The determination of the concentration of aqueous smoke solutions used in restoration projects

By
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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

Signature Date

Abstract

It is well known that smoke and aqueous smoke solutions promote the germination of certain seeds. This has considerable practical implications for restoration in fire prone areas like the Cape fynbos. The aqueous smoke solution (more commonly known as smoke water) can be used in restoration projects to stimulate seeds to germinate faster so that a wide diversity of plants can be established rapidly. Smoke water is made using different methods and different plant materials. This inevitably results in different concentrations of smoke water. Although made in different ways, different smoke waters may all have an enhancing effect on seed germination.

In this study, the germination of Grand Rapids lettuce seed was used to determine the differences between five different types of smoke water. Germination was done in a controlled environment, using through-flow germination boxes (patent no. ZA2000/1832, registered 11/4/2000) instead of traditional petri dishes. The differences in the concentrations were determined using bioassays. A very strong concentration of smoke water damaged the seed and a very weak concentration did not have any enhancing effect on germination. The concentrations of the different smoke waters were compared to a standard smoke solution (the first smoke solution ever made, that of De Lange & Boucher (1990)). The different concentrations of the smoke solutions were determined by comparing them to the standard, using a best fit line on the germination graphs. Each of the smoke solutions tested is given a "**delb**" rating (after **De** **L**ange & **B**oucher), with the standard smoke water being 1 delb. The delb value is used to determine the dilution factor for each smoke solution.

It is concluded that the five smoke solutions tested all differed from each other emphasizing the need for quality control in commercial and experimental applications.

Uittreksel

Dit is wel bekend dat rook en vloeibare rook oplossings (rookwater) die ontkieming van sekere sade bespoedig. Dit het groot praktiese implikasies vir hervestiging in gebiede met gereelde vuur, soos die Kaapse fynbos. Die rookwater kan in hervestiginsprojekte gebruik word om sade te stimuleer om vinniger te ontkiem om sodoende 'n groot diversiteit van plante vinnig te vestig. Rookwater word op verskillende maniere en met verskillende materiaal vervaardig. Dit kan lei tot verskillende konsentrasies rookwater, alhoewel al die verskillende rookwaters 'n stimulerende effek op saadontkieming kan bewerkstellig.

In hierdei studie is Grand Rapids slaai saad gebruik om die verskille tussen vyf verskillende rookwaters te ondersoek. Ontkieming was in 'n beheerde atmosfeer gedoen en deurvloei ontkiemingsbakke (patent nr. ZA2000/1832, geregistreer 11/4/2000) is gebruik, i. p. v. traditionele petri bakkies. Die verskille in konsentrasies is gemeet m. b. v. biotoetse. 'n Baie serk konsentrasie het die sade beskadig en 'n baie flou konsentrasie het geen stimulerende effek op ontkieming gehad nie. Die konsentrasie van die verskillende rookwaters is vergelyk teenoor 'n standaard rookwater (die eerste rookwater ooit gemaak, die van De Lange en Boucher (1990)). Die verskillende rookwater konsentrasies is bepaal deur dit met die standaard te vergelyk m. b. v. 'n regressie lyn op die ontkiemingsgrafieke. Elke rookwater getoets kry dan 'n "**delb**" waarde (n. a. v. **De** **L**ange & **B**oucher), met die standard gelyk aan 1 delb. Die delb waarde word gebruik om die optimale verdunning van elke rookwater te bepaal.

Daar word opgesom dat al die rookwaters getoets wel van mekaar verskil en dit beklemtoon die waarde van kwaliteits beheer in kommersiële en eksperimentele toepassings.

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Chapter 1

INTRODUCTORY REVIEW OF RESTORATION ECOLOGY AND THE USE OF SMOKE

1.1 Restoration ecology: Terminology and definitions

The problem of ecosystem damage is international and probably no country in the world is unaffected (Urbanska *et al.* 1997). As long as we need the comforts of civilization (many of these comforts originate from the ground, e.g.: mineral ores, fossil fuels and building materials), ecosystems will be damaged (Bradshaw 1983). Ecological restoration is the process of repairing damage caused by humans to the appearance, diversity and dynamics of indigenous ecosystems (Jackson *et al.* 1995). Where previous conservationists wrote off damaged areas as a lost cause, restorationists are actually opening a new frontier of conservation in these damaged areas. When damage occurs it is the best to repair it as quickly as possible, however the best cure for any disturbance is prevention of the disturbance. This should always be remembered and restoration ecology should complement and not be used instead of conservation.

Restoration ecology includes any form of ecological healing or rehabilitation (MacMahon & Jordan 1994). It can include terms such as restoration, rehabilitation, reclamation, re-creation and ecological recovery (MacMahon & Jordan 1994). Technically every term has a different meaning and can lead to different end results. Restoration ecology is a vast field of research and the meaning applied to the term restoration generally depends on the anticipated end product.

Restoration ecology aims to provide a scientifically sound basis for the repair of damaged or destroyed ecosystems to a state where they are self-sustainable (Urbanska *et al.* 1997) with little or no intervention by man (MacMahon & Jordan 1994). Restoration ecology can be defined as the full or partial placement of structural or

functional characteristics that have been extinguished or diminished and the substitution of alternative qualities or characteristics, to the ones originally present. The prerequisite being that they have more social, economic or ecological value than existed in the disturbed or displaced state (Cairns 1988). This definition combines all the above mentioned terms neatly into one definition.

1.2 The focus of restoration

The most commonly used type of restoration is habitat restoration. It simply refers to the place where organisms live and implies less than ecosystem restoration. It puts more emphasis on the restoration of place rather than on important ecological functions. Our attention should always be to focus on the restoration of processes, of functions and of biological potential, because without these components communities of organisms, in which we are interested, cannot persist (Bradshaw 1997).

1.3 The process of restoration ecology

The ultimate goal of restoration ecology is to restore a degraded landscape to its original, undisturbed condition. Restoration ecology is consciously or unconsciously mimicking the natural process of succession. This is done by manipulating the succession, either to speed it up or to stop it in an earlier state (MacMahon & Jordan 1994). Man restores ecosystems, because the natural succession of disturbed areas is usually a very slow process and requires some assistance (Bradshaw 1983).

To ensure the success of any restoration project social complications such as legislative and economic contingencies, community opinion and risk evaluation must be clearly understood (Cairns & Heckman 1996). Public opinion should never be underestimated, because the public have a lot of influence on local governments.

An ecosystem has many attributes. These can be simplified into two main components, structure and function. The damage to an ecosystem that has been disturbed can then

be represented on a graph with structure and function on each axis respectively (Fig.1.1). Both components need to be restored, because both would have suffered through the disturbance. Sometimes emphasis can be given to only one of the components, although the ideal is to incorporate both components (Cairns & Heckman 1996). Rehabilitation, in which progress has been made but the original state not achieved and reclamation, to something different (refer to above), can be represented on the same figure. This shows that restoration may not be easy, because it may be possible to restore function but the structure may not be achieved or *vice versa*. Ecosystems are in a dynamic equilibrium and are not static. Therefore, restoration of function may be more important than structure (Bradshaw 1997).

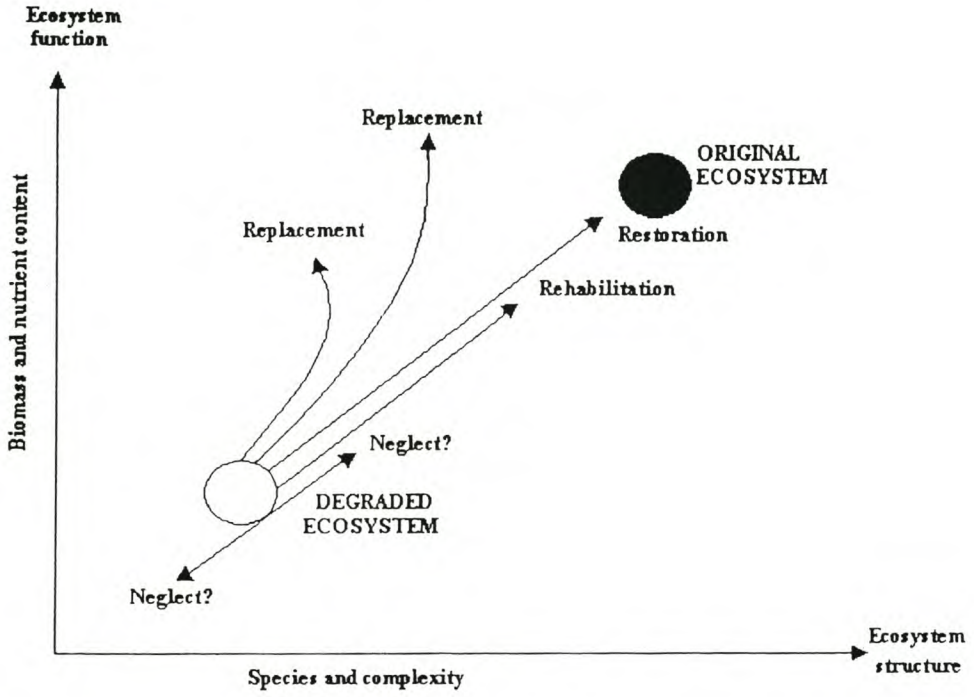

Figure 1.1 The different options for the improvement of a degraded ecosystem expressed in terms of structure and function. Restoration implies returning a system to its original state in both function and structure. A number of other alternatives are also possible, like rehabilitation where the original system is not reconstructed entirely, and replacement where the original is replaced by something different (after MacMahon & Jordan 1994).

According to Bush (1997) ecosystem restoration is still a very young science and most of the projects take place on a relatively small scale. The size of a restoration project is an essential question that has great relevance to its potential success or failure. The

ideal is to have a high degree of detail on a large spatial scale with a long time-scale in mind. This is usually not very practical and in most cases the temporal and spatial scales are much smaller and the detail is also sacrificed (MacMahon & Jordan 1994) (Fig.2.2).

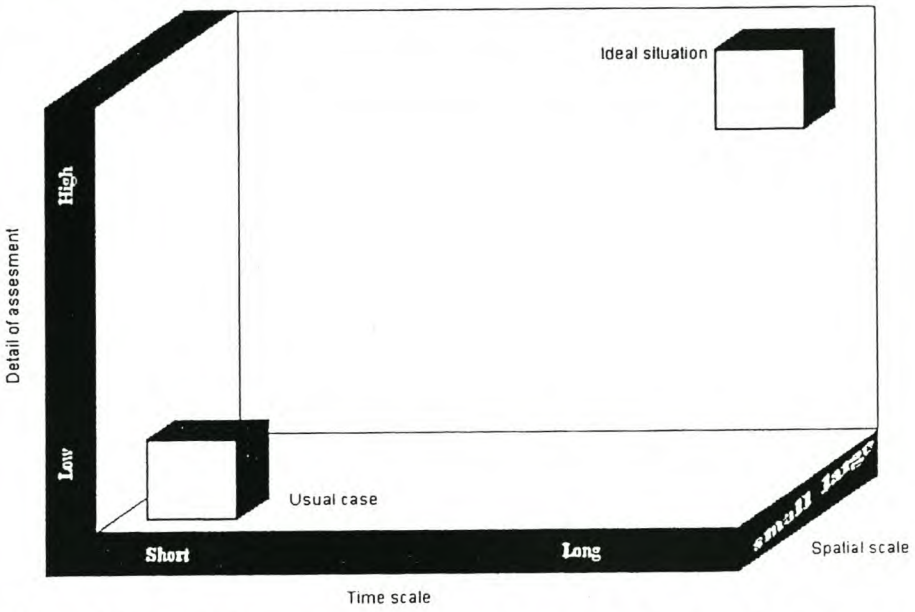

Figure 1.2 The ideal situation in restoration is to work on large spatial scales and with a high degree of detail with a long time scale in mind. The usual cases involve working on a smaller spatial and temporal scale and sacrificing detail (after MacMahon & Jordan 1994).

When restoring a given area, the first step is to return the topsoil (Bradshaw 1983). It may contain some seed and vegetative fragments as well as a lot of organic material (Ward *et al.* 1997). Failing this, the topsoil must be re-created. In fire-prone Mediterranean-type ecosystems dormant viable seed stored in the soil seed bank is a major source of new recruits for many plant species, such as in the southern Australian kwongan (Bell *et al.* 1993), South African fynbos (Bond *et al.* 1990) and Californian chaparral (Keeley *et al.* 1985; Enright *et al.* 1997). The topsoil is the "gold" of a restoration project. The "silver" is the seed that can be collected from nearby vegetation and sown unto the disturbed site. There are differences in the rate and abundance of seed production among populations of a given plant species (Handel *et al.* 1994) and this may influence the amount of seed collected of each plant species.

The apparent re-appearance of a plant species, after it appears to have disappeared, may depend on the persistence of its seed in the soil. These plants form a category that

includes species that are persistent in the soil for at least five years (Bakker *et al.* 1996). That is why the topsoil of a site to be restored is very important and must be stored for post disturbance utilization. The germinating seed can be used as an exellent initiator of the restoration process.

