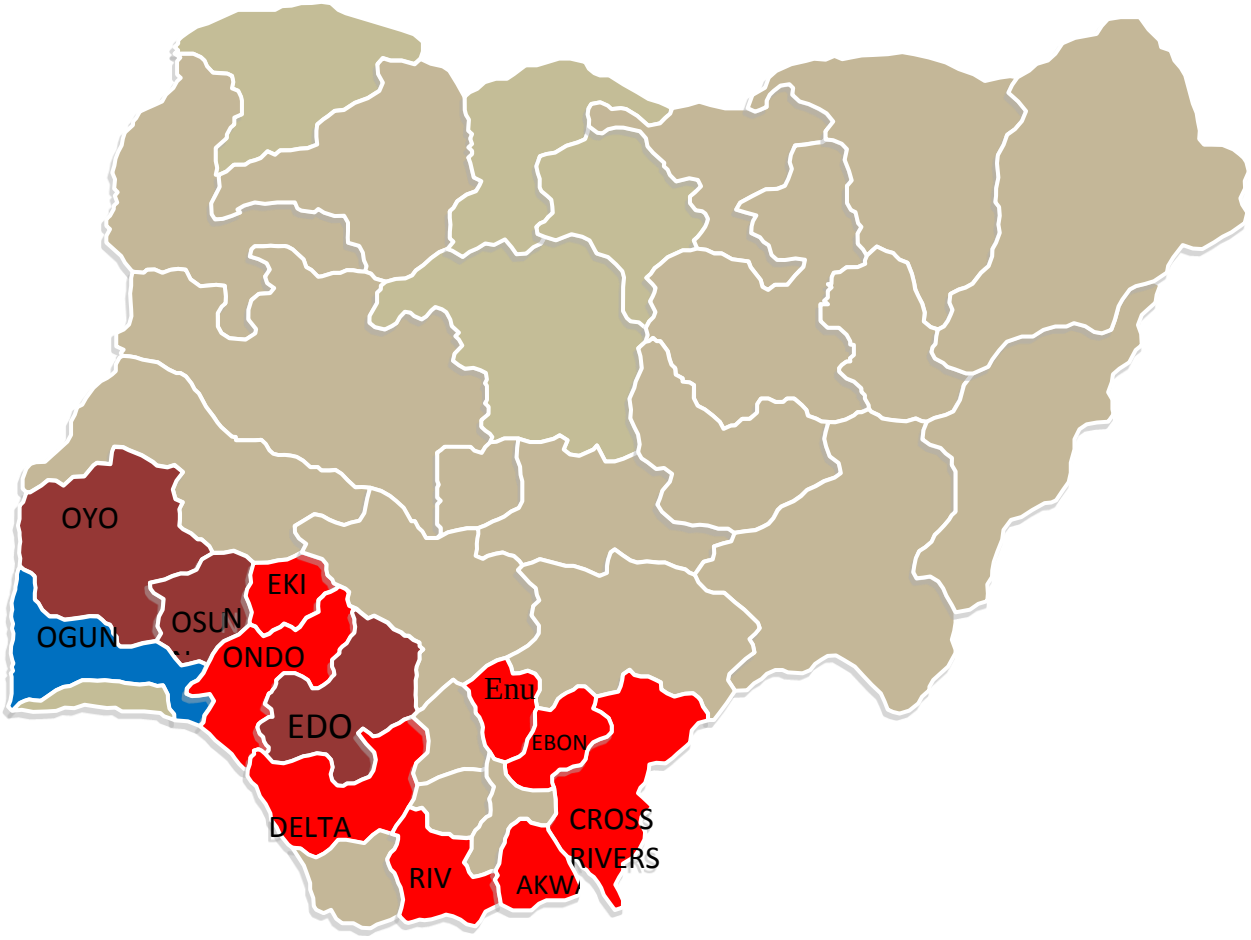

Figure 1.3: Map of Nigeria showing states where *Napoleonaea vogelii* and *Diospyros barteri* have been reported

- Areas marked red indicates states where *Napoleonaea vogelii* have been reported
- Areas marked blue indicates states where *Diospyros barteri* have been reported
- Areas marked brown indicates states where both plants have been reported

1.7 RESEARCH ISSUE

Despite the important influences of environmental factors on seed germination, there has been little research in the effects of these parameters on many species of terrestrial shrubs including *Napoleonaea vogelii* and *Diospyros barteri* which have ecological and economic potentials.

Little is known about the ecology of these plants. Although the conservative state of *Napoleonaea vogelii* is still unknown. *Diospyros barteri* is vulnerable and on the verge of genetic erosion (IUCN, 2015). Most *Napoleonaea* species are experiencing same problem of genetic erosion. While *Napoleonaea egertonii* is vulnerable, *Napoleonaea imperialis* is near threatened. *Napoleonaea alata*, *Napoleonaea lutea* and *Napoleonaea reptans* are critically endangered (IUCN, 2015)

1.8 RESEARCH QUESTIONS

1. Could germination be a factor in the current place of the plants distribution?
2. How long does it take these plants to germinate?
3. What are the requirements needed for seedling establishment?
4. How does priming, bush fire, and other factors affect germination of these plants

1.9 AIM OF THE STUDY

The aim of this research is to study the germination and seedling establishment of *N. vogelii* and *D. barteri* under different experimental conditions

1.10 OBJECTIVES OF THE STUDY

1) To evaluate the effects of physical and chemical factors on the germination of *N. vogelii* and *D. barteri.*

2) To evaluate the germination percentage and speed of germination in the treatments.

3) To promote the use of physical and chemical factors in achieving higher germination percentage and greater speed of germination.

4) To strengthen plant diversity in our ecosystem.

CHAPTER TWO

LITERATURE REVIEW

In many experiments concerned with seed treatments, the pattern of germination, both in time and extent is the key consideration. Not just the final germination percentage attained, but also the speed and distribution of this germination are often used to judge the agronomic relevance of treatments. The reinforcement of wild plant populations using individuals raised *ex situ* is considered a valid means of reducing the risk of extinction of threatened species or populations. Enhancing seed germination has expanded from agricultural to horticultural, medical, and weedy species and has shown promise for stimulating germination and seedling growth in other economically valuable species

2.1 EFFECTS OF PRIMING ON GERMINATION

Seed priming is a pre-sowing strategy for influencing seedling development by modulating pre-germination metabolic activity prior to emergence of the radicle and generally enhances germination rate and plant performance (Ghasemi-Golezani *et al*., 2008).

Seed priming allows some of the metabolic processes necessary for germination to occur without germination actually taking place. In priming, seeds are soaked in

different solutions with high osmotic potential. This prevents the seeds from absorbing enough water for radicle protrusion, thus suspending the seeds in the lag phase (Taylor *et al*., 1998). Priming is one of the most important physiological method which improves the seed performance and provides faster and synchronized germination (Javid *et al.*, 2013). Seed priming has contributed to increase in speed and synchrony of seed germination (Pirasteh-Anosheh *et al*., 2011).

Seed priming has been commonly used to reduce the time between seed sowing and seedling emergence and to synchronize emergence (Parera and Cantliffe, 1994). Hussian *et al*. (2006) compared halo- and hydro priming and indicated that both priming with NaCl and water resulted in lower time taken to 50% emergence and mean emergence time and higher final emergence, energy of emergence, plant population, achene yield and yield contributing factors and achene proteins. Primed crops grow more vigorously, flowered and matured earlier and produced bigger cobs and higher yield and an independent measurement on a subset of 35 trials showed a mean increase in cob weight of 6% in maize (Harris *et al*., 2001). Primed crops also grew more vigorously, tolerated dry spells better and flowered earlier (typically 7-10 days earlier) and matured earlier (8-10 days earlier) in maize (Harris *et al*., 1999).