Restoration consists of different processes that usually include the replacing of topsoil, the sowing of seed, the planting of plants and the landscaping of the part to be restored so that it blends into the rest of the surrounding area. Successful restoration should be judged not only by the biomass and productivity of the restored site but also by the diversity of the plant community (Thorhaug 1980). This diversity can be achieved in various ways, for instance, by sowing seeds from wild species, by transplanting some individuals to help with recolonization or by using topsoil that may contain dormant seed or vegetative fragments (Bradshaw & Chadwick 1980). A large number of different operations have to be carried out correctly for a restored ecosystem to function like a natural one. There is no single overriding principle to be observed (Bradshaw 1983). That is why thought, before action, about the questions being asked, prior to and during a restoration project, are essential (Fairweather 1993).

Maximum return for investment in seed acquisition is vital. Synchrony and speed of emergence and gross quantitative success of establishment continue to be of industrial and ecological interest (Roche *et al.* 1997). Smoke and aqueous smoke solutions play a very important role in stimulating seed to germinate faster or stimulating seed with retarded germination under natural conditions. This has been proven for fire prone, Mediterranean ecosystems through the world (Brown 1993b; Dixon *et al.* 1995).

1.4 Smoke research in respect of restoration ecology

The use of smoke (in particular aqueous smoke solution) is a very important tool in restoring degraded land in the Fynbos Biome. The smoke solution is often made by the restorationist himself, but the concentration needed for optimal germination potentially differs between batches produced, because of variables such as heat of the fire, the amount of foliage burnt, type of plant material used etc. A way of measuring

the germination potential of the smoke solution is therefore needed. In this way uniform concentrations of smoke solution can be applied in every restoration project to get the optimum germination stimulus from the smoke solutions applied.

1.5 Smoke usage in restoration ecology

1.5.1 Introduction

In Mediterranean ecosystems, fire is a very important part of the ecosystem. The fynbos of the southwestern Cape, South Africa, is part of such an ecosystem. Periodic fires, with a frequency of five to 40 years, occur (Kruger & Bigalke 1984) and the fynbos plants are adapted to recurrent fire cycles. Fire stimulated germination has been reported for a number of fynbos species (Le Maitre & Midgley 1992). Characteristically there is an intense recruitment of plants immediately after fire, with little or no recruitment between fires (Brown 1993b). In the past a number of factors have been proposed as being responsible for the effect of fire on germination and are summarized by Brown (1993b). After investigation of the endangered plant *Audouinia capitata*, De Lange & Boucher (1990) found that heat does not stimulate the seed of this species to germinate. They did find, however, that smoke from burnt fynbos plant material had a stimulatory effect on seed germination.

Fynbos merits high conservation status due to its restricted distribution in the southwestern and southern parts of the Cape, its floristic richness and because of its high degree of endemism (Peterson 1988). Many of the fynbos plants are of great horticultural value and propagation, from seed, of some of these plants, is very difficult and the seed usually requires very specific environmental conditions (Brown & Botha 1993), of which fire or smoke may be one. Fynbos can be regarded as a moderately stressed vegetation type (Davis *et al.* 1994) which makes its restoration very difficult. The use of smoke (in particular aqueous smoke solution, or as it is more commonly known, smoke water) has helped a lot in the commercial restoration of fynbos (it has been used in the restoration of the Du Toit's Kloof N1 road project (Boucher *et al.* 1996) and at the Saldanha Steel factory restoration project (Mr. J.D. van Eeden,

VULA Environmental Services, tel. 0825645748, pers. com. 1999). Smoke is known to be a germination cue for fire-dependent and non fire-dependent plant species (Jäger *et al.* 1996a). That is why it is important to use smoke as an effective germination stimulant in fynbos restoration projects to hasten the recovery process.

The mechanism of smoke stimulated germination is a chemical stimulus rather than a mechanical one, in contrast to the effect of the heat of fires on seeds. In restoration, this distinction may be a very important factor in the stimulation of germination of certain species. The usual practice in restoration projects was the utilization of hardy, well known and rapid growing species (Peterson 1988), that are often pioneer species. The problem with this is that the ecological diversity of the site is greatly reduced. At Fermilab (in the U.S.A.) attempts to restore 250 hectares of prairie have resulted in sacrifices in quality and diversity of native species and underrepresentation of species that are difficult to propagate (MacMahon & Jordan 1994). To avoid the same problems in the fynbos, smoke (including aqueous smoke solution) has done a lot to raise the diversity of plants in the restored areas as well as getting seeds to germinate that were previously considered to be "difficult" to germinate.

The smoke water is applied to the seed in the natural vegetation instead of normal water, because it mimics the reaction of the smoke in a wild fire and stimulates certain species to germinate without the hazards associated with a fire.

1.5.1.1 History and general characteristics

The first researchers to point that other factors than the heat of a fire may be responsible for seed germination were Keeley *et al.* (1985), Keeley & Pizzorno (1986) (charred wood) and Van de Venter *et al.* (1988) (ethelyne), but De Lange & Boucher (1990) were the first to record that smoke stimulates seeds to germinate. The species they investigated, *Audouinia capitata* (L. f.) Brongn., is known to be difficult to germinate under normal nursery conditions, but there is usually moderate recruitment in nature, after wild fires.

Positive germination response to smoke occurs over a wide range of plant species from all life forms (trees to herbs) and reproductive strategies (annual seeders, bradysporous species and long-lived resprouters) (Brown & Van Staden 1997). Smoke and smoke solution acts as a seed germination cue in fynbos species *inter alia* in the families Asteraceae, Ericaceae, Restionaceae, Proteaceae and many more (Brown 1993b; Brown *et al.* 1993). Out of 32 Restionaceae species tested, 25 showed improved germination in the presence of smoke or smoke water. This shows the importance of smoke from fynbos fires as a germination cue in many Restionaceae species (Brown *et al.* 1994). Out of 94 native Australian species tested, 45 were positively influenced by smoke derived from burning native vegetation. This included 15 families and 26 genera of dicotyledons, five families and eight genera of monocotyledons and the gymnosperm *Actinostrobus acuminatus* Parl. Of these, 23 taxa have previously proven to be difficult to germinate using conventional methods. This indicates that the products of fire, rather than the direct influence of the heat, may stimulate seed germination (Dixon *et al.* 1995).

Instead of being stimulated to germinate, some plants (like *Helichrysum aureonitens* Sch. Bip) are inhibited by smoke solution (Afolayan *et al.* 1997). This may show that the stimulus of smoke cannot be associated with specific families, although other Asteraceae have been shown to germinate in reaction to smoke solution (Brown 1993b).

Smoke has other plant stimulation properties as well. Smoke is known to stimulate flowering in the fire-lily, *Cyrtanthus ventricosus* (Jacq) Willd. (Tompsett 1985, Keeley 1993), and in the Australian plant, *Xanthorrhoea australis* R. Br. (Gill & Ingwersen 1976). Aqueous smoke solutions are also known to have a positive effect on radicle emergence and lateral root development (Taylor & Van Staden 1996). Although the active compound(s) that stimulate(s) flowering and root development is/are not known, they may be the same as those that stimulate germination in seed.

Smoke is not the only germination stimulant known and it can be used in combination with other stimulants such as hormones, heat, darkness etc. (Keith 1997, Strydom *et al.* 1996). Rapid aging and smoke work together to enhance germination in 7% of the

native Australian plant species tested. All the groups tested by Roche *et al.* (1997) gave increased germinability after 1 year of soil storage after being treated with smoke solution. Germination can sometimes be higher in the second year of soil storage after treatment with smoke than in the first year (Roche *et al.* 1997). This may be explained by the fact that different species require different smoke concentrations to stimulate germination. Smoke may stimulate germination, but other factors may put the seed into secondary dormancy, which will only be lifted after soil storage for a certain period of time.

Aqueous leachate of charred foliage has also been found to be very effective in stimulating germination of certain fire–following chaparral herb species (*Emmenanthe penduliflora* and *Eriophyllum confertiflorum*) (Keeley & Pizzorno 1986).

1.5.1.2.1 Methods of production of aqueous smoke solution

Introduction

Both aqueous smoke solutions and direct applications of smoke are used to stimulate germination. The first documented technique to make smoke for germination stimulation purposes was that by De Lange & Boucher (1990). This was done by generating smoke in a 130 *l* drum, using a mixture of dry and wet fynbos vegetation. The smoke was then blown through a pipe into plastic tents, set over earth in the field, for 30 min. The system allowed the smoke to cool before coming into contact with the soil. Roche *et al.* (1997) and Brown (1993b) both used the same system, exposing the seed containers to smoke for an hour.

Heating and charring of foliage

Before the reaction of smoke on germination was known Keeley & Pizzorno (1986) found that unblackened *Adenostoma* wood heated to 175°C for 30 minutes was as effective in stimulating germination in *Emmenanthe penduliflora* and *Eriophyllum confertiflorum* as was wood that was fully charred. Pure cellulose heated to 175°C did not stimulate germination of the two above mentioned chaparral species, although cellulose heated to lower temperatures did stimulate germination (Keeley & Pizzorno

1986). To find an alternative to smoke, dry heating of leaf and stem material was found to release the same active germination stimulants as are found in smoke. This method was used by Brown (1993a) using *Passerina vulgaris* Thoday. plant material heated to 80°C and by Baxter *et al.* (1995) using *Themeda triandra* Forssk. plant material heated to 175–225°C. At lower temperatures the active compound(s) in *T. triandra* were not released (Baxter *et al.* 1995).

The generation of smoke from materials heated to different temperatures appears to have an impact on the volume of active compound(s) released. The greatest promotive effects on germination were found in smoke generated between 160–200°C (Jäger *et al.* 1996a). At temperatures greater than 220°C the promotive effects on germination were lost, possibly through the volatilisation of the active compound(s) (Jäger *et al.* 1996a). This finding is of ecological importance when considering the effects of wildfires on plant regeneration where temperatures above the soil can reach up to 600°C (De Lange 1992). This highlights the argument that very high temperatures may have an influence on the formation of the active compounds present in smoke.

Bubbling

Smoke can be bubbled through water to obtain an aqueous smoke solution (De Lange & Boucher 1990; Enright *et al.* 1997). This will be referred to as the "Bubbling Method" and the product will be called aqueous smoke solution or simply smoke solution or smoke water. De Lange & Boucher (1990) burnt a mixture of fynbos material. Sutcliffe & Whitehead (1995) derived smoke from dried seedpods (they do not mention whether the seedpods were heated or burnt) and bubbled it through 1 *l* quantities of distilled water for 15 min. They claim that this water is saturated with smoke. Keeley & Fotheringham (1998) combusted foliage and wood from a chaparral shrub (*Adenostoma fasciculatum*) on a hot plate (no fire) and funneled the smoke through a hose into a 70 *l* glass chamber. The duration of the process is unspecified.

Brown (1993b) ignited a mixture of dry and green fynbos material in an 18 *l* metal drum. The smoke was bubbled through a glass column containing 2.5 *l* of distilled water for 30 min. using compressed air. This smoke water concentrate was then diluted with water into different dilutions. There is uncertainty as to the variation in

active compounds being produced using different species to generate smoke, consequently Brown (1993b) only burnt *Passerina vulgaris* to produce smoke solutions for test purposes.

Drewes *et al.* (1995) generated smoke in a standard beekeeper's smoker using burnt *Themeda triandra* leaf material. This smoke was bubbled through distilled water in a glass jar until "the water was yellow in colour" (Jäger *et al.* 1996a & c; Thomas & Van Staden 1995). The duration of the process required to make this solution is not given. This suggests a lack of repeatability, particularly as the concentration is not specified. This smoke solution was used in the development of a bioassay using *Lactuca sativa* L. Grand Rapids seed (lettuce). According to the Oxford Dictionary of Plant Sciences (1998) a bioassay is "the use of living organisms to make quantitative and/or qualitative measurements of the amounts or activity of substances."

Dixon *et al.* (1995) used De Lange & Boucher's (1990) method but used a mixture of native Australian vegetation from the *Banksia-Eucalyptus* Woodlands. This is now sold commercially by Kings Park and Botanic Garden, in Australia (West Perth). The solution is obtained by bubbling smoke through water for 90 min.

The results presented in this thesis are based on the standard smoke solution produced by De Lange & Boucher (1990) (bubbling method), which is termed the De Lange Standard. This smoke solution was stored below freezing point in the interim (1990 – 1998).

Distilling

Applying aqueous smoke solution in restoration projects usually requires considerable volumes of the concentrated aqueous smoke solution. It is a very labourious operation to mix 80 *l* of standard smoke solution into a 20 000 *l* tank. A method to make a more concentrated smoke solution to reduce distribution effort was needed. J. D. van Eeden of Vula Environmental Services (tel. 082 564 5748) a horticulturist and restorationist, developed a technique to obtain a distillate from smoke by condensation. This smoke distillate which is potentially more concentrated will be evaluated below.

Discussion

There are clearly a number of different methods available to extract the germination stimulants present in smoke derived from plant materials. Each method has many variables that can influence the concentration or affectivity of the end product. The Bubbling Method should give the same concentration each time if the amount of plant material always has the same moisture content, the rate of combustion is constant; the rate of bubbling is constant; the duration of burning and bubbling is constant and if the temperature of the fire and the solute are constant. The same plant species must also be used for combustion. These are variables that are clearly very difficult to regulate.

The principle aim of this thesis is to quantify the concentration (therefore determining the optimum dilution level) of smoke solutions, because it is virtually impossible to control the different variables during the production process.

1.5.2 Physiological review

There is strong suggestion that the same active compound(s) occur in all the different kinds of smoke solutions made locally or internationally (Baxter *et al.* 1995). Smoke used in germination experiments has been obtained from a variety of sources including gymnosperms and a number of different angiosperm leaves, even from tissue paper and from cellulose (Jäger *et al.* 1996a). It is therefore suggested by Baxter *et al.* (1995) and Jäger *et al.* (1996a) that the active compound(s) in plant derived smoke originate from components which occur in many different plant taxa and are effective on many different types (species) of seeds (Van Staden *et al.* 1995c). Bioactive compounds breaking dormancy in *Syncarpha vestita* (L.) B. Nord. have been found in smoke and aqueous solutions of smoke and charred wood derived from burning leaf and stem material of *Passerina vulgaris* (Brown 1993a; Brown *et al.* 1994). Baxter *et al.* (1995) note that there is a variation in the amount of promotion of germination by the smoke from different plant species. This can either be, because of the different amount of the active compound(s) in each of the plants or through differences in heat and amount of smoke produced when the smoke was generated etc. The concentration of different smoke solutions may therefore differ from each other, because different

proportions of active compounds may be released. These active compounds could be heat breakdown products of cellulose or hemicellulose (Baxter *et al.* 1994; Jäger *et al.* 1996a; Jäger *et al.* 1996b; Taylor & Van Staden 1996).

The present study was undertaken, to try and develop an universal smoke dilution assay that can be used internationally and thereby enabling the production of comparable results.

Mechanisms of smoke induced germination

Three classes of mechanisms participating in smoke-induced germination have been proposed (i) nutritive mediated stimulation, a role ascribed to nitrate-induced germination; (ii) chemical scarification of the seed coat and (iii) a "signal–mediated" stimulation of germination involving a diversity of different chemical signals (Baldwin *et al.* 1994). Three classes of compounds could also be involved in the induction of germination: (i) oxidizing compounds, (ii) protons and (iii) weak acids (Keeley & Fotheringham 1998).

Octaonic acid

According to Sutcliffe & Whitehead (1995) the active compound in smoke saturated water is octaonic acid. However, from its chromatographic behaviour, octaonic acid is unlikely to be the active compound in smoke that stimulates the germination of lettuce seed (*Lactuca sativa* L. var. "Grand Rapids") (Jäger *et al.* 1996c). Sutcliffe & Whitehead (1995) tested *Cyclopia* seed and found that variations in the active compounds that stimulate germination of different seeds may occur.

Ethylene & fatty acids

When vegetation is burnt, both ethylene and short-chain fatty acids are released in the smoke. The presence of these compounds in the smoke could stimulate seed from various species to germinate (Sutcliffe & Whitehead 1995, Van de Venter & Esterhuizen 1988). Through different experiments it can be shown that ethylene is not the principal active compound in smoke (De Lange & Boucher 1990). Ethylene does not stimulate the fire-lily, *Cyrtanthus ventricosus*, to flower (Keeley 1993), despite smoke having a stimulatory effect on the flowering of *C. ventricosus* (Tompsett 1985).

Hormones

Smoke may contain compounds that have similar effects to cytokinins (Thomas & Van Staden 1995). This would explain the synergistic effect caused by the cytokinin, benzyl adenine (BA) and smoke solution on the germination of Grand Rapids lettuce seed, in a study done by Strydom *et al.* (1996).

High temperatures present in surface soil layers during fires may cause a decrease in cytokinin levels in the seed. Seeds need these cytokinins to release thermodormancy. Smoke may then increase the sensitivity of the seed to cytokinins (Strydom *et al.* 1996). There is no heat involved when seeds are treated with smoke solution and the above mentioned process may therefore be relevant to certain selected seeds only. The sensitivity of seed to smoke stimulation for germination increased relative to the degree of imbibition of the seed in smoke solutions (Baxter *et al.* 1994). This suggests, according to Baxter *et al.* (1994) and Taylor & Van Staden (1996), that smoke may work on an enzyme system or on phytohormone metabolism. In light sensitive lettuce seed, there was a synergistic effect with the hormone gyberillic acid (GA_3) when combined with smoke. Of all the hormones tested, the gibberellins had the greatest interaction with smoke to promote germination (Brown & Van Staden 1997).

Oligosaccharins

The bioactive compound in charred wood may be an oligosaccharide type molecule, which is a thermal breakdown product of xylan or other hemicellulose having glucuronic acid side chains. Oligosaccharins may be released by the alterations produced by heating xylan and its glucuronic acid side chains. This may stimulate germination and the subsequent growth of dormant seeds (Keeley & Pizzorno 1986).

Combination of compounds

Keeley & Fotheringham (1998) suggest that the nitrate(s) in the smoke (and smoke solutions), although they may play a part, are not primarily responsible for the germination. In a study done by Van Staden *et al.* (1995b), twelve compounds were identified in one particular smoke solution and seven of these compounds occurred in another, different smoke solution. These compounds are fairly stable and can be

detected after a series of chromatographic procedures (Van Staden *et al.* 1995b). Van Staden *et al.* (1995b) postulated that the active compound(s) might therefore be one or more of the seven compounds that occurred in both solutions. After obtaining some of these active compounds commercially it was found that not one of them alone stimulated germination. Therefore, at the moment, it is believed that more than one of these active compounds in combination are involved in the stimulation of germination of seed (Brown 1993b; Brown *et al.* 1994; Van Staden *et al.* 1995a; Van Staden *et al.* 1995b).

The effective compound(s) may persist in the soil until other conditions are favourable for germination (De Lange & Boucher 1993). In a study done by Keith (1997) the combined effect of heat shock, smoke and darkness gave significantly enhanced germination to a fire prone Australian shrub (*Epacris stuartii* Stapf.). Seeds therefore, may need different combinations of stimuli for germination to be initiated (De Lange & Boucher 1993). These results suggest that smoke may only be one of a combination of factors that may stimulate germination. It must be remembered that low germination percentages when using smoke may not be the result of low viability, but may be because of the absence of other factors needed as germination cues (Brown *et al.* 1994).

The active compound(s) in smoke will probably depend less on the type of material being burnt, than on the extent and speed of the combustion. It seems that a slow, smouldering natural fire will be more effective in producing the active compound(s) (Brown & Van Staden 1997) or this may simply be a concentration effect. Smoke is as effective as smoke solution in stimulating *A. capitata* seed to germinate (De Lange & Boucher 1993) but smoke solution is much easier to handle and can be applied to a larger area under more controlled conditions.

Researchers are currently investigating the chemical characteristics of the active compounds in smoke. In the industry, guidelines are needed urgently for the assessment of different smoke solutions in restoration projects. Until the active compound(s) are identified another guideline as to the strength of smoke solutions is required. This study attempts to develop such guidelines.

1.6 Methods to compare different smoke solution dilutions with each other

Different smoke solutions were compared with each other by means of different criteria in an attempt to find a method for the consistent comparison of the different smoke solutions. The criteria tested are pH, resistance, colour and bioassay.

1.6.1 pH

Brown & Van Staden (1997) found that the pH of the smoke solution does not influence the effect of the active compounds in the smoke solution. In contrast Prof. K. W. Dixon (Director – Plant Science, Kings Park and Botanic Garden, West Perth, Australia, pers. com. 1999) found, using an Australian produced smoke solution, that the pH did have an effect on the germination of Grand Rapids lettuce seed. More than 80% of the seed of the chaparral annual *Emmenanthe penduliflora* germinated using very acid solutions (< pH 4) while smoke solutions buffered to pH 7 (more alkaline) were less effective (<10% germination) (Keeley & Fotheringham 1998).

The question to be addressed is whether there is a correlation between pH value and the concentration of the smoke solution? In this study the pH of the smoke solution was not found to be a very reliable method of comparison, because the smoke solutions tested in this study all had a pH of less than 5 (between 3 and 4) (Appendix 1). A pH of less than five (more acid) has been shown only to have a different effect on seed germination than a pH of more than five (more alkaline) (K. W. Dixon pers. com. 1999).

1.6.2 Resistance

Another option was to use resistance as an indication of the concentration of the smoke solutions. The resistance of the different smoke solutions tested were not constant and no comparison could be drawn between concentration and resistance (Appendix 2).

1.6.3 Colour

Colour of the aqueous solution was another option. I suggest that colour is unreliable and will not give a clear indication of the concentration of the active compounds in the solution. A solution with a darker colour may be a stronger solution, but could equally be a weaker but contaminated aqueous solution containing ash and incompletely burnt materials that would darken the solution out of proportion to the active compounds present (Appendix 3). These contaminents could be filtered out, but this may be time consuming and may not be a rapid method of testing a smoke solution. A solution can also be darkened artificially before it is tested, therefore giving an unreliable result.

1.6.4 Bioassay

The comparison of germination results using different smoke solutions to a standard solution (e. g. the De Lange Standard) should give a reliable and precise result, because various dilutions of the smoke solution can be tested. It was decided to attempt to develop a bioassay for the determination of the optimum dilution level of active compound(s) in aqueous smoke solutions as it was thought that this method held the most promise of success. The use of a bioassay for experiments on smoke solutions has been used extensively in the literature (Drewes *et al.* 1995; Jäger *et al.* 1996a, b & c; Van Staden *et al.* 1995a, b & c) in efforts to determine the active compound(s) in the smoke. Here it will be used to determine the optimum dilution level of smoke solutions. Other questions that may arise from the use of a bioassay are:

1) Which seed should be used for the bioassay?

2) What factors influence the reaction of the chosen seed?

1.7 Critical questions

In restoration ecology there is a need to provide a means to quantify the quality of aqueous smoke solution particularly for quality control in commercial restoration projects. The cost of commercial restoration projects can be very high. The seeds being used can amount to over R1 million (Boucher *et al.* 1996), therefore the

germination of the seeds can be a very important quality control measure. Under these circumstances the lack of quality control can lead to the use of inferior products. The following critical questions will be addressed as a first attempt to solve these problems.

1. Can the determination of the concentration of the active compound(s) in aqueous smoke solutions be standardized?
2. Is there a difference in germination between Through - flow Germination boxes (see next chapter for discription) and petri dishes when used to determine smoke solution concentrations?
3. Is there a difference in the concentration of active compound(s) in smoke solutions made in different ways?
4. Can seeds be exposed to concentrated aqueous smoke solutions for lengthy periods without being harmed?

Chapter 2

GENERAL METHODS USED IN THIS STUDY

2.1 General methods for germination used in this study

Disposable plastic petri dishes and Whatmann No. 1 filter papers were used in experiments done by other researchers testing smoke solution (Baxter & Van Staden 1994; Van Staden *et al.* 1995a, b & c).

Special germination boxes (Fig. 2.1 & 2.2) were used in this study. These boxes are made of Perspex® and function as and are called the Through-flow System (patent no. ZA2000/1832, registered 11/4/2000). This concept is similar to "slants" (germination paper held at 20° off the vertical) described by Korkmaz & Pill (1999). Some seed (up to one fourth of a given population) of certain herbaceous ornamental plants (Atwater 1980) will germinate readily when moist, but the remainder may have inhibitors that must be leached out before the remaining seed will germinate. The use of the Through-flow System enables any inhibitory excretions from the seed to be removed from the vicinity of the seed. The moisture gradient on the filter paper remains constant throughout. With the Through-flow Germination Boxes, a constant flow of the germination medium, which in this case is aqueous smoke solution, is supplied to the seed. The effect of leaching can also be measured using these germination boxes.

Germination of quiescent seeds, may be divided into three stages namely, (1) activation of metabolism, (2) preparation for elongation and (3) seedling growth (Obroucheva & Antipova 1997). Following the literature seed germination is deemed successful when either:

a) the radicle becomes exposed (Keeley & Pizzorno 1986); or
b) the radicle length reaches 1 mm (Sutcliffe & Whitehead 1995 and Pierce *et al.* 1995); or
c) the radicle length reaches 2 mm (Drewes *et al.* 1995).

Estimation of 2 mm as a suitable length for germination proved impractical as it was very time consuming to measure each radicle when it was approximately this length. It was therefore decided to classify a Grand Rapids lettuce seed as having germinated once the radicle length equalled the seed length (the average length for ten seeds are 2.7 mm).

Glass microfibre filterpaper was used, as it is neutral and it does not degenerate, thereby releasing acids, an important consideration when using unbuffered solutions. Use of glass microfibre filterpaper also removed possible germination stimulatory or inhibitory effects from the degradation of cellulose in conventional filterpaper. Furthermore, glass microfibre filterpaper is considered to give a better, more constant flow of the germination solution.

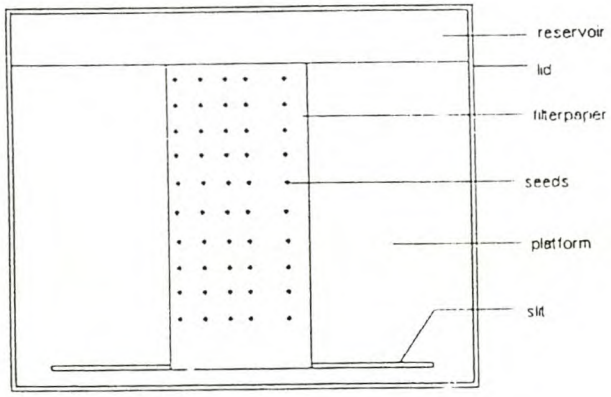

Figure 2.1 A germination box viewed from above, showing the placement of the seeds.

The Through-flow System germination boxes (which will be referred to as germination boxes hereafter) were used repeatedly and were washed with an ammoniated soap (Neptune®) and sterilised with Snobrite® liquid bleach after which they were rinsed in distilled water and then drip-dried.