Huang *et al.* (2006) studied the effect of hydropriming in cucumber. Three cucumber seed lots *viz*. Bingo-I, Bingo-II and HB-128 were subjected to hydropriming for 1,

2 and 3 days. It was found that hydropriming increased the speed of germination of all seed lots. It was found that hydropriming increased the germination in HB-128

2.2 SEED PRIMING TECHNIQUES

Various types of seed priming techniques for improving the performance of growth, emergence, and yield of the crop include hydropriming, halopriming, osmopriming and smoke-water priming.

2.2.1 HYDROPRIMING

Hydropriming involves soaking the seeds in water before sowing (Pill and Necker, 2001) and may or may not be followed by air-drying of the seeds. In many agricultural areas, a major cause of poor stand establishment and low crop yield is unfavorable environmental conditions for seed germination and seedling emergence (Javid *et al.*, 2013).

However, rapidly germinating seedlings could emerge and produce deep roots before the upper layers of the soil are dried out and crusted, which may result in good crop establishment and higher crop yield (Javid *et al.*, 2013). Rafiq *et al.* (2006) reported that seed priming reduces the effect of salinity on the morphological parameter of the plants. Any factor that facilitates rapid germination may contribute to establishment of a successful crop. A low cost approach, designated as on farm

seed priming was proposed by Harris (1992) and involve soaking of seed in water before sowing. This pre sowing seed treatment, known as hydropriming, allows the seed to imbibe water and go through the first phase of germination in which pre-germination metabolic activities are started while the latter two phases of germination are inhibited (Pill and Necker, 2001). Although soaking seed in water and drying before sowing is the easiest way to achieve hydration, a major disadvantage is that it may result in uneven hydration and non-uniform germination. Soaking is not suitable for some plant species, as rapid hydration may cause leakage of essential nutrients out of the seed, resulting in seed damage.

Hydropriming showed pronounced effect on field emergence, rate and early seedling growth of maize crop and it improved the field stand and plant growth, both at vegetative stage and at maturity of maize (Nagar *et al.*, 1998).

Organic and inorganic substances have major role in the plant growth and development of storages bodies of plants. Presoaking of seeds in water may alter the mobilization of both inorganic and organic substances from the storage organs to the developing embryo in some species (Javid *et al.*, 2013). In sugar beet and pigeon pea, it was determined that the effect of hydropriming on improving seed germination was closely related to the solubilization of P-subunit of 11-S globulin storage protein and were very effective in the mobilization of compounds such as

proteins, free amino acids, and soluble sugars from storage organs to growing embryonic tissues under salt stress (Jyotsna and Srivastava, 1998).

Similar to other priming techniques, hydropriming plays an important role in the seed germination and radicle and plumule emergence in different crop species. It also enhances seed germination and seedling emergence under both saline and non-saline conditions, although there are exceptions (Javid *et al.,* 2013). Roy and Srivastava (1999) reported that soaking wheat kernels in water improved their germination rate under all conditions; no such improvement was obtained in similar research conducted by Ashraf and Iram (2002).

2.2.2 HALOPRIMING

Halopriming refers to soaking of seeds in solution of inorganic salts i.e. NaCl, KNO_3 $CaCl_2$, $CaSO_4$, etc. A number of studies have shown a significant improvement in seed germination, seedling emergence and establishment, and final crop yield in salt affected soils in response to halopriming. Khan *et al.* (2009) evaluated the response of seeds primed with NaCl solution (1 mM) at different salinity levels 0, 3, 6 and 9 dSm^{-1} in relation to early growth stage and concluded that seed priming with NaCl has been found to be better treatment as compared to non-primed seeds in case of hot pepper for improving the seedling vigour and stand establishment under salt stressed conditions. Priming with NaCl and KCl was helpful in removing the

deleterious effects of salts (Iqbal *et al.*, 2006). Rice seed treated with a mixed salt solution germinated faster than unprimed seed under salt-stress conditions (Chang-Zheng *et al.*, 2002). Seed germination is promoted by halopriming but also stimulate subsequent growth, thereby enhancing final crop yield (Sallam, 1999).

2.2.3 EFFECTS OF SMOKE WATER TREATMENT AS A METHOD OF IMPROVING GERMINATION IN PLANTS

Smoke, as a fire by-product, in fire-prone areas, was identified as a seed germination promoter in 1990 (de Lange and Boucher, 1990). Research addressing germination response to smoke has occurred in fire prone areas, such as Fynbos in Africa (Brown *et al.*, 2003), California chaparral in America (Keeley and Fotheringham, 1998), dry deciduous forest in India (Singh and Raizada, 2010), and Eucalyptus woodland in Australia (Enright and Kintrup, 2001). However, a considerable number of studies have indicated that smoke application also can influence seed germination in fire-free areas (Figueroa and Cavieres 2012).

Plant-derived smoke application research has expanded to agricultural and horticultural species (Kulkarni *et al.*, 2011), medicinal species, and weeds (Stevens *et al.*, 2007), and has shown promise for stimulating germination and seedling growth in other economically valuable species (Kulkarni *et al.*, 2011). Smoke affects the water uptake process in seed germination by changing the permeability of the

internal cuticle via increased the number and size of permeates (Jain *et al.*, 2008). Three active compounds in plant-derive smoke have been identified: karrikins, a family of butenolides related to 3-methyl-*2H*furo[2,3-c]pryan-2-one (Flematti *et al.*, 2004); cyanohydrins (Flematti *et al.*, 2011); and 3,4,5-trimethylfuran-2(*5H*)-one (Light *et al.*, 2010). Karrikins and cyanohydrins are both germination promoters; on the contrary, 3,4,5-trimethylfuran is a germination inhibitor with unclear dynamics (Soos *et al.*, 2010 and Flematii *et al.*, 2011).

Smoke has been tested and recognized as having potential as a restoration treatment for years, and has proven fruitful for this in Australia. Rokich and Dixon (2007) identified smoke-stimulated seed germination across phylogeny, life form, seed dormancy class, habitat type, and fire regions; summarized multiple restoration studies in *Banksia* woodlands; compared physical restoration techniques such as soil-stabilizers (like polymer gels, bitumen, oil-shale solid waste, and jute-matting) to topsoil seed banks, application of seed-coating, earlier sowing time, soil raking and others, and found smoke played a significant role in seed germination. Some *in-situ* smoke-stimulated seed germination tests have shown that smoke application has a potential role in large-scale restoration (Read *et al.*, 2000, Rokich and Dixon 2007). Studies of the effect of smoke on soil seed banks produced similar management suggestions (Ablla, 2009).

2.3 IMPACT OF BUSH FIRE ON GERMINATION

Fire is an integral component of many ecosystems worldwide. Bush fires of natural or anthropogenic origin are an essential feature in the regeneration and modification of vegetation communities (Kozlowski, 2000). In the tropics, bush fire has led to the elimination of sensitive species, which have been driven back into wetter habitats and areas of discontinuous growth, and a predominance of resistant species (Danthu *et al.*, 2003). Many plant species require fire-related cues, primarily heat and smoke, to trigger germination (Penman *et al.*, 2008). One important factor which influences distribution and abundance of plant species in relation to fire is the response of the seedbank. Heat has been shown to stimulate germination in a wide range of species, from a number of families including Fabaceae (Auld and O'Connell, 1991), Convolvulaceae (Read *et al.*, 2000) and Cyperaceae (Thomas *et al.*, 2003). Heating fractures the seed coat enhancing germination, particularly in hard-seeded species. The optimum range of temperatures for germination varies between species (Read *et al.*, 2000), with short term exposures (of only a few minutes) to high temperatures (>100°C) resulting in seed mortality in some species (Auld and O'Connell, 1991). The protective effect exerted by the soil during the passage of spreading fire is well documented depending on a number of factors: fire intensity, depth of seed burial, soil type and water content

2.4 EFFECTS OF LIGHT ON GERMINATION AND SEEDLING ESTABLISHMENT

Plants have been classified in terms of their responses to light for germination as follows: (i) those that require light to germinate, (ii) those that require darkness to germinate and (iii) those that have a large percentage of seeds neutral to light. These groups have been named positive photoblastic, negative photoblastic and neutral photoblastic by Baskin and Baskin (2014).