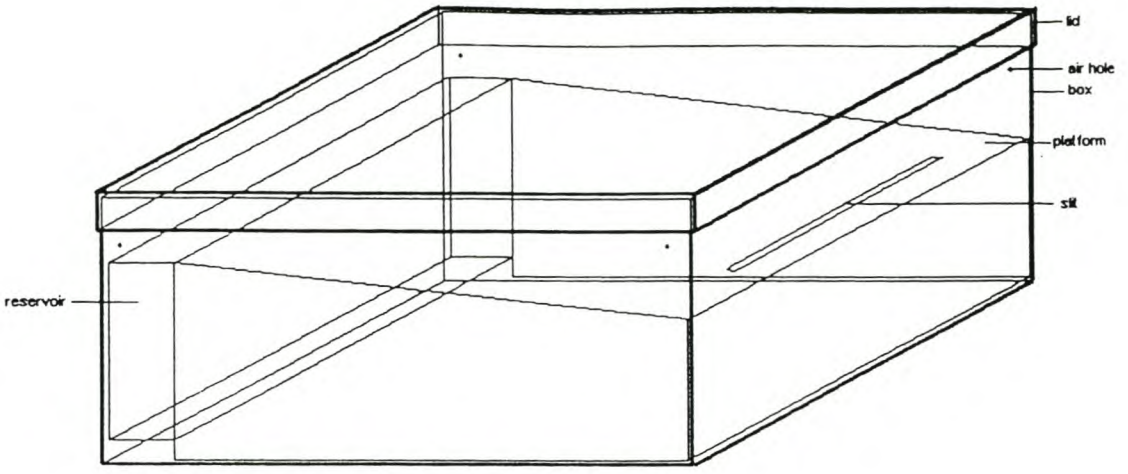

Figure 2.2 A 3-dimensional view of a germination box.

Seeds were stored in a cool, dry, dark room prior to use in the various experiments. Tweezers were used to place the 50 seeds per replicate (Thomas & Van Staden 1995, Drewes *et al.* 1995) in five lines of ten seeds each on the germination box platform, to prevent contamination or moisture transfer from the fingers influencing results. Successful germination was expressed as a percentage of the total number of seeds used.

Drewes *et al.* (1995) used a green safe light during inspections to prevent exposure of seed to light. During all the present series of experiments seeds were handled under normal laboratory light while placing them in the germination boxes, as dry lettuce seed does not germinate or respond to light. They are only imbibed sufficiently after 16 hours for light to have an effect on germination (Noggle & Fritz 1976, Mr. M. A. van der Merwe, Dept of Botany, University of Stellenbosch, South Africa, pers. com. 1998). Dim lighting was used prior to subjection of the first germination box to moisture (Keith 1997). Black refuse bags were used to cover the germination boxes immediately after either the distilled or the smoke water was added and the germination boxes were placed in a darkened Labcon Growth chamber maintained at 25°C ±0.5°C following optimum conditions as recommended by Drewes *et al.* (1995). The refuse bags excluded light and also prevented possible cross-contamination by air movement from one germination box to another.

Experiments were performed randomly through the day beginning at 6h00 and ending at 3h00. Timing of each experiment was started once the last box was loaded. The time discrepancy between first and the last box was discounted by randomising the placement of the boxes in the growth chamber.

The growth chamber had space for 12 germination boxes at a time thus eleven different dilutions and one control could be evaluated simultaneously. Germination boxes were randomly placed in the growth chamber and each experiment was repeated a minimum of 3 times (the minimum repetitions needed for statistical evaluation) at different time intervals. In some cases up to six replicates at different time intervals were performed to ensure the results were accurate. The data were arcsine transformed and then subjected to a one way analysis of variance test by means of a least significant difference test (LSD) using Statgraphics (Statgraphics version 5 1991). Bars marked with different letters are significantly different from each other at the 95% confidence level.

All experiments were done over 24 hours with the standard smoke solution (De Lange Standard) at a dilution level of 1:1 600 (this dilution level constantly gave good results), unless otherwise indicated. Distilled water was used in control experiments, unless otherwise indicated.

To determine if the Through-flow Germination Boxes were the better option in this study, the following experiment was performed.

2.2 Differences between petri dishes and Through-flow Germination boxes

2.2.1 Introduction

The following critical question is evaluated here: Are the Through-flow Germination Boxes better to germinate seed, when exposed to smoke solutions, than petri dishes?

2.2.2 Methods

The general methods was applied in these experiments with the following differences: In this study glass petri dishes were compared to Through-flow Germination Boxes to determine whether the equipment gave equivalent results. The same glass micro fibre filter paper was used throughout this experiment.

Twenty five seeds were placed in each of the petri dishes (Drewes *et al.* 1995) as well as in each of the germination boxes. Each trial was repeated six times. Each petri dish was placed in a zip lock bag to prevent any air movement from one petri dish to another. Both the petri dishes and the Through-flow Germination Boxes were placed in black refuse bags for the duration of the experiment to prevent light influencing the trial. In preliminary tests it was determined that 2 ml of solution, as used by Drewes *et al.* (1995), was insufficient to keep the substrate wet because fairly large petri dishes were used. The filterpaper in the petri dishes was therefore wetted using 3 ml of smoke solution or distilled water. A 1:1 600 smoke solution dilution was used in this experiment. Comparisons between the different types of apparatus were made using (a) the same smoke solution in the dark, (b) with distilled water in the dark and (c) with distilled water in the light. Germination was expressed as a percentage of total seed over a period of 24 hours.

2.2.3 Results

The average germination percentage of the germination boxes in the light was 89.33% and that of the petri dishes 84% (Fig. 2.3). An LSD test on the data indicated that there was no significant difference.

In the dark the average germination percentage of the germination boxes was 30% and that of the petri dishes 15.5% (Fig. 2.3). The LSD test indicated no statistically significant difference.

In the dark, with a smoke solution, the germination percentage of the germination boxes (58%) was significantly higher than that of the petri dishes 26.5% (Fig. 2.3).

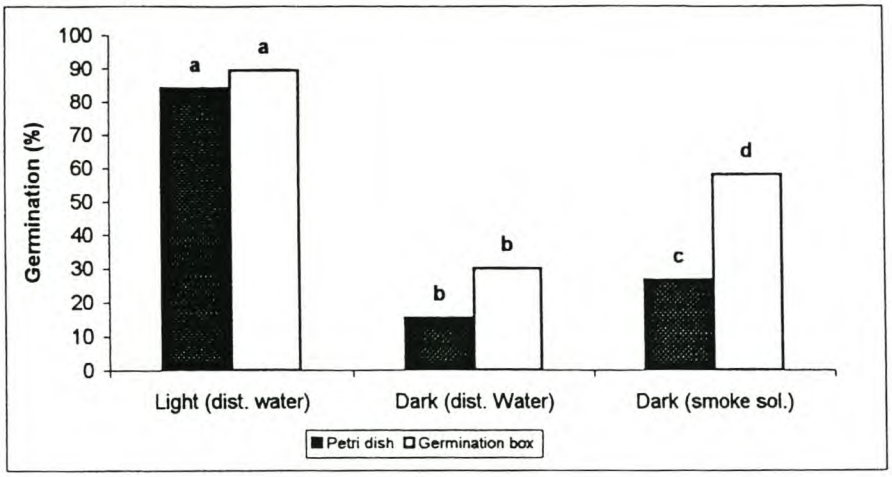

Figure 2.3 The comparison of germination percentages (over 24 hours) between germination boxes and petri dishes in the light using distilled water, in the dark using distilled water and in the dark using a 1:1 600 smoke solution (n = 25). The same letter indicates no statistical difference.

2.2.4 Discussion

The germination percentage in the light compared to the dark was high for both the germination boxes and the petri dishes (Fig. 2.3). The fact that the untreated Grand Rapids lettuce seed did not germinate very well in the dark confirms the argument that this seed has photomorphogenetic germination inhibition (Noggle & Frits 1976, Drewes *et al.* 1995). There is no significant difference between the germination boxes and petri dishes in the light or in the dark using distilled water. This suggests that there are apparently no inhibitory substances being released.

In contrast, the germination boxes show a significantly higher germination percentage than the petri dishes in the dark when the seed is exposed to smoke solution (Fig. 2.3). Based on the premise that the Through-flow Germination Boxes and the petri dishes provide uniform and constant wetting and uniform gas exchange, this suggests that the Through-flow Germination Boxes differ from the petri dishes in that each seed is continuously exposed to a fresh supply of uniform smoke concentration. In the petri dishes the smoke concentration immediately around the seed possibly decreases as the seed absorbs the active elements from the smoke solution. The seed will not germinate at too low concentrations of smoke solution (Jäger *et al.* 1996c). The germination

boxes were found to have more consistent accurate results (Appendix 4) and are recommended for this type of germination experiments.

2.3 Seed type used in the determination of the bioassay

2.3.1 *Phaenocoma prolifera* seed

Seeds from the everlasting, *Phaenocoma prolifera* are also known to have a positive response to smoke (up to 80% germination compared to 12% in the control (Dr. C. Boucher, Botany Dept., University of Stellenbosch, pers. com. 1998)). The latter species was found to be unsuitable for extensive bioassay purposes because of a poor seed set in five locations sampled during the 1998 season. Administrative difficulties were also experienced with Cape Nature Conservation who caused a delay in the granting of permission to collect the seed to the extent that it became a restriction to the research program.

2.3.2 *Anigozanthos manglesii* seed

Anigozanthos manglesii is used in bioassay tests in Australia by Kings Park and Botanic Garden to test for toxic or negative effects of smoke on germination (Dr. K. W. Dixon, Director – Plant Science, Kings Park and Botanical Garden, West Perth, Australia, pers. comm. 1998). Attempts to obtain these seeds for comparative smoke solution bioassay tests were unsuccessful (import permits were not granted) so these seeds could not be included in the tests.

2.3.3 *Syncarpha vestita* seed

Seed from the fynbos ephemeral, *Syncarpha vestita* (Brown 1993a) and the fire-climax grass, *Themeda triandra* (Baxter & Van Staden 1994) have been used in previous studies with smoke solutions due to their positive germination responses to smoke solutions. *S. vestita* is used by Brown (1993a) as a bioassay.

S. vestita seed obtained from Dr. N. A. C. Brown of the National Botanical Institute, Kirstenbosch which was collected in Febr. 1998 was tested here with the De Lange

Standard smoke solution to determine what dilution level of smoke solution should be used in fynbos restoration projects. The *S. vestita* seeds were handsorted and only dark grey and black seeds were used (Brown 1993a). The best germination percentages were obtained at a dilution of 1:250 of the Standard solution (Fig. 2.4). The germination percentages obtained, in contrast to those of Brown (1993a), were not found to be significantly different when compared to the distilled water control. These seeds could have been old and have lost their response to smoke or could have been from a batch of poor quality with low viability. From this activity it was found that *S. vestita* seed are difficult to sort, subject to seasonal supply and take a fairly long time (at least 20 days) to germinate and would therefore be unsuitable for extensive bioassay purposes.

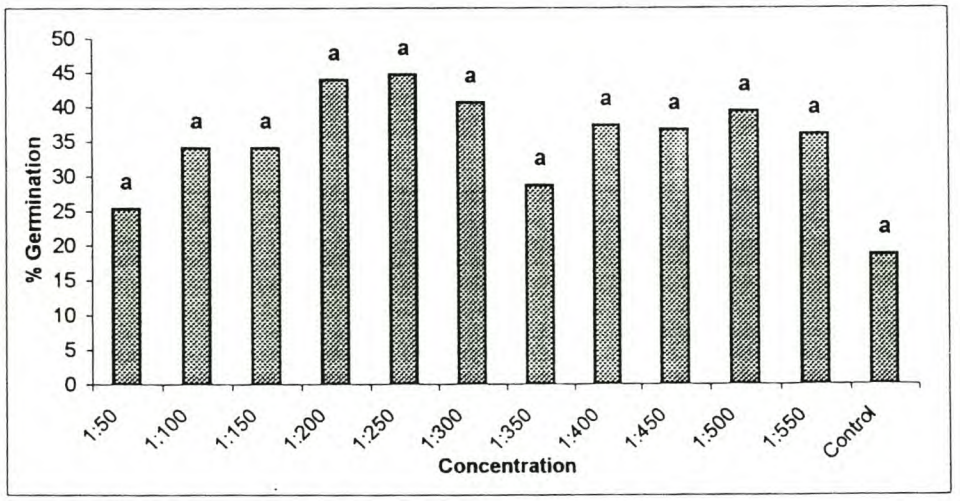

Figure 2.4 Bioassay for the standard smoke solution using *Syncarpha vestita* seeds (n=30). Bars with same letters indicate no significant difference.

2.3.4 *Lactuca sativa* L. (cv. Target) seed

Seed from the commercially available lettuce cultivar "Target" was tested for smoke solution bioassay purposes, because it is readily available. However no difference in germination was found between the control and the treated seed. The germination percentage was high (above 80%) in both cases. This may be due to pre-treatment of

seed with plant growth regulators or reflect genetic modifications to remove the inhibitory influence of darkness on germination (Korkmaz & Pill 1999).