The knowledge of light effects on germination is relevant in propagation of wild species for restoration purposes (Khurana and Singh 2001) but also to better understand germination ecology.

Light can be an important key to determine safer conditions for germination in environments where seeds are likely to be burried in the soil or to grow under the shade of other plants (Flores and Jurado 2003). The influence of light on germination has also been associated with plant growth form. Light promotes the germination of annual species (de Villiers *et al.,* 2002); seeds from shorter plants have a stronger light requirement for germination than those from taller plants (Flores *et al.,* 2011); Seeds requiring light are small (Flores *et al.* 2011). Positive photoblastism is also considered to be associated with phylogeny (Flores *et al.,* 2011) and with temperature variation during seed development (Rojas-Aréchiga *et al.,* 1997).

It has also been suggested that light response and seed mass coevolved as an adaptation to ensure germination of small-seeded species only when they are close enough to the soil surface as to be able to emerge (Milberg *et al.*, 2000). Also, Wright *et al*. (1998) studied the effect of substrate and canopy gap position on the regeneration of six tree species in the cedar-hemlock forest and found that the emergence of all species was strongly affected by gap position seedbed substrate and year.

CHAPTER THREE

MATERIALS AND METHOD

3.1 SEED COLLECTION

Napoleonaea vogelii and *Diospyros barteri* are found in small populations in Edo and Delta States. Once suitable populations were located they were monitored for flowering and fruit development until mature seeds could be collected. *Napoleonaea vogelii* begins to flower just after the rains (November to February) and fruits just into the raining season. Fruits were collected from a reserve in Oria, Abraka, Delta State. *Diospyros barteri* fruits were collected during the raining seasons in the company of indegenes of various localities from the understorey of forests and plantations around Eko-Abetu in Ovia-North East Local Government Area of Edo State. For each species, fruit collection was performed at multiple sites for sufficient quantity of seeds. Seeds were collected at maturity either by direct harvest of the fruit from the plant or handpicking of fallen fruits around the plants.

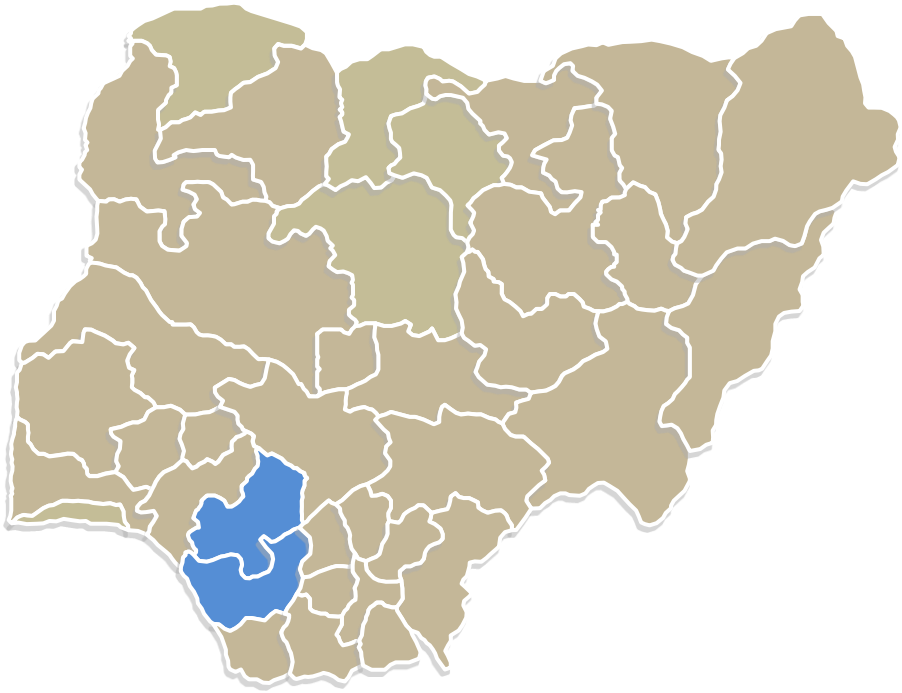

Figure 1.4: Map of Nigeria showing states where *Napoleonaea vogelii* and *Diospyros barteri* were collected

3.1.1 Coordinates of the seed collection points

1. *Napoleonaea vogelii*- Latitude- 5.75609 N 5°45’21.922”

   Longitude- 5.6088 E 6°2’6.215”

2. *Diospyros barteri*- Latitude- 6.49954 N 6°29’58.356”

   Longitude- 5.6088 E 5°36’31.692”

3.2 SEED GERMINATION

Seeds were sown at approximately 2 cm depth using a measured dibber to minimize errors resulting from the effect of sowing depth on seedling emergence and establishment. Seed germination during all experiments was defined as the emergence of the plumule or radicle from the surface of the soil. To quantify germination time and total number of germinated seeds, the number of germinated seeds was counted daily and recorded.

3.2.1 CALCULATING GERMINATION PERCENTAGE, SPEED OF AND COEFFECIENT OF RATE OF GERMINATION

3.2.1.1 $\boldsymbol{Total\ germination\ percentage} = \frac{N \times 100}{N_T}$

Where $N =$ Number of germinated seeds

$N_T =$ Total number of seeds planted

3.2.1.2 $\boldsymbol{Germination\ Speed}$ (Bewley *et al.,* 2013)

$$= (N_1 \times 1) + (N_2 - N_1) \times \frac{1}{2} + (N_3 - N_2) \times \frac{1}{3} + (N_4 - N_3) \times \frac{1}{4} \dots\dots\dots + (N_n - N_{n-1}) \times \frac{1}{n}$$

Where N_1= Germinated seeds observed at first day of germination

N_2 = Germinated seeds observed at second day of germination

N_3 = Germinated seeds observed at third day of germination

N_4 = Germinated seeds observed at fourth day of germination

N_n = Germinated seeds observed at nth day of germination

N_{n-1} = Germinated seeds observed at the day before the nth day of germination

3.2.1.3 ***Time taken to reach* 50%** ***of total germinated seeds*** (Bewley *et al.*, 2013).

$$= \frac{(N_1 \times T_1) + (N_2 \times T_2) + (N_3 \times T_3) + (N_4 \times T_4) \ldots . + (N_n \times T_n)}{N_1 + N_2 + N_3 + N_4 \ldots \ldots . + N_n}$$

Where

N_1= Germinated seeds observed at first day of germination

N_2 = Germinated seeds observed at second day of germination

N_3 = Germinated seeds observed at third day of germination

N_4 = Germinated seeds observed at fourth day of germination

N_n = Germinated seeds observed at nth day of germination

T_1= First day of germination

T_2 = Second day of germination

T_3 = Third day of germination

T_4 = Fourth day of germination

T_n = nth day of germination

3.3 PRIMING TECHNIQUES (SEED PRIMING)

Ghasemi-Golezani *et al*., (2008) described seed priming as a technique in which seed are partially hydrated until the germination process begins, but radicle emergence does not occur. Procedures were as follows:

3.3.1 HYDRO AND HALOPRIMING

Required number of seeds were soaked for 24 hours each in distilled but cool water for hydropriming, NaCl solution for halopriming, and smoke water priming at around room temperature for each treatment. After priming, seeds were wiped and air dried for 48 hours before sowing.

The salinity experiment was a factorial with two factors which are salinity at 4 levels (0, 2, 4, and 6) g/L and priming with 2 levels (unprimed and NaCl primed seeds), arranged in a completely randomized design with three replications and 40 seeds per replicate. Seed germination was recorded daily up to day 20 after the first germination was recorded. A seed was considered as germinated when the radicle emerges from the surface of the soil

3.3.2 SMOKE WATER EXPERIMENT

3.3.2.1 CONSTRUCTION OF COMBUSTION CHAMBER AND PREPARATION OF AQUEOUS SMOKE SOLUTIONS

Aqueous smoke solution was produced by burning dry mixed litter materials (leaves and sticks) of local plant remains in a combustion chamber and directing the resultant smoke into distilled water (Baxter and Van Staden., 1994). Mixed litter materials were collected from the natural habitat of the species. A combustion chamber was constructed by assembling.

- an electric ring heater,
- a metal can (2500g peak milk container),
- an air supply hose, and a water can.

The rubber end of the electric ring heater was firmly fitted to the metal can, leaving room for the power cord to exit from under the can. Two holes were drilled on opposite sides of the metal can; one directly connected to an air supply hose and the other connected to a water can.

The dry plant materials were then put in the metal container with the electric ring heater right inside, such that the ring heater and the dry plant materials are enclosed inside the can. Electric power was supplied to the ring heater, and air was also

pumped into the combustion chamber through the air hose. Smoke generated was passed through the attached tubing into 4 L of distilled water in a container thereby producing aqueous smoke solution. Two solutions were made by continuing this process for different time periods (10 and 20 minutes). These smoked water solutions were applied to seeds of *Napoleonaea vogelii* and *Diospyros barteri*. This application was compared to a control. *Napoleonaea vogelii* and *Diospyros barteri* were tested for the effect of aqueous smoke extract (smoked water) on germination**.**

3.4 LIGHT EXPERIMENTS:

Plants grown in continuous light or alternating light and darkness were illuminated by two standard white 20-watts fluorescent rechargeable lamps. The lamps were placed in a position directly on the open top of the incubation box. The planting bowls were arranged on the floor of the box, 30 cm from the light source. Plants grown in darkness were incubated inside light-tight, closed box. An effort was made to maintain uniform light conditions when monitoring germination.

The light conditions investigated were:

- Germination in continuous light (24 hours daily)
- Germination in continuous darkness (24 hours daily)
- Germination in 12 hours light and 12 hours darkness

3.5 BUSH FIRE METHOD

This experiment tested the effect of fire on the germination and seedling establishment of *N. vogelii* and *D. barteri* seeds. Controlled bush fire was made by burning dry mixed litter material (leaves and sticks) of local plant remains in a small open space. After the passage of fire, seeds were sown directly and germination was compared to that of intact seeds.

The effect tested was based on seed position during the bush fire. The seed position could be buried in the soil, on the soil or above the soil. The range tested (taking soil level as reference 0 and negative values as buried) was as follows: -5 cm depth , 0, and 10 cm.

0 cm- This experiment concentrated on subjecting naked seeds on the soil to high fire intensity. They were sown afterwards to assess their survival and germination capacity.

-5 cm depth- This experiment concentrated on subjecting naked seeds buried 5 cm in the soil to high fire intensity. They were sown afterwards to assess their survival and germination capacity

10 cm height- This experiment was more specifically concentrated on subjecting seed pods to high fire intensity then dehull to remove the seeds. The seeds were sown later and seedling survival were then assessed.

3.6 PLANT HEIGHT

Plant height was taken by measuring the height of five randomly sampled plants from the base to the highest point with a meter rule and the mean height calculated.

3.7 NUMBER OF LEAVES

The leaves of the five sampled plants were counted and the mean determined.

3.8 STATISTICAL ANALYSIS

Treatment effects of each variable in the individual experiments were determined using Analysis of Variance (ANOVA). All experiments were set up using a completely randomized block design.

CHAPTER FOUR

RESULTS

The results obtained in this study, are shown in Tables 4.1-4.6, Figures 4.1-4.4 and Plate 4.1

Table 4.1 shows the percentage germination of seeds of *Napoleonaea vogelii* Hook. and Planch. observed under the different treatments to initiate germination. The highest and least percentage germination, was obtained in seeds subjected to hydropriming and bush fire (0 cm depth) respectively

Table 4.1: Percentage germination of seeds of *Napoleonaea vogelii* under different treatments, thirty-five days after planting.

TREATMENTS	PERCENTAGE GERMINATION(%)
Control (no treatment applied)	60.00±8.16
Bush fire (0 cm depth)	32.50±4.79
Bush fire (5 cm depth)	75.00±8.66
Bush fire (10 cm height)	70.00±4.08
Smoke water treatment (10 minutes)	80.00±4.08
Smoke water treatment (20 minutes)	77.50±4.79
24 hours light treatment (continuous light)	75.00±6.45
24 hours light treatment (continuous darkness)	67.50±6.29
Alternating light/dark regime (12hrs L/12hrs D)	70.00±7.07
Priming with water (hydro)	90.00±4.08
Priming with NaCl (2g/l)	85.00±6.45
Priming with NaCl (4g/l)	85.00±2.89
Priming with NaCl (6g/l)	77.50±7.50
Seeds sown with persistent floral parts (No priming)	60.00±4.08
Seeds from decaying fruits on tree	35.00±8.66

Values= mean ± standard error

Table 4.2 shows the percentage number of seeds of *Napoleonaea vogelii* observed under the different treatments that germinated per week. Germination of seeds was observed under all treatments given to seeds. Germination began at week 3 with hydropriming showing the highest percentage germination (30%) in the first week, while control (no treatment applied), bush fire (5 cm depth), 24 hours light treatment (continuous darkness), alternating light/dark regime (12hrs L/12hrs D), and seeds sown with persistent floral parts (No priming) all showed delayed germination. Week 4 shows hydropriming also having the highest percentage germination (90%) while seeds from decaying fruits on tree showed the lowest percentage germination (30%). Week 5 shows that hydro and halo primed seeds reached their total germination earliest with no germination still taking place while seeds sown with persistent floral parts had the highest percent germination with 25% germination still taking place.

Table 4.2: Number of seeds of *Napoleonaea vogelii* that germinated per week under different treatments 5 weeks after planting.

TREATMENTS	NO OF SEEDS SOWN	WEEKS AFTER PLANTING (WAP)				
		1	2	3	4	5
Control (no treatment applied)	40	0	0	0	19(47.5)	24(60)
Bush fire (0 cm depth)	40	0	0	4(10)	13(32.5)	13(32.5)
Bush fire (5 cm depth)	40	0	0	0	21(52.5)	30(75)
Bush fire (10 cm height)	40	0	0	4(10)	26(65)	28(70)
Smoke water treatment (10 minutes)	40	0	0	6(15)	32(80)	32(80)
Smoke water treatment (20 minutes)	40	0	0	6(15)	29(72.5)	31(77.5)
24 hours light treatment (continuous light)	40	0	0	1(2.5)	27(67.5)	30(75)
24 hours light treatment (continuous darkness)	40	0	0	0(0)	20(50)	27(67.5)
Alternating light/dark regime (12hrs L/12hrs D)	40	0	0	0(0)	23(57.5)	28(70)
Priming with water (hydro)	40	0	0	12(30)	36(90)	36(90)
Priming with NaCl (2g/l)	40	0	0	9(22.5)	34(85)	34(85)
Priming with NaCl (4g/l)	40	0	0	10(25)	34(85)	34(85)
Priming with NaCl (6g/l)	40	0	0	10(25)	31(77.5)	31(77.5)
Seeds sown with persistent floral parts (No priming)	40	0	0	0(0)	14(35)	24(60)
Seeds from decaying fruits on tree	40	0	0	2(5)	12(30)	14(35)

Percentage germination in parenthesis

Table 4.3 shows the number of days taken to reach 50% seed germination (T50) of *Napoleonaea vogelii*. T50, was negatively influenced by bush fire (0 cm depth) and decaying fruits on tree. Seeds conditioned to priming showed the least number of days to get to 50% of the total germinated seeds. Seeds treated with 2g/l NaCl, 6g/l NaCl and hydropriming reaching their T50 mark 23 days after planting.