2.3.5 *Lactuca sativa* L. (cv. Grand Rapids) seed

Grand Rapids lettuce seed has previously been employed as an aqueous smoke solution bioassay (Drewes *et al.* 1995) to determine the active germination stimulants present in smoke (Van Staden *et al.* 1995b). It was found that germination occurred with smoke water dilutions between 1:100 and 1:10 000 (v/v ratio i.e. 1 part smoke to 99 parts water = 1:100), with the best germination response detected at dilutions between 1:500 and 1:1000 (Van Staden *et al.* 1995b). The Grand Rapids cultivar will be used in this study to determine the optimum dilution level of smoke solutions.

Grand Rapids lettuce seed is readily available commercially, it germinates within 24 hours, enabling rapid repetition of experiments and it is responsive to different smoke solution dilutions (Drewes *et al.* 1995). These characteristics make it ideal to use as a bioassay in the determination of the optimum dilution level of a smoke solution. Seed was sourced from Hygrotech Seed Company (Stellenbosch, South Africa).

Seed batch 1

The first batch of Grand Rapids lettuce seed obtained in February 1998 was from an unknown source.

Seed batch 2

A second batch of Grand Rapids was obtained in January 1999, because insufficient quantities of the first batch were obtained by Hygrotech. This was marked as being Grand Rapids TBR which could indicate that the seeds were either "Tip Burn Resistant" or "Tip Burn Tolerant M1" (Drewes *et al.* 1995). According to Drewes *et al.* (1995) both cultivars are suitable for use in bioassay experiments.

Different germination percentages obtained with the two seed batches indicate that seed from the same cultivar, but of different origins, may react differently to smoke solutions. This supports Drewes *et al's* (1995) findings. Experiments showed,

however, that the reaction of the different batches to variations in smoke concentrations followed similar patterns. Both batches showed germination peaks at a 1:1 600 dilution level (Fig. 2.5), although the germination percentage of the second batch was considerably lower than that of the first.

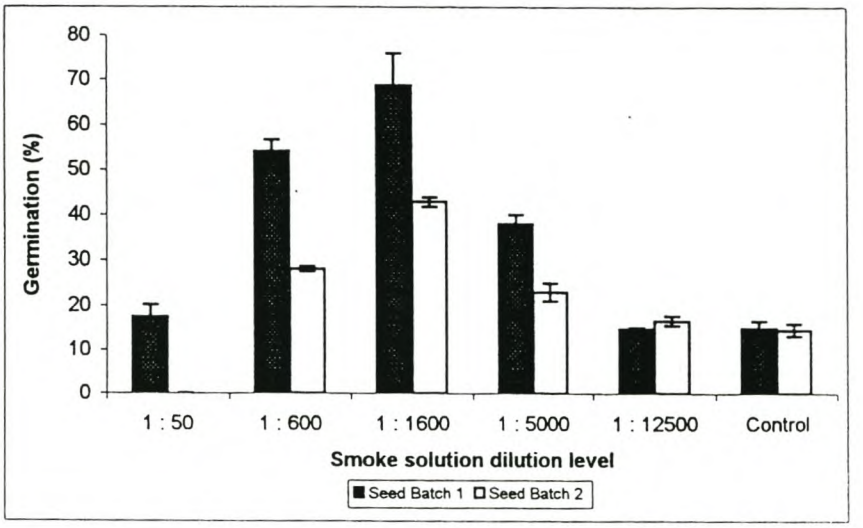

Figure 2.5 Preliminary testing for the suitability of Grand Rapids lettuce seed for bioassay purposes using different smoke solution dilutions. Maximum germination for both seed batches are at the same dilution level (n=50).

2.4 Variables to be addressed using grand rapids lettuce seed for bioassay purposes

2.4.1 Viability of seed

The seed batches were first tested by germinating them in light to determine what the natural viability of the seed was. Experimental variation can then not be attributed to seed quality.

The same methods were used as outlined in the general methods, except that distilled water was used and the seeds were monitored over a 48 hour period. There was no

need for black refuse bags or a darkened growth chamber. The rest of the methods are performed as described in the general methods for germination (2.2).

After 48 hours 149 of the 150 seeds tested in the three time replicates, had germinated. The Grand Rapids lettuce seed used in this study therefore has a viability of 99.3% (Appendix 5). The viability of this seed batch is acceptable for experimental purposes.

2.4.2 Light sensitivity

2.4.2.1 Introduction

Grand Rapids lettuce seed in general will not germinate or respond to light if it is dehydrated, however, once sufficient imbibition has occurred these seeds respond positively to light i.e. they exhibit a photomorphogenic response (Noggle & Fritz 1976). This response was tested in the seed batch used in this study.

2.4.2.2 Methods

The comparison of seed germination at different times (3 replicates were performed) under light versus dark conditions was undertaken over 24 hours. This tested the light sensitivity of Grand Rapids lettuce seed and for this experiment only distilled water was used and no smoke solution was added. The rest of the methods are performed as described in the general methods.

2.4.2.3 Results

There was a significant difference ($p = 0.001$) between Grand Rapids lettuce seed germination in the light (88.5%) and in the dark (18.5%) (Fig. 6.1) after 24 hours.

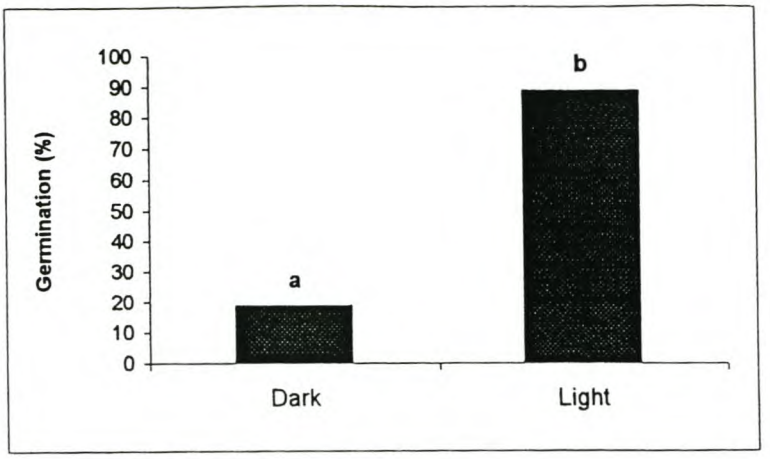

Figure 2.6 Effect of light versus dark treatments on Grand Rapids lettuce seed germination (n = 50) assessed after 24 hours of imbibition.

2.4.2.4 Discussion

Grand Rapids lettuce seed is capable of an average of 88.5% germination within 24 hours when exposed to light compared to 18.5% in the dark (Fig. 6.1). This supports Flint and McAlister findings (in Noggle & Fritz's 1976) that Grand Rapid lettuce seed are partially photomorphogenic. If the smoke solution acts as a partial substitute for light and stimulates the lettuce seed to germinate as reported by Brown & Van Staden (1997) then the germination response of these seeds should be acceptable as a smoke solution dilution bioassay as long as the bioassay determination is done in the dark.

2.4.3 Time

2.4.3.1 Introduction

For bioassay purposes the difference between a successful treatment and a control should preferably be as large as possible to provide robustness (Dr. C. Boucher, Botany Department, University of Stellenbosch, South Africa pers. com. 1998). The measurement of time must be logical, consistent and easy to perform. A time of 24 hours has previously been determined as optimal (Drewes *et al.* 1995). To determine whether 24 hours was the optimum time to use germination experiments were performed comparing the smoke solution to the control.

2.4.3.2 Methods

Different exposure times following treatment with smoke solution were assessed to determine the optimal time for a bioassay determination, namely 12, 18 – 30 (with one hour intervals) and 36 hours. The rest of the methods are performed as described in the general methods.

2.4.3.3 Results

No germination was observed after either 12 or 18 hours following experiment initiation for both the control or for the smoke treated seed. Germination started at 20 hours and a statistically significant difference between smoke solution and control could only be observed after 24 hours. At a 24 hours germination 12.12% of the control compared to the 27.33% of the smoke treated seed had germinated. The line graph (Fig. 6.2) indicates a steep rise in germination of seed in the smoke solution at 24 hours (standard error bars represent comparison of the germination of each smoke solution and its respective control). Fig. 6.3 illustrates the percentage difference between smoke solution and control for each time interval. Bars with the same letter do not differ significantly from each other.

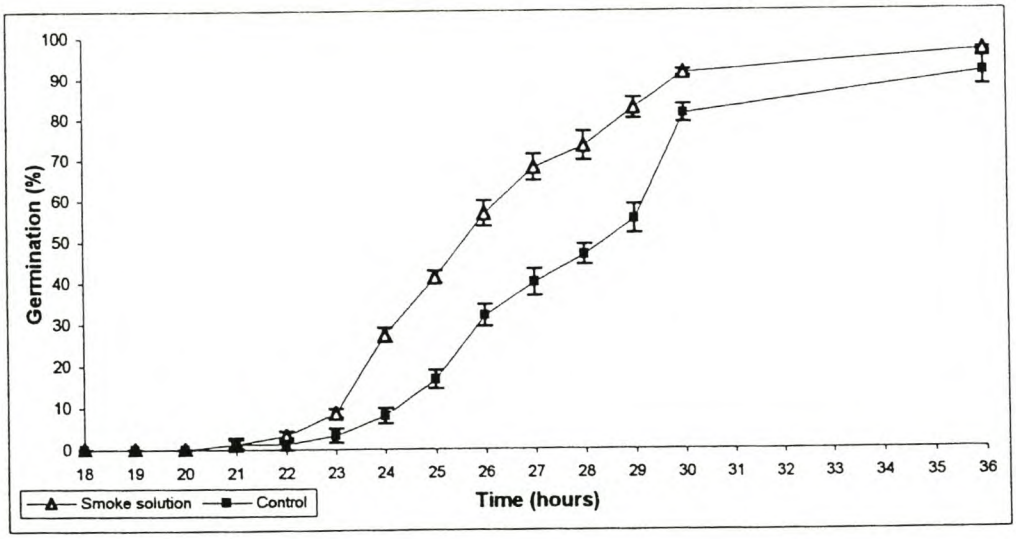

Figure 2.7 Line graph of germination differences from 12 hours to 36 hours between smoke solution and distilled water (control) (n = 50).

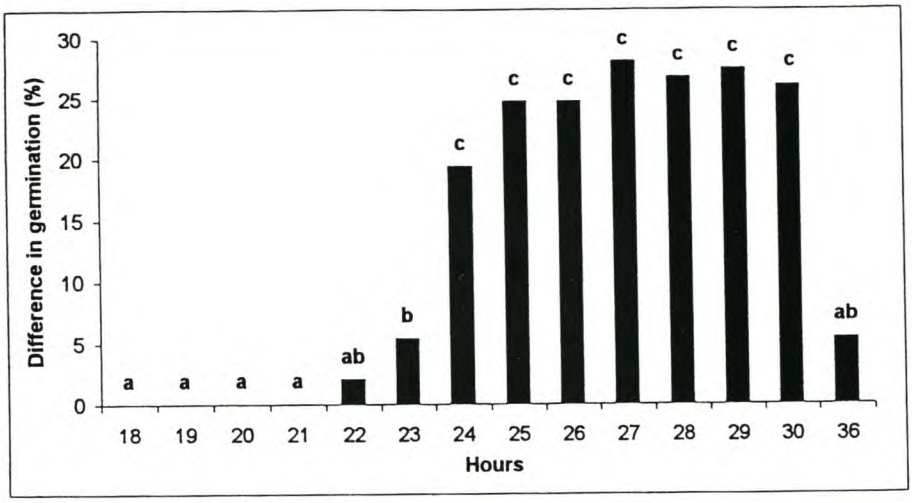

Figure 2.8 The difference in germination percentage (%) between treated seeds and the control over a 36 hour period (n = 50). Bars with the same letter(s) do not differ significantly from each other.

2.4.3.4 Discussion

Treating seed with smoke solution results in more rapid germination of Grand Rapids lettuce seed. The best time to use for bioassay determination, using Grand Rapids lettuce seed, is 24–25 hours. At this time the difference in germination percentage between the smoke solution and the control is the largest (Fig. 6.2) and the difference between the smoke solution germination and the respective control is significantly different from the shorter time intervals tested (18 – 23 hours) for the first time (Fig. 6.3).

Because there is no significant difference in germination differences (between smoke solution and control) between 24 – 29 hours, any of these times could be used. It is more convenient in practical terms and less liable to error to standardise on a 24 hour interval between experiment initiation and response assessment. This period has been used in previous experiments in the literature (Drewes *et al.* 1995). After 30 hours, the effect of the time the seed are exposed to moisture overcame the negative effect of darkness on germination and no significant differences were found between the control and seed exposed to the smoke solution. The seeds are partially photomorphogenetic (Gill & Ingwersen 1976).

Chapter 3

DETERMINATION OF THE OPTIMUM DILUTION LEVEL OF AN AQUEOUS SMOKE SOLUTION BY MEANS OF A BIOASSAY

3.1 Introduction

The effect of smoke produced from different plant materials has been shown to be either stimulatory or to inhibit germination dependent on the concentration applied (Jäger *et al.* 1996c) and the seeds being tested (Afolayan *et al.* 1997). Germination was inhibited in all cases where a smoke solution dilution of 1:1 and even (in some cases) of 1:10 was used. In contrast Jäger *et al.* (1996a) found that dilutions of 1:10 and lower stimulated germination. Smoke solution can result in a concentration dependent increase in germination with a complete inhibition at too high concentrations (Jäger *et al.* 1996c).