Table 4.3: Number of days taken to reach 50% of total germinated seeds

Treatments	Number of days
Control (no treatment applied)	27.67±0.67
Bush fire (0 cm depth)	0.00±0.00
Bush fire (5 cm depth)	27.00±1.00
Bush fire (10 cm height)	26.00±0.58
Smoke water treatment (10 minutes)	24.00±0.00
Smoke water treatment (20 minutes)	24.33±0.33
24 hours light treatment (continuous light)	27.00±1.00
24 hours light treatment (continuous darkness)	27.00±0.58
Alternating light/dark regime (12hrs L/12hrs D)	26.67±0.88
Priming with water (hydro)	23.00±0.00
Priming with NaCl (2g/l)	22.33±0.33
Priming with NaCl (4g/l)	23.33±0.33
Priming with NaCl (6g/l)	23.00±0.00
Seeds sown with persistent floral parts (No priming)	26.33±3.18
Seeds from decaying fruits on tree	0.00±0.00

Values= mean ± standard error

Table 4.4 shows the average number of leaves of *Napoleonaea vogelii* observed under the different treatments, with light treatment (24 hours darkness) showing significantly lower mean number of leaves (2.37). Number of leaves in the other treatments were not significantly different from the control.

Table 4.4: Average number of leaves per plant of *Napoleonaea vogelii* under different treatments, sixty-three days after planting.

Treatment	Mean of number of leaves
Control (no treatment applied)	9.3607^{b}
Bush fire (0 cm depth)	9.8^{b}
Bush fire (5 cm depth)	9.7571^{b}
Bush fire (10 cm height)	10.2143^{b}
Smoke water treatment (10 minutes)	10.0286^{b}
Smoke water treatment (20 minutes)	9.9857^{b}
24 hours light treatment (continuous light)	2.3714^{a}
24 hours light treatment (continuous darkness)	9.8857^{b}
Alternating light/dark regime (12hrs L/12hrs D)	10.2714^{b}
Priming with water (hydro)	10.1429^{b}
Priming with NaCl (2g/l)	10^{b}
Priming with NaCl (4g/l)	10.0571^{b}
Priming with NaCl (6g/l)	10^{b}

Means with the same alphabet(s) shows that there is no significant difference between them ($P<0.05$).

Table 4.5 shows the mean height of seedlings of *Napoleonaea vogelii* observed under the different treatments. Seedlings exposed to complete darkness showed the highest mean growth in height (32.42 cm) while those in 24 hours light showed lowest growth in height (18.88 cm). Hydropriming and halopriming (2g/l) also showed significantly higher growth in heights (24.12 cm and 24.08 cm respectively)

Table 4.5: Average height of *Napoleonaea vogelii* plants grown from seeds subjected to different treatments 52 days after planting

Treatment	Plant height (cm)
Control (no treatment applied)	22.48^{bc}
Bush fire (0 cm depth)	22.1^{bc}
Bush fire (5 cm depth)	22.12^{bc}
Bush fire (10 cm height)	21.32^{b}
Smoke water treatment (10 minutes)	22.16^{bc}
Smoke water treatment (20 minutes)	21.36^{b}
24 hours light treatment (continuous light)	18.88^{a}
24 hours light treatment (continuous darkness)	32.42^{d}
Alternating light/dark regime (12hrs L/12hrs D)	22.48^{bc}
Priming with water (hydro)	24.12^{c}
Priming with NaCl (2g/l)	24.08^{c}
Priming with NaCl (4g/l)	22.22^{bc}
Priming with NaCl (6g/l)	22.44^{bc}

Means with the same alphabet(s) shows that there is no significant difference between them ($P<0.05$)

Figure 4.1 shows the effects of various treatments on the speed of germination of *Napoleonaea vogelii* observed under the different treatments to initiate germination. All forms of priming showed improved speed of germination with hydropriming being superior with a mean speed of 2.99 day^{-1} (Appendix 4.7). Control (seeds without treatment) showed the least speed with 0.39 day-1. All treatments of priming showed a germination rate ($P < 0.05$) higher than control

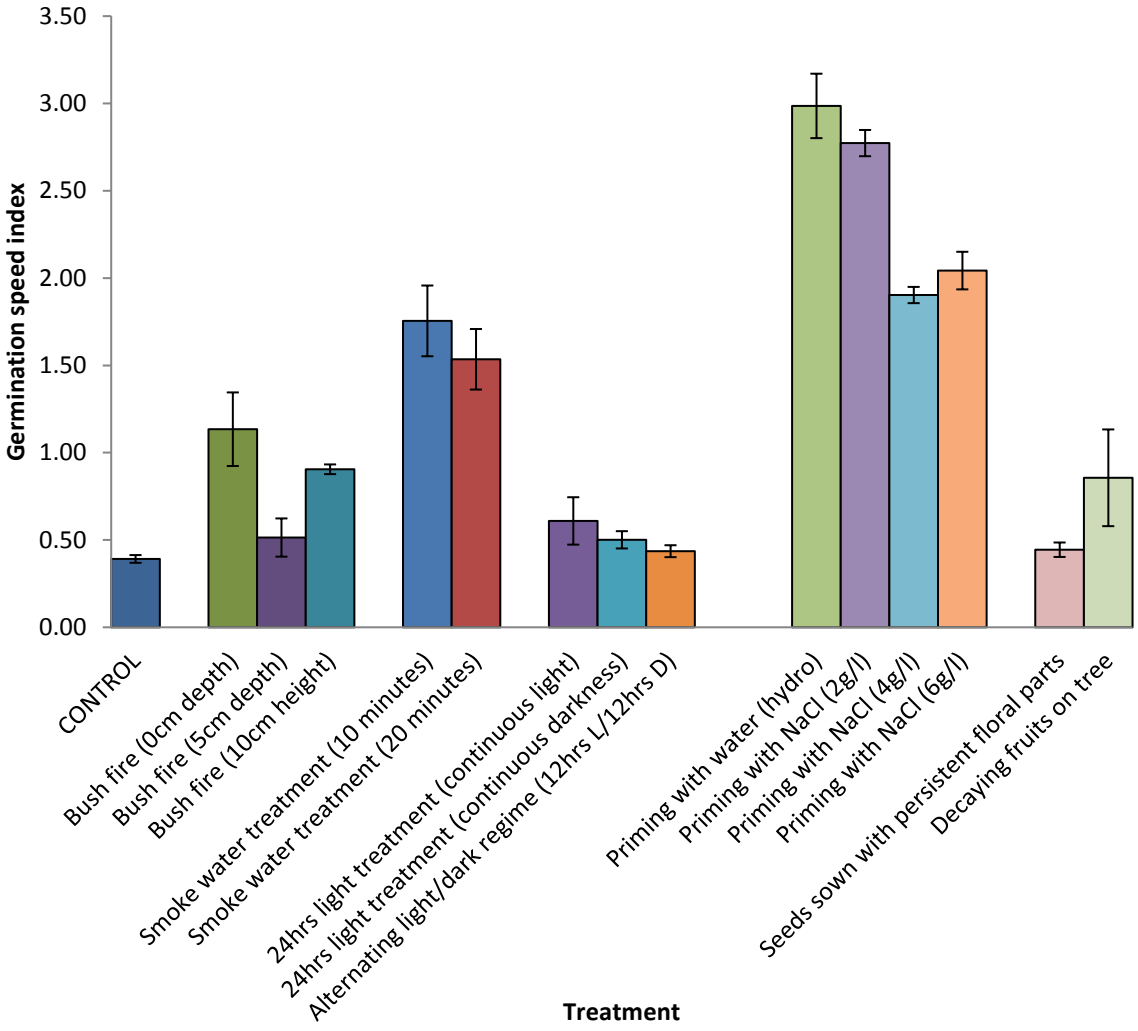

Figure 4.1: Shows effects of various treatments on the speed of germination of *Napoleonaea vogelii* 35 days after planting. Bars represent (±) standard error

Plate 4.1- *Napoleonaea vogelii* plants grown from seeds in the study. Pot A shows plants from seeds given alternating 12hours dark/ 12 hours light condition, pot B shows plants from seeds given continuous darkness condition and pot C shows plants from seeds given continuous light condition.