The variability in the published maximum germination percentages, with different smoke solutions is possibly due to the different smoke solutions having different concentrations. Germination peaks for Grand Rapids lettuce seed were found at smoke solution dilutions of 1:100, 1:500 and 1:1 000 (Drewes *et al.* 1995), at 1:10000 – 1:100 000 (Jäger *et al.* 1996b) and at 1:10 – 1:100 (Jäger *et al.* 1996a). These results illustrate the point that research performed with different smoke solutions is not comparable unless they are related back to a standard.

3.2 Methods

Fifty-one smoke solution dilutions were tested to determine the reactions of Grand Rapids lettuce seeds in the present bioassay assessment experiments. This was deemed necessary, as the dilution levels used by Drewes *et al.* (1995) did not give repeatable responses. Unfortunately the Statgraphics program did not allow the simultaneous comparison of the 52 different dilutions. The results from 11 evenly distributed dilution levels (starting at 1:50) were subjected to statistical testing. The 52 dilutions were simultaneously subjected to a standard error (SE) test. The smoke solution used

in this experiment is that made by De Lange & Boucher (1990) and the rest of the methods are as described in the general methods (Chapter 2). The smoke solution was stored below 0°C.

3.3 Results

The results of the experiments to determine the effect of smoke solution dilution on the average germination responses of Grand Rapids lettuce are illustrated in Fig. 3.1. The magnitude of the standard errors for each dilution level used is represented by the lengths of the I – bars.

The Grand Rapids lettuce seed has a significantly lower germination percentage than the control at a smoke solution dilution of 1:50 and 1:100 (Fig. 3.1). There was a significantly higher germination percentage with smoke solution dilutions between 1:400 and 1:12 000. A significant decline in the percentage of germination occurred between 1:10 000 and 1:16 000 dilution levels resulting in a final germination percentage lower than the control at a dilution level of 1:16 000. Maximum germination occurred at a smoke solution dilution of between 1:1 150 and 1:1 700.

These data show a type of plateau being reached between dilutions 1:1 000 and 1:10 000. The data are subjected to a regression trendline to be able to predict a normal curve (Fig. 3.2). Using the normal curve, the maximum germination is predicted to be at 1:2 000. Maximum germination is predicted to be 36% at a dilution of 1:2 000.

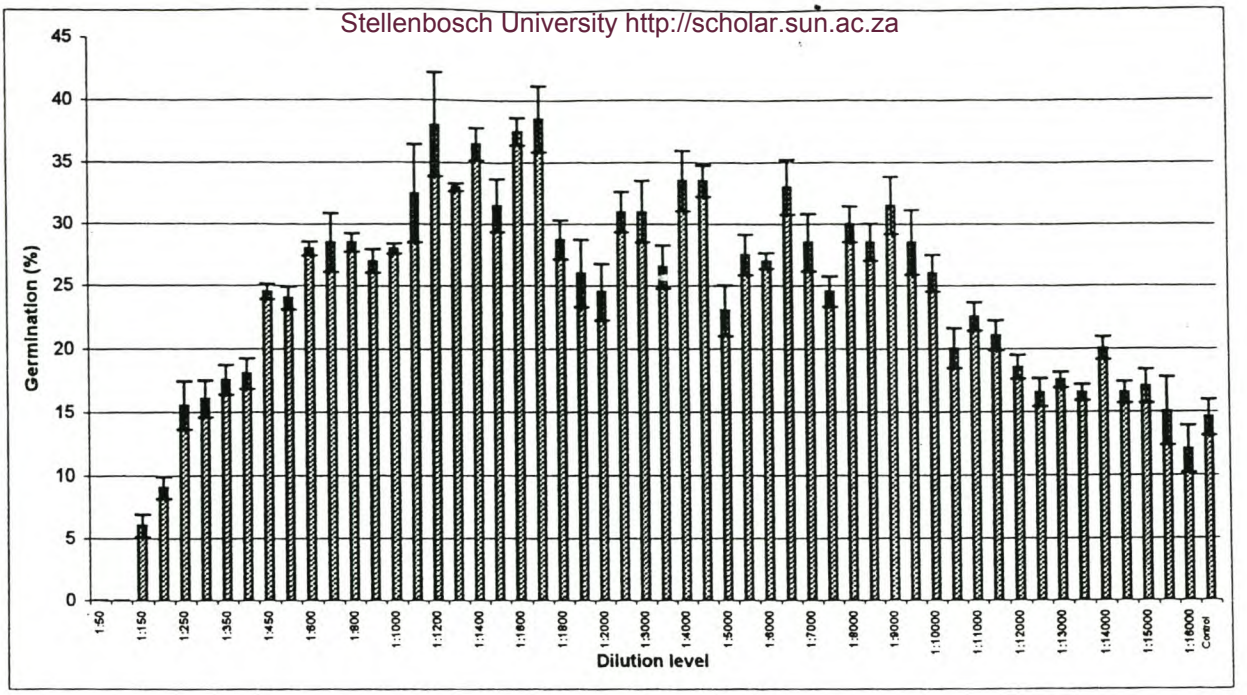

Figure 3.1 The effect of 24 hours exposure to smoke solution (standard smoke solution) at different concentrations on the germination percentage of Grand Rapids. I-bars represent the standard error (n = 50).

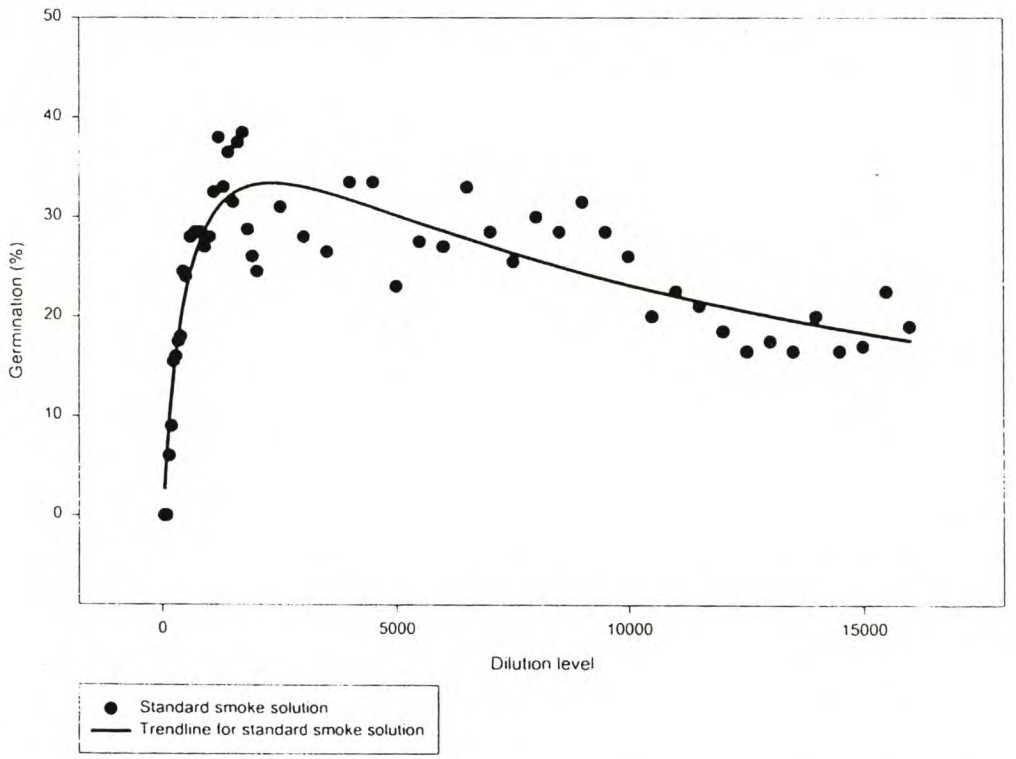

Figure 3.2 The polynomial regression line predicted from the smoke solution concentration bioassay of Grand Rapids lettuce seed. The maximum germination from the predicted normal curve is at 1:2000.

3.4 Discussion

Examination of the graph (Fig. 3.1) indicates that the germination pattern varies inconsistently at different dilution levels. The variation in germination responses may possibly be ascribed to the presence of different active compounds in the smoke. The highest germination peak however, is present at a smoke solution dilution of between 1:1 200 and 1:1 700 if many intervals are compared or at 1:1 600 if wider intervals are taken. This may indicate that either the principal compound or the joint effect of a number of compounds results in a general consistent peak at this interval. The subsidiary peaks could be attributed to the optimum dilution levels for certain individual active compounds.

Although different seeds from the same crop may have different germination responses (Milberg *et al.* 1996) this variation should not be present in this study, as the seeds would have been mixed in the reaping and packing process.

It is presumed that the germination percentages are low in the more concentrated dilutions, because the seeds have been damaged. The germination percentages are low in the more diluted solutions, because the active compounds are insufficient to have an effect on germination.

A concentrated smoke solution may inhibit germination (Fig. 3.1) because:

a) it is very acid (pH 3–4) and this may damage the seed physically;
b) it might impact on certain physiological processes; or
c) at higher concentrations a previously active or inactive compound or a complex of compounds may become inhibitory. At low concentrations the stimulus supplied by the active compound(s) has an insignificant effect on germination (Fig. 3.1). Use of the correct dilution is required as a too high concentration might inhibit seed from germinating and a too low concentration might not stimulate the seed to germinate.

The comparison of smoke solutions from different batches and different sources requires the standardised expression of smoke solution dilution. This may be

optimised by comparing all other aqueous smoke solution bioassays to that of a standard bioassay. A standard bioassay has been determined and is presented here. The smoke solution that was used for this bioassay is the first smoke solution that was made by De Lange & Boucher (1990). It is proposed that it be known as the De Lange Standard. Germination start being significantly stimulated at a dilution level of 1:1 100 and continues to a dilution level of 1:10 000. Optimal germination using this standard occurs at a dilution of between 1:1 200 and 1:1 700 using Grand Rapids lettuce seed. Using the predicted regression line the optimum germination occurs at a dilution level of 1:2 000.

At too high concentrations the acidity of the smoke solution may inhibit the germination (compared to the inhibiting effect of hypochlorite (Baldwin *et al.* 1994)) or the concentration of the active compounds may be too high and at a too low concentration it may be the amount of active compounds that is not enough to stimulate germination. The activity should thus be measured at the point where the active compounds optimally stimulate germination. A normal curve must be predicted, because the plateau between dilutions 1:1 000 and 1:10 000 makes it necessary. It is proposed that a concentration equivalent to this optimum predicted dilution level (1:2000) be termed 1 "delb" (derived from De Lange & Boucher). Hence, smoke solution from a different source, which gives maximum germination at 1:1 000 level would be of weaker concentration equivalent to 0.5 delbs whilst a germination peak at 1:8 000 level would indicate a stronger smoke solution equivalent to 4 delbs. Other smoke solutions could now be subjected to a bioassay and then compared to the normal curve predicted for the Standard. Bioassay results using the smoke of different origins are related back to the results using the Standard predicted dilution of 1:2 000. Each smoke solution will then be given a "delb" value.

Chapter 4

EVALUATION OF DIFFERENT AVAILABLE AQUEOUS SMOKE SOLUTIONS

4.1 Bubbled aqueous smoke solution

4.1.1 Introduction

As mentioned in Chapter 1, the first documented technique to obtain a smoke solution was a bubbled aqueous smoke solution (De Lange & Boucher 1990) made from a mix of fynbos plants. Bubbled aqueous smoke solution is commonly used in research, because:

1) it is storable and transportable in containers,
2) it is safe (versus lighting a fire in dangerous locations), and
3) it is controllable (a repeatable process).

There are a few factors that potentially may influence the concentration of the smoke solution.

- The time that the smoke is bubbled through the water should have an influence on the concentration. A longer bubbled time should give a more concentrated smoke solution.

- The fuel that is used also plays a very important role. Wet plant material will smoke more than dry plant material but not ignite as easily, therefore to keep plant material smouldering the ratio between wet plant material and dry plant material should be monitored and pre-selected. In research, only one plant species should be used, in South Africa either *Passerina vulgaris* (an ericoid shrub from the fynbos) or *Themeda triandra* (an ubiquitous African grass) have been used as standards. According to Van Staden *et al.* (1995b) an aqueous smoke solution made from *Themeda triandra* contains more biologically active material than that obtained from burning fynbos plants. Logically if a fuel consists of a mixture of different plants occurring in the same area (like different fynbos plants) it should

contain a wider variety of compounds and therefore represent a more natural smoke.

- The rate of combustion and the heat of the fire should also be taken into account. The faster the plant material burns, the less smoke will be available. Burning the plant material will give less smoke and use more fuel than smouldering it. If the rate of bubbling is too fast it will not be effective enough to dissolve the smoke in the water and some of the smoke may escape. The size of the bubble will probably also influence the effectiveness of the absorption process.

Smoke solutions prepared by different scientists at different times are thus probably totally incomparable without quality control.

The Standard used in this study (De Lange Standard) is a bubbled aqueous smoke solution. Another bubbled solution, received from the Kings Park and Botanical Garden in West Perth, Australia was tested. The Australians make their own aqueous smoke solution according to the same method of production that was used by De Lange & Boucher (1990) (Prof. K. Dixon , King's Park and Botanical Garden, Perth, Australia, pers. com. 1998) and it is being sold commercially.

4.1.2 Methods

The methods used in the smoke solution bioassay for the Australian smoke solution are as described in Chapter 2 except that the Standard smoke solution is replaced by the Australian smoke solution.

4.1.3 Results

The Australian smoke solution was tested starting with the same 11 dilutions that were used for the bioassay determination of the Standard (Chapter 3), although stronger dilutions had to be tested as well. Fig. 4.1 illustrates the bioassay determined for the Australian smoke solution from a dilution of 1:10 to a dilution of 1:15 000. Optimum germination is reached at 1:300 although dilutions 1:600 to 1:1 600 are not

significantly different from 1:300. However, the highest germination percentage is reached at 1:300 (30.67% compared to the 11.33% of the control).

In the graph (Fig. 4.1) a few minor peaks can be observed, at 1:1 600 and at 1:12 500. The minor peaks are evident in both the Australian smoke solution and in the Standard smoke solution. When subjecting the Australian smoke solution bioassay to a regression line and then comparing it to the Standard (Fig. 4.2), it is evident that the Australian smoke show a maximum germination level at a higher dilution level (1:550) than the Standard (1:2 000). The overall germination percentage (Fig. 4.2) is lower in the Australian smoke solution than in the Standard. The Australian smoke solution is weaker than the Standard.

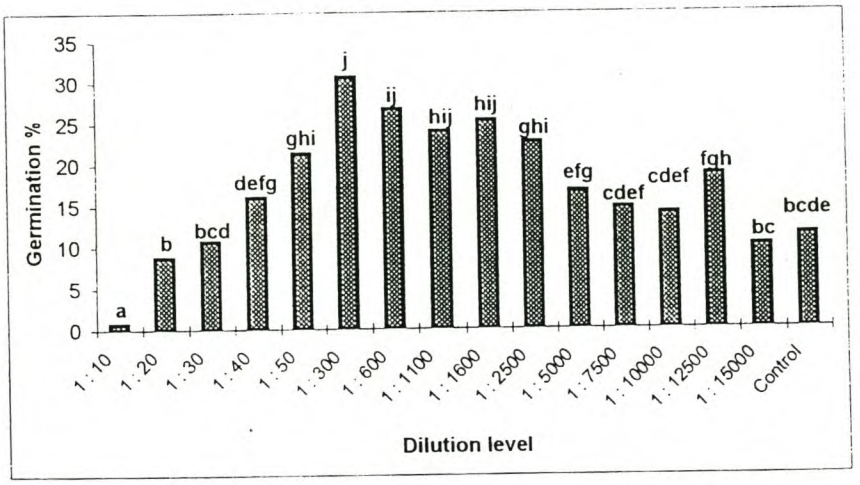

Figure 4.1 Bioassay of Grand Rapids lettuce seed, using the Australian smoke solution (n = 50). Bars with the same letter(s) do not significantly from each other.

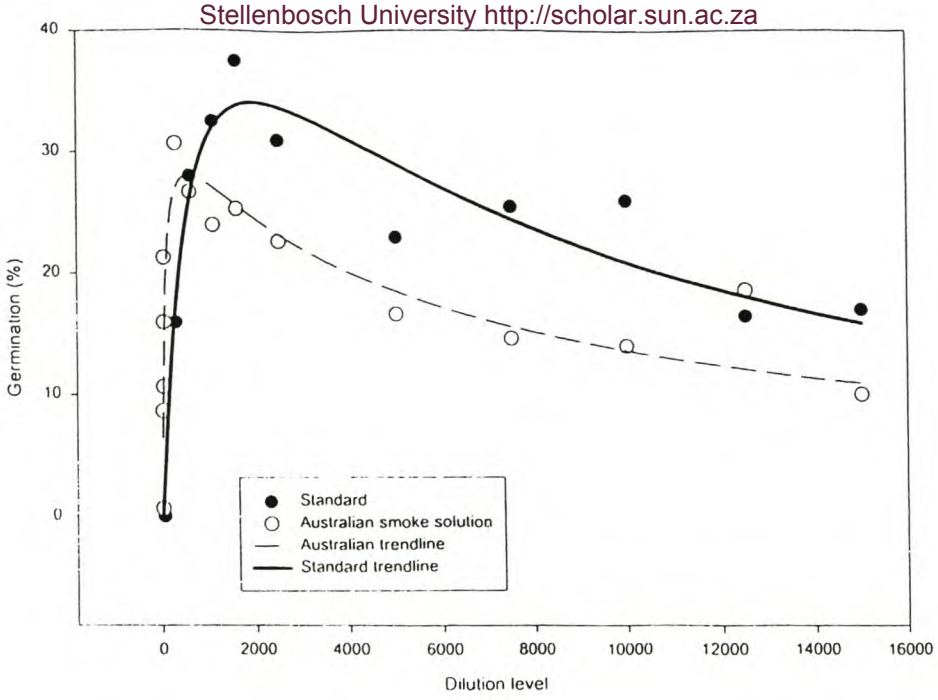

Figure 4.2 Best fit line graph comparing the Australian smoke solution to the Standard smoke solution (n = 50). The maximum germination for the Australian smoke solution from the predicted normal curve is at 1: 550.

4.1.4 Discussion

Smoke solution is most frequently made by bubbling smoke through water. From Fig. 4.2 it is evident that, although made using the same method, smoke solutions differ from each other in the amount (concentration) of active compounds present, which will have an effect on germination. The Standard solution has a higher overall germination percentage than the Australian smoke solution. Australian plants may have the active ingredients in smaller amounts than fynbos plants. It is tempting to postulate that fynbos may be adapted to shorter intervals of fire and may produce more of the active compound(s). The same reasoning may be used for the fact that smoke derived from the grass *Themeda triandra* has more biological active compounds than smoke derived from fynbos (Van Staden *et al.* 1995b), because grasslands in South Africa are subjected to shorter fire intervals than fynbos. There is, of course, a lot of other factors that may influence the concentration of a smoke solution.

The concentration of the active ingredients in a smoke solution will be influenced by the burning rate of the fuel, the rate of the bubbling of the smoke through the water and the amount of active ingredients present in the fuel. Both the Australian smoke

solution and the Standard have minor peaks present at different dilution levels (Fig. 3.1 & 4.1). This supports the theory that different plants contain the active compounds, differing only in amounts (Jäger *et al.* 1996a). The active compounds must be concentration dependent, because too high concentrations inhibit and too low concentrations have no effect on germination.

The germination peak of the Australian smoke solution is at 1: 550 (Fig. 4.2) and the germination peak of the Standard is at 1:2 000 (Fig. 4.2). The concentration of the Australian smoke solution tested is 0.28 delbs.

4.2 Distilled aqueous smoke solution

4.2.1 Introduction

Smoke solution prepared by distillation has not been recorded in the literature yet. The process is more time consuming than bubbling the smoke, but the product is proposed to be more suitable for industrial applications than the typical aqueous solution as outlined above (J. D. van Eeden Vula Environmental Services (tel. 082 564 5748), pers. com. 1998). A single species, *Willdenowia incurvata* (Thunb.) H. P. Linder was used to produce the distillate solutions. Two distillate smoke solutions prepared by Mr. van Eeden were evaluated. They will be referred to as Distillate 1 and 2.

4.2.1.1 Distillate solution 1

No filter was used in the preparation of Distillate 1 consequently it contains more impurities, than Distillate 2. Both distillates were distilled for the same time.

4.2.1.1.1 Methods

The general experimental methods applied here are described in Chapter 2 except that the Standard smoke solution is replaced by Distillate 1.

4.2.1.1.2 Results

The optimum germination percentage for Distillate 1 could not be determined using the same dilution range as that of the Standard. The germination curve had not reached a peak at 1:15 000 and the dilution range had to be extended to even weaker dilutions (up to 1:40 000). The maximum germination percentage of 54% was only reached at a dilution of 1:18 000 compared to the 10% of the control (Fig. 4.3). Minor germination peaks are observed at dilution levels of 1:12 500, 1: 16 000 & 1:25 000, but the germination percentage falls again towards the control at 1:40 000 (Fig. 4.3). When the predicted normal curve of this smoke solution is compared to that of the Standard (1:2 000) (Fig. 4.4) the maximum germination is reached at a more diluted level (1:13500). The germination percentage at 1:13 500 of the distilled smoke solution is higher than that of the Standard (1:2 000).

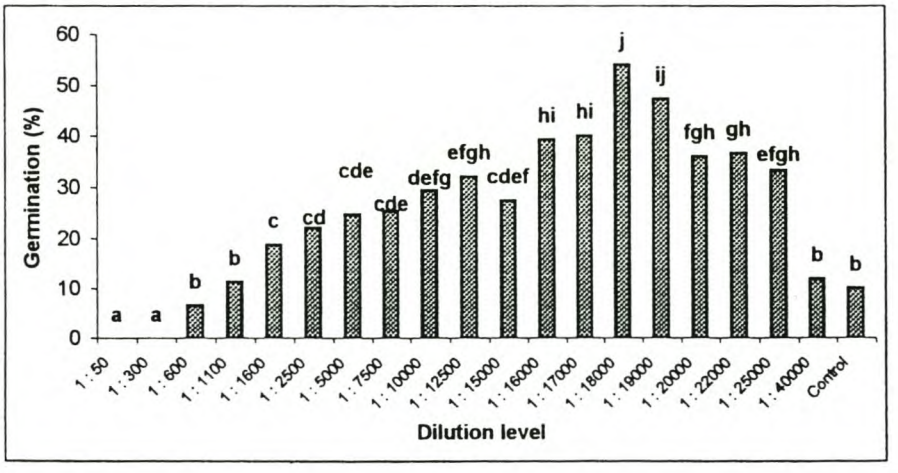

Figure 4.3 Bioassay of the Grand Rapids lettuce seed, using Distillate 1 (n = 50). Bars with the same letter(s) do not differ significantly from each other.

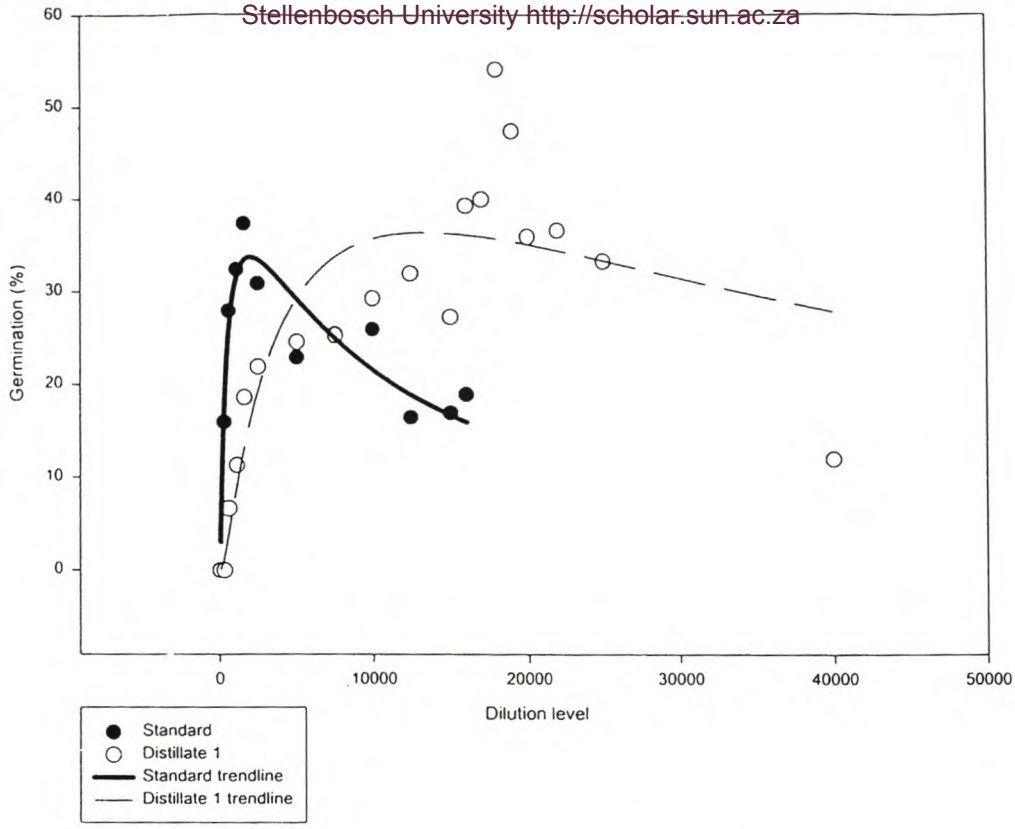

Figure 4.4 Best fit line graph comparing Distillate 1 to the Standard smoke solution (n = 50). The maximum germination from the predicted normal curve is at 1:13 500.

4.2.1.1.3 Discussion

Distillate 1 (Fig. 8.4) gives a significantly higher germination percentage than the control. The distilled smoke solution inhibits germination at very high concentrations and has no effect on germination at very weak concentrations. The active compounds must therefore be concentration dependent and may even damage the seed at too high concentrations (Chapter 5).

The predicted maximum germination of this smoke solution is at 1:13 500, while the predicted maximum germination of the Standard is at 1:2 000 (Fig. 4.4), showing that Distillate 1 contains the active compounds in much higher concentrations. Not all of the active compounds present in the smoke may dissolve in the water during the bubbling process and this will result in a lower concentration of active compounds being present in bubbled smoke solutions. By distilling smoke, more of an active compound is dissolved and results in a more concentrated smoke solution.

The minor peaks present show that the different active compounds that are present in the Standard are also present in Distillate 1 (Fig. 4.3). The germination percentage at 1:13 500 is higher than that of the Standard at 1:2 000. A single plant species was used to produce the Distillate 1 solution. In the Standard a whole mix of species were used. This may have an influence on the comparison of the two smoke solutions.