GERMINATION OF *Diospyros barteri.*

Table 4.6 shows the percentage germination of seeds of *Diospyros barteri* observed under the different treatments to initiate germination. No germination of seeds was observed under all treatments given to seeds. Results showed no germination in all treatments after 5 weeks

Table 4.6: Shows the number of *Diospyros barteri* seeds that germinated given different treatments 5 weeks after planting.

TREATMENTS	NO OF SEEDS SOWN	WEEKS AFTER PLANTING (WAP)				
		1	2	3	4	5
Control (no treatment applied)	20	0	0	0	0	0
Bush fire (0 cm depth)	20	0	0	0	0	0
Bush fire (5 cm depth)	20	0	0	0	0	0
Bush fire (10 cm height)	20	0	0	0	0	0
Smoke water treatment (10 minutes)	20	0	0	0	0	0
Smoke water treatment (20 minutes)	20	0	0	0	0	0
24 hours light treatment (continuous light)	20	0	0	0	0	0
24 hours light treatment (continuous darkness)	20	0	0	0	0	0
Alternating light/dark regime (12hrs L/12hrs D)	20	0	0	0	0	0
Priming with water (hydro)	20	0	0	0	0	0
Priming with NaCl (2g/l)	20	0	0	0	0	0
Priming with NaCl (4g/l)	20	0	0	0	0	0
Priming with NaCl (6g/l)	20	0	0	0	0	0

CHAPTER FIVE

DISCUSSION

5.0 DISCUSSION

The tropical forests occupy 7% of the earth's area with about half of the world's forest cover and 65% of global biodiversity (Whitmore, 1990). In Nigeria and the tropics, tropical forests have been foremost victims of anthropogenic pressure to the extent that most areas either replaced by secondary vegetation or denuded completely (Menon *et al*., 2001).

For sound management and continued economic gains from tropical forests, it is desired to assess reproductive seed biology of native tree species that contribute considerably to local biodiversity as well as also valued by the indigenous people (Deb and Sundriyal, 2008). There is considerable advancement in the understanding of tropical tree seeds over the past two decades (Panna and Sundriyal, 2013), however only selected species are used in afforestation because of the ease in their seed collection and management (Smith *et al*., 2002).

Tropical rainforest trees and shrubs exhibit wide heterogeneity in their layers, fruit types and seeds. Information on the germination and seedling growth of

these trees and shrubs is of enormous use to understand species distribution and management in any forest stand (Panna and Sundriyal, 2013).

5.1 GERMINATION: Seed germination is a test indicating the potential of the seed to produce normal seedlings under ambient conditions. During seed germination, various stored substrates are reactivated, repaired if damaged, and transformed into new building materials necessary for the initial growth of the embryo, its subsequent growth and seedling establishment in its natural habitat (Koller and Hadas, 1982). The seed lot having higher germination index is considered to be more vigorous. Proper germination of seeds, seedling emergence and establishment, therefore are critical processes in the survival and growth cycle of plant species in general.

The use of seed priming allows the seed to imbibe water slowly, permitting the early stages of germination to begin without radicle protrusion through the seed coat.

The percentage increase in germination over control of *Napoloenaea vogelii* Hook. and Planch. is evident of the positive effect of priming on seeds. The highest percentage germination was obtained in seeds subjected to hydro, halo and smoke water priming with already decaying seeds and seeds subjected to bush fire (0 cm depth) showing the least percentage germination.

Similar results have also been recorded by Demir and Oztokat (2003), Javid *et al.* (2013) and Pirasteh-Anosheh *et al*. (2011), whereas, in asparagus, Evans and Pill

(1989) reported that faster germination of primed seed is independent of treatment and conditions and that priming has no effect on germination percentage.

Increase in germination percentage due to priming in this study may be due to mechanisms involved in which seeds complete first two phases of germination during the priming process, hence primed and dehydrated seeds enter immediately into phase III of imbibition once rehydrated during sowing (Bradford, 1986).

Much of the germinative metabolism that occurs in Phase II continued, including DNA and mitochondrial repair, degradation of stored mRNAs, and transcription and translation of new proteins. Replicative DNA synthesis, cell division and embryo expansion are prevented and desiccation tolerance is maintained, allowing the seeds to be dehydrated following the priming treatment before planting.

Generally, if radicle emergence has not occurred, seeds retain desiccation tolerance and can be dried following the priming treatment without damage, although extending treatments too long (overpriming) can result in damage to radicle tips and poor subsequent seedling growth (Bewley *et al.*, 2013). Following planting, primed seeds imbibe water rapidly and exhibit shortened Phase II durations, moving relatively quickly from hydration to radicle emergence and growth. This considerably reduced the time from planting to seedling emergence, and improves the uniformity (decreases the spread) of emergence over time. Priming can also

enhance the ability of seeds to germinate under stressful conditions such as low temperatures or salinity.

Seeds which passed through bush fire treatments had significantly lower percent germination. This is probably due to the deleterious effects of heat on some seeds (Auld and O'Connell, 1991). There is no previous report on the deleterious effects of bush fire on the germination of species in the Lecythidaceae family. Though previous studies show that fire related cues which includes heat, promotes germination of seeds (Read *et al.,* 2000; Thomas *et al.,* 2003). This study shows *Napoleonaea vogelii* had negative deleterious response to heat shock agreeing with Auld and O'Connell (1991) when they stated that short term exposure to high temperatures results to seed mortality in some species

There was no significant difference in germination of the buried seeds (5 cm depth) compared to the control. When *Napoleonaea vogelii* seeds are buried up to 5 cm depth in the soil, there was no effect of temperature on the seeds. This finding is in line with earlier work by *Danthu et al.* (2000) who reported that there was no improved germination in seeds buried at 5 cm.

Fruits of *Napoleonaea vogelii* are sometimes found firmly attached to the stem of the trees even when they are ripe. These fruits decay on the plant. Seeds collected

from decaying fruits on trees had significantly lower percent germination while those planted with their floral parts intact had delayed germination

Absence of germination in seeds of *Diospyros barteri*, is likely caused by a layer of hard tissue that acts as an impermeable barrier to water uptake, respiratory gas exchange or both. Hence the embryo tissues are either unable to hydrate or respire or both. This condition is known as 'hard–seededness' and because it is an in-built physical barrier to germination, some people do not acknowledge that it is a form of dormancy- but the result is the same: poor or non-existent germination, until the seed coat is 'pretreated'(Gosling, 2007)
Gosling, (2007) stated that in seeds like this, there is no fundamental, physiological block to embryo growth, merely a physical barrier which excludes one or more of the essentials for growth from the tissues.

Speed of germination is the first character which is observed while evaluating the seed during germination process. It is an important criterion to know the fastest stand establishment in the field which is considered to be an important component of seed vigour index (Perry, 1984). The pooled analysis of data pertaining to the effect of different seed priming treatments showed that speed of germination was maximum in the hydro primed seeds followed by those which were primed with NaCl and smoke water. Minimum speed of germination was observed in control, seeds sown with persistent floral parts (no priming) and alternating light/dark regime.

The present findings are in agreement with those of Pereira *et al*. (2009), Javid *et al.* (2013) and Lima and Filho (2010). The possible reason for fastest speed of

germination of the primed seeds, seems to be the completion of pre-germinative metabolic activities, enabling the seeds ready for radicle protrusion, thus making conditions favorable for early and fast germination compared to untreated dry seeds (Arif, 2005).

The primed seeds may have rapidly imbibed and revived the seed metabolism resulting in higher and faster germination rate and reduction in the inherent physiological heterogeneity in germination

Another possible reason for higher speed of germination in the primed seeds seems to be increase in peroxidase activities and respiration rate which may have resulted in higher speed of germination and overall percentage of germination besides repair mechanisms that occur during seed inhibition (Bray, 1995).

Faster speed of germination of the primed seeds may also have led to higher seedling emergence rate which is also related to seed weight and seed coat thickness. Also, there may be an increase in protein, sugar and RNA content which may have resulted in quicker germination of seed besides extensive accumulation of nucleic acid which may also have been involved in the acceleration of germination process resulting into faster speed of germination.

The speed of seedling emergence from non-primed seeds was low, independent of the initial physiological seed quality These results revealed the benefits of priming

seed lots of normal physiological quality and have been attributed to membrane repair, increased protein synthesis and more efficient mobilization of sugars or proteins (Srinivasan *et al.*, 1999). Similar are the findings of Arif *et al*. (2008).

Persistent floral parts lead to dormancy in some species. This could be a reason for the relatively slower speed in germination of *Napoleonaea vogelii*

Days taken to 50 per cent emergence of seed is an important character which is directly correlated with early and total germination. This character is also an indicator of fastest stand establishment in the field which indicates its positive correlation with seed vigour index (Perry, 1984). It is evident from the pooled data recorded on days to 50 per cent emergence after planting that hydro and halo primed seeds took minimum number of days to 50 per cent emergence followed by smoke water primed seeds as compared to control. The early and fast emergence of the primed seeds as in the present investigations may be due to early completion of pre-germinative metabolic activities enabling the seeds ready for radicle protrusion and such seeds germinated soon after planting as compared to untreated seeds (Arif, 2005).

These positive effects are probably due to stimulatory effect of priming on the early stages of germination process by mediation of cell division in germinating seeds (Sivritepe *et al*., 2003). Enhancement of emergence in primed seeds may also be attributed to metabolic repair processes, a buildup of germination metabolites or osmotic adjustments during priming treatment (Bray *et al*., 1989). These findings agree with those of Brocklehurst *et al*. (1987) and Harris *et al*. (2001b) who also reported faster emergence of primed seed in vegetables and wheat, respectively. Several reasons have been proposed to explain the observed stimulation in early and total germination. When seeds imbibe, the water content reaches a plateau and changes little until radicle emergence (Bradford, 1986). In addition to this, priming may advance germination by inducing a wide range of biochemical changes in the seed. Gallardo *et al*. (2001) also observed an increase in polypeptides during the priming treatment in *Arabidopsis thaliana* (L.) Heynh. Priming has also been reported to cause hydrolysis of abscisic acid (ABA) and leaching of cytokinins, coumarin and phenolic compounds from the seeds to the aqueous solution which can act as germination inhibitor (Hopkins, 1995)

Maximum number of days to 50 per cent emergence was recorded in control, light treatment, bush fire(5 cm depth and 10 cm height) and seeds sown with persistent floral parts which may be due to the presence of growth inhibitors which did not leach and the enzymes like catalase, peroxidase, amylase and invertase which

remained inactive. The present results are in agreement with those of Nath *et al*. (1991) and Owen and Pill (1994).

Light is essential for all aspects of plant life. Plants regulate growth and development in response to light. Analysis of variance of the obtained data revealed that there were significant differences in seedling growth of *N. vogelii*, with light treatment showing significant variations. Plants in the complete darkness showed the highest mean growth in height (32.42 cm) while those in 24 hours light showed lowest growth in height (18.88 cm). The absence of light was characterized by long, weak stems which were pale yellow in colour. This plant strategy increases the likelihood of the plant to reach a light source often from under the soil, leaf litter, or shade of competing plants. This elongation is controlled by auxins which are produced by the growing tip to maintain apical dominance

Analysis of variance of the obtained data revealed that there were significant differences in the number of leaves of *N. vogelii*, with light treatment (24 hours darkness) showing significant lower mean number of leaves (2.37). Number of leaves in the other treatments were not significantly different from the control. These leaves which were developed in complete darkness were smaller in sizes and pale yellow due to the absence of products of photosynthesis from these plants.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

Any factor that facilitates rapid germination may contribute to establishment of a successful crop. There is an urgent need for afforestation in Nigeria and a need to maintain the diversity of these forests, which calls for germination studies of forest shrubs and trees. This study will be useful in maintaining global plant diversity. It is recommended that further studies be carried out on *Diospyros barteri* to ascertain the best treatment for breaking its dormancy or best conditions needed for germination. Understanding species response to fire-derived cues (heat and smoke water treatments) will result in improved predictions regarding the impacts of fire management practices on individual species, hence communities.

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APPENDIX

Appendix 1: Anova summary table of the effects of various treatments on germination of *Napoleonaea vogelii* accross treatments using three replicates

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1575.244	14	112.517	25.316	.000
Within Groups	133.333	30	4.444		
Total	1708.578	44			

Appendix 2: ANOVA SUMMARY TABLE OF LEAF NUMBERS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	969.461	13	74.574	5.928	.000
Within Groups	2289.455	182	12.579		
Total	3258.916	195			

Appendix 3: ANOVA SUMMARY TABLE OF PLANT HEIGHT AT GROWTH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	586.723	12	48.894	21.867	.000
Within Groups	116.268	52	2.236		
Total	702.991	64			

Appendix 4: Multiple comparisons between treatments for total germination of *Napoleonaea vogelii*

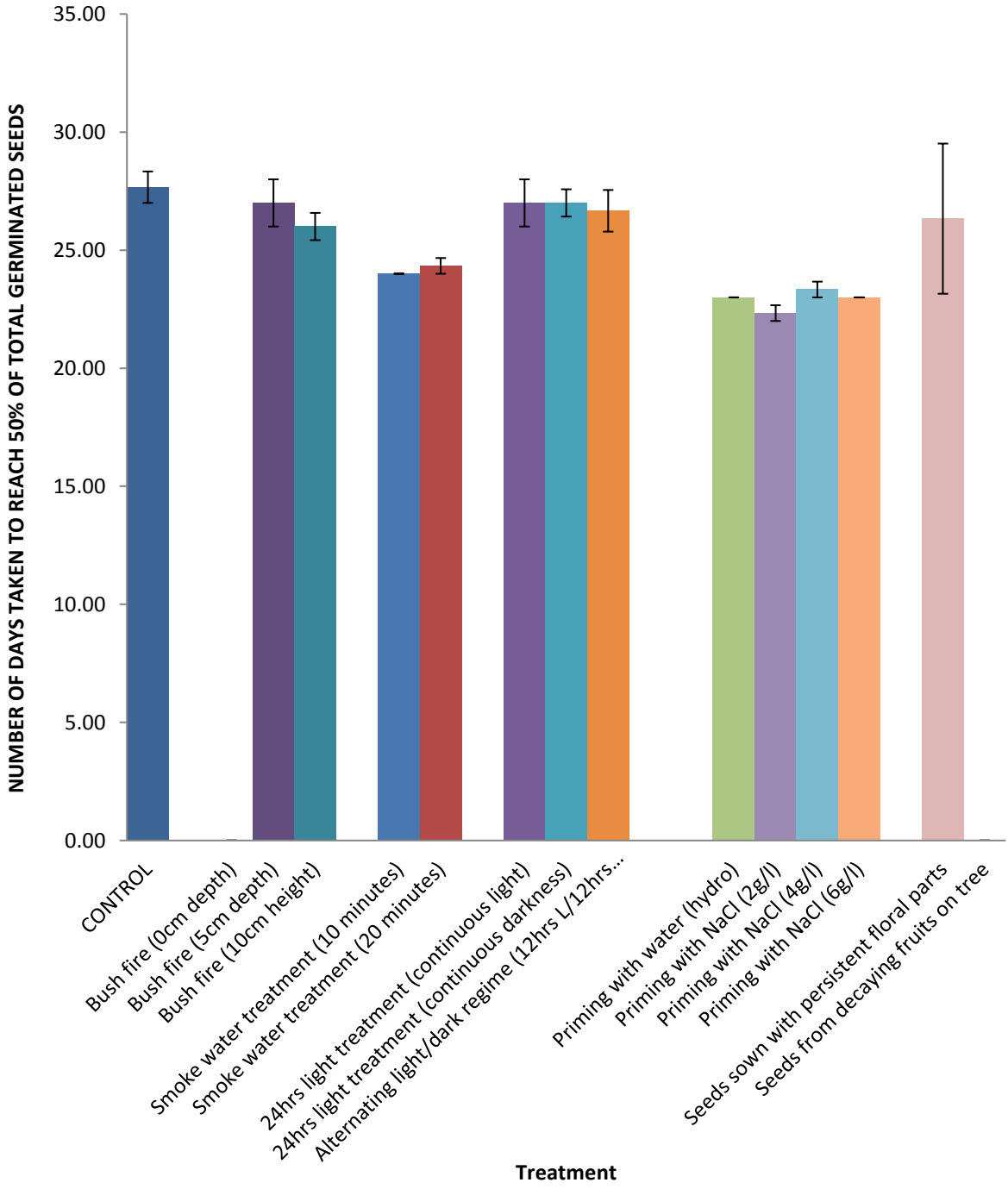
Treatment	Mean of total germination
Control (no treatment applied)	26.33^{bcd}
Bush fire (0 cm depth)	15.33^{a}
Bush fire (5 cm depth)	29.33^{def}
Bush fire (10 cm height)	25.33^{bc}
Smoke water treatment (10 minutes)	33^{fg}
Smoke water treatment (20 minutes)	31^{ef}
24 hours light treatment (continuous light)	29^{cdef}
24 hours light treatment (continuous darkness)	25^{b}
Alternating light/dark regime (12hrs L/12hrs D)	28.67^{bcd}
Priming with water (hydro)	34.33^{g}
Priming with NaCl (2g/l)	33^{fg}
Priming with NaCl (4g/l)	33^{fg}
Priming with NaCl (6g/l)	31.33^{ef}
Seeds sown with persistent floral parts (No priming)	26^{bcd}
Seeds from decaying fruits on tree	13.67^{a}

Means with the same alphabet(s) shows that there is no significant difference between them ($P<0.05$).

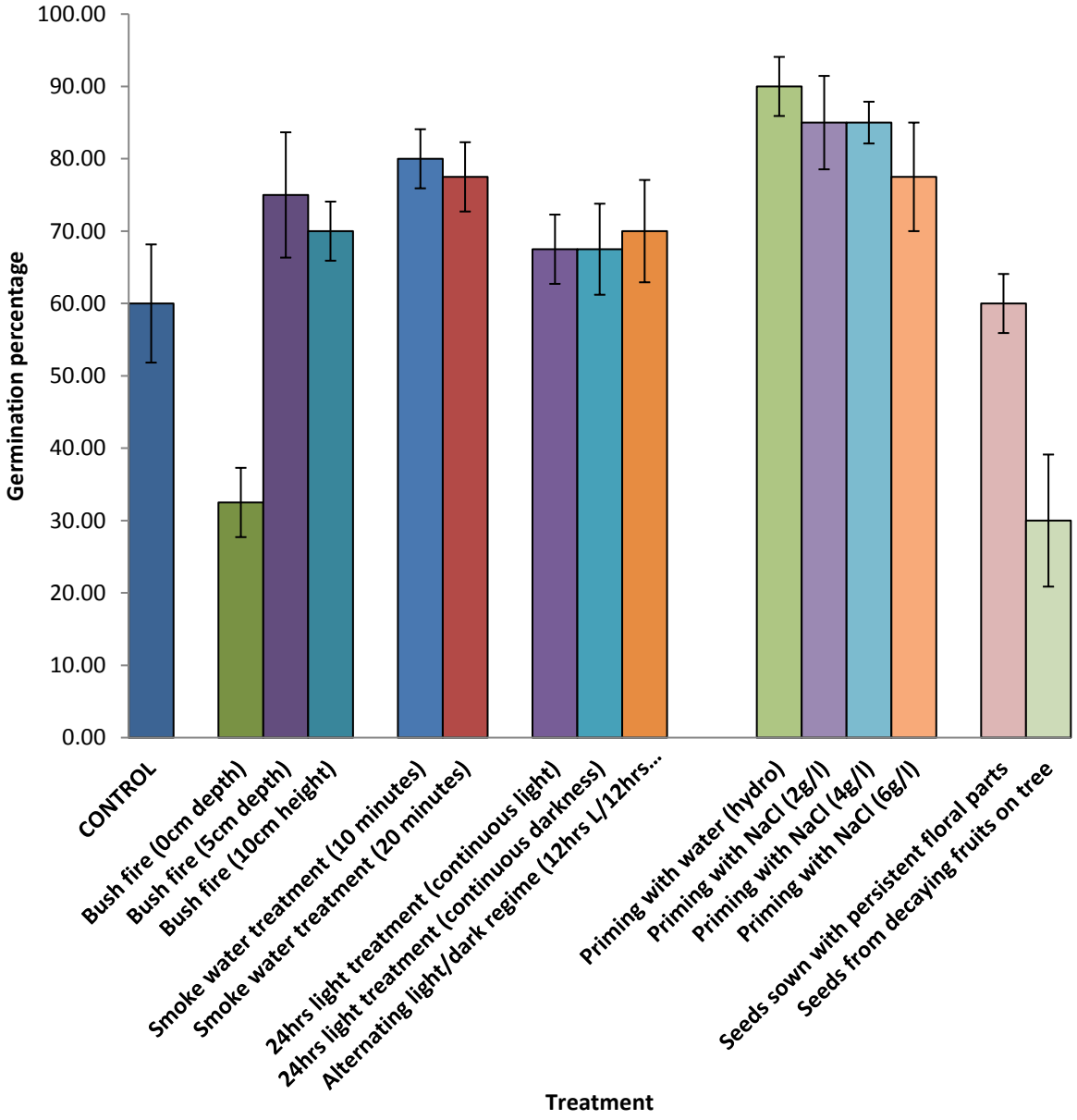
Appendix 5: Shows effects of various treatments on the speed of germination of *Napoleonaea vogelii* 35 days after planting.

SPEED OF GERMINATION

TREATMENTS	Speed of germination
Control (no treatment applied)	0.39±0.02
Bush fire (0 cm depth)	1.13±0.21
Bush fire (5 cm depth)	0.51±0.11
Bush fire (10 cm height)	0.90±0.03
Smoke water treatment (10 minutes)	1.75±0.20
Smoke water treatment (20 minutes)	1.53±0.17
24 hours light treatment (continuous light)	0.61±0.14
24 hours light treatment (continuous darkness)	0.50±0.05
Alternating light/dark regime (12hrs L/12hrs D)	0.44±0.03
Priming with water (hydro)	2.99±0.18
Priming with NaCl (2g/l)	2.77±0.08
Priming with NaCl (4g/l)	1.90±0.05
Priming with NaCl (6g/l)	2.04±0.11
Seeds sown with persistent floral parts (No priming)	0.44±0.04
Seeds from decaying fruits on tree	0.86±0.28



Appendix 6: Number of days taken to reach 50% of total germinated seeds



Appendix 7: Effects of various treatments on the percentage germination of *Napoleonaea vogelii* 35 days after planting. Bars represent (±) standard error.