Distillate 1 has a concentration of 6.75 delbs.

4.2.1.2 Distillate solution 2

This distillate (Distillate 2) is the refined product that is due to be marketed in the near future. It is filtered, has a better appearance and most of the undissolved impurities have been removed.

4.2.1.2.1 Methods

The general experimental methods used here are described in Chapter 2 except that the Standard smoke solution is replaced with Distillate 2.

4.2.1.2.2 Results

This solution was used to test the method developed. Distillate 2 has a maximum germination percentage at 1:10 000 (53.33%) compared to the 22.67% of the control (which is higher in this case than in some of the other experiments, but still show statistically significant differences between smoke and control) (Fig. 4.5), with minor peaks at between 1:600 and 1:1 100, and at 1:5 000 (Fig. 4.5).

When the predicted normal curve of this smoke solution is compared to that of the Standard (1:2 000) (Fig. 4.6) the maximum germination is reached at a more diluted level of 1:6 000. The germination percentage at this dilution (1:6 000) of the distilled smoke solution is higher than that of the Standard (1:2 000).

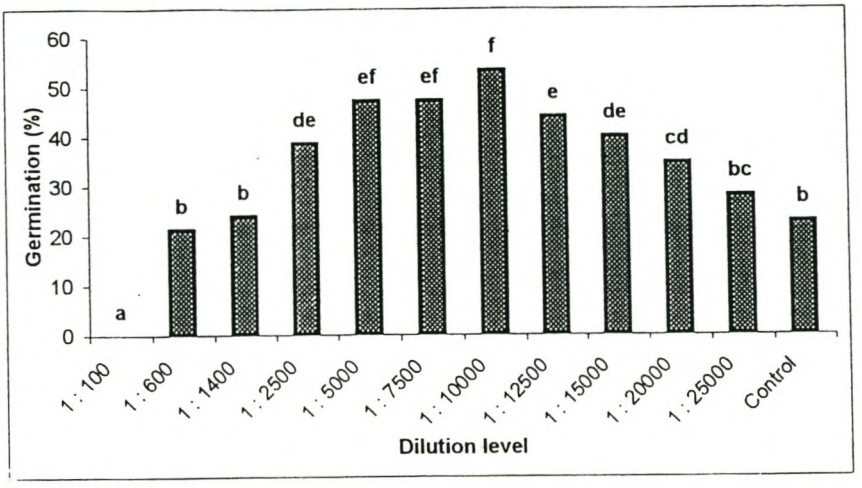

Figure 4.5 Bioassay of the Grand Rapids lettuce seed, using Distillate 2 (n = 50). Bars with the same letter(s) do not differ significantly from each other.

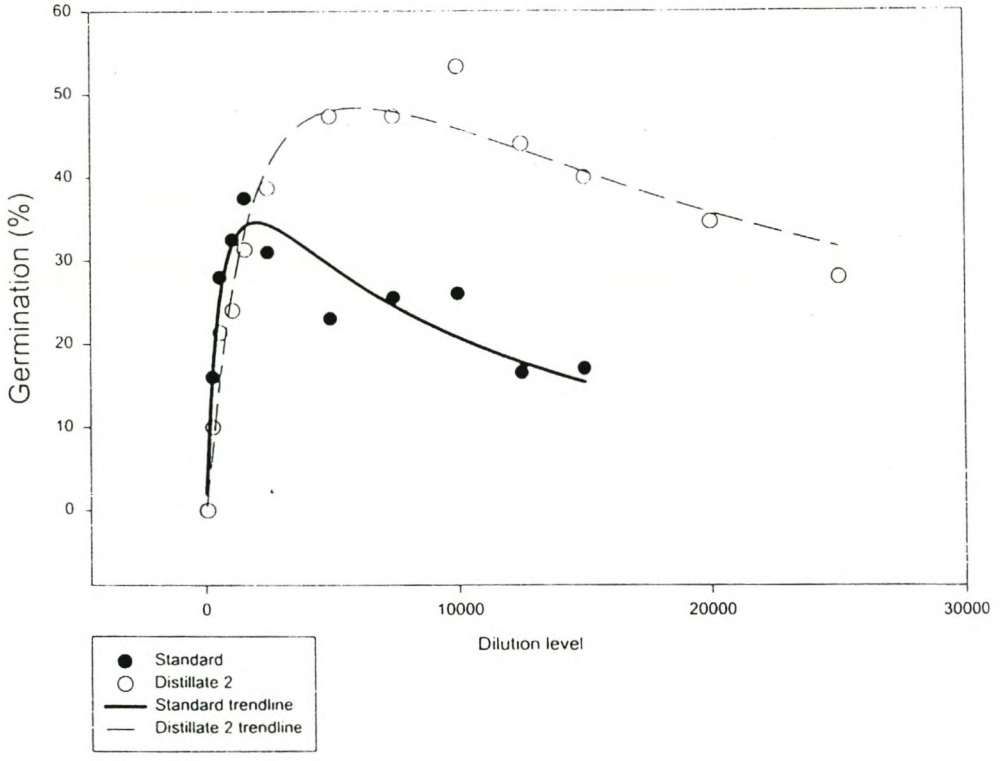

Figure 4.6 Best fit line graph comparing the distilled smoke solution (Distillate 2) to the Standard smoke solution (n = 50). The maximum germination from the predicted normal curve is at 1:6 000.

4.2.1.2.3 Discussion

Distillate 2 resulted in a significantly higher germination percentage than the control (Fig. 4.6). A very concentrated solution of Distillate 2 inhibits germination and a very weak concentration has no effect on the percentage of germination. A dilution level of 1:6 000 of Distillate 2 gave the predicted maximum germination, compared to a predicted dilution of 1:2 000 in the Standard (Fig. 4.6). The minor peaks on the graph (Fig. 4.5) with Distillate 2 show that a combination of different active compounds is also present in this smoke solution. The predicted total germination percentage is higher for Distillate 2 than for the Standard, so there should be a higher concentration of the active compounds present in Distillate 2. The concentration of Distillate 2 is 3 delbs.

Distillate 1 (6.75 delbs) is more concentrated than Distillate 2 (3 delbs) and both are significantly more concentrated than the Standard solution. This shows that even distilled solutions will differ from each other.

4.3 Smoke food flavourant

4.3.1 Introduction

The industrial use of smoke food flavourants is to give a smoky flavour to food such as meat, fish or vegetables (e.g. potato chips). The smoke food flavourant used in this study is known as Pyroligneous acid 621053 and was obtained from Haarmann & Reimer (SA) (Pty) Ltd. [tel. (011) 921 – 5478] following the findings of Jäger *et al.* (1996) who claim this type of smoke to be highly concentrated. The smoke flavouring tested was imported from Germany. The exact method of production is a trade secret, but distilling plays an important part in the manufacturing process. After importing the smoke solution from Germany different substances, like alcohols etc., are added to the solution to make the solution; either more or less viscid, depending on the proposed application (Mr. J. Esterhuizen, Haarmann & Reimer, Johannesburg, Technical Manager – flavours, pers. com. 1999). This commercially available smoke food flavourant is therefore a smoke solution with additives.

4.3.2 Methods

The general methods used here are described in Chapter 2 except that the Standard smoke solution is replaced with the smoke food flavourant.

4.3.3 Results

Maximum germination percentage is reached at 1:80 000 (Fig. 4.7). At a dilution of 1:1 500 000 the germination percentage is the same as that of the control. Two minor peaks can be observed, one at 1:200 000 and the other at 1:1 000 000. The germination percentage at 1:80 000 (45.33% compared to 18.67% of the control) is similar to that of the Standard (38%) (Fig. 4.7). The additives may play a role in the germination stimulation activity of this solution and should be excluded experimentally during further research with this material.

When the predicted normal curve of this smoke solution is compared to that of the Standard (1:2 000) (Fig. 4.8) the maximum germination is reached at a much more diluted level of 1:170 000.

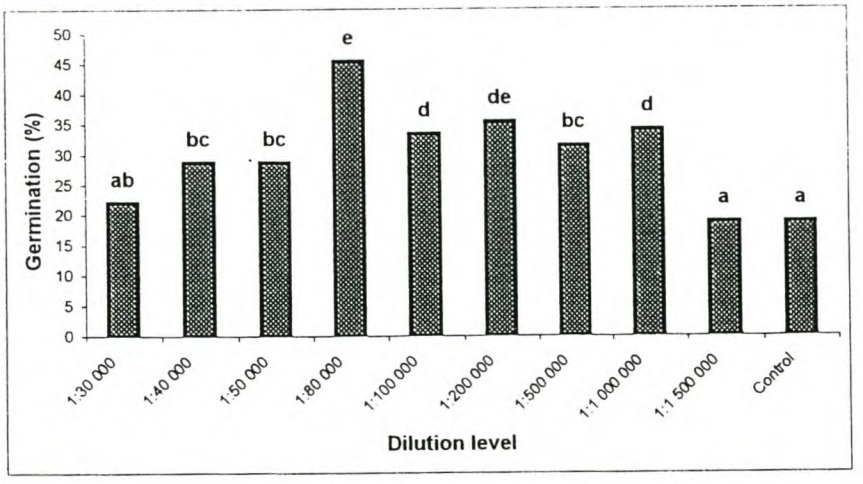

Figure 4.7 Bioassay of the Grand Rapids lettuce seed, using the food flavourant (n = 50). Bars with the same letter(s) do not differ significantly from each other.

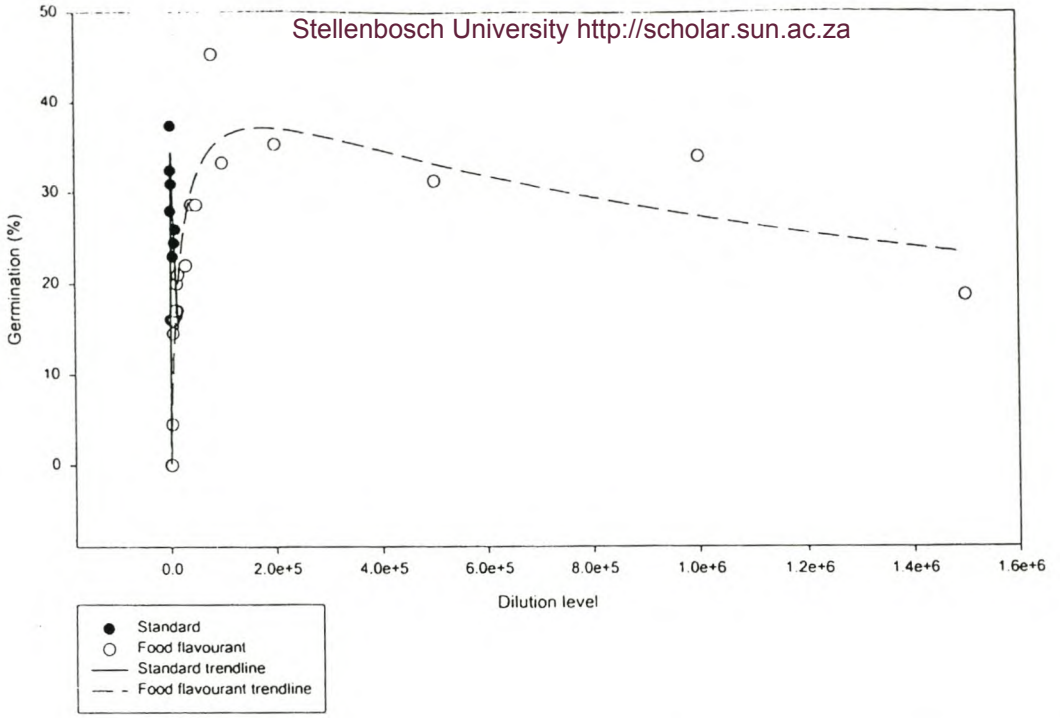

Figure 4.8 Best fit line graph comparing the food flavourant smoke solution to the Standard smoke solution (n = 50). The maximum germination from the predicted normal curve is at 1:170 000.

4.3.4 Discussion

The food flavourant gave a significantly higher germination percentage than the control (Fig. 4.7). Jäger *et al.* (1996b) found the maximum germination percentage for a food flavourant they tested to be between 1:10 000 and 1:100 000. The use of the Through-flow Germination Boxes has made it possible to narrow the maximum germination percentage down to around 1:80 000 (Fig. 4.7). The minor peaks that are present support the concept that there are different active compounds present in the smoke and the maximum germination percentage (at 1:80 000) could then be the compounded effect of all the active compounds or of the principle compound involved alone.

Using the predicted regression line, the food flavourant smoke solution (with a predicted peak at 1:170 000) has a concentration of 85 delbs.

4.4 Comparison of smoke solutions

4.4.1 Results

Based on the bioassay results, the different aqueous smoke solutions differ remarkably from each other representing a range of concentrations varying from 0.28 to 85 delbs. Compared to the control (Fig. 4.9) all of the smoke solutions show a significantly higher total germination percentage. The total germination percentage of the two distilled solutions do not differ significantly from each other or from that of the food flavourant smoke solution, but differ from the Standard and the Australian smoke solutions. The total germination percentage of the food flavourant differs significantly from the Standard and the Australian smoke solutions. The total germination percentage of the Australian smoke solution does not differ significantly from the Standard although the concentration differs (Fig. 4.9).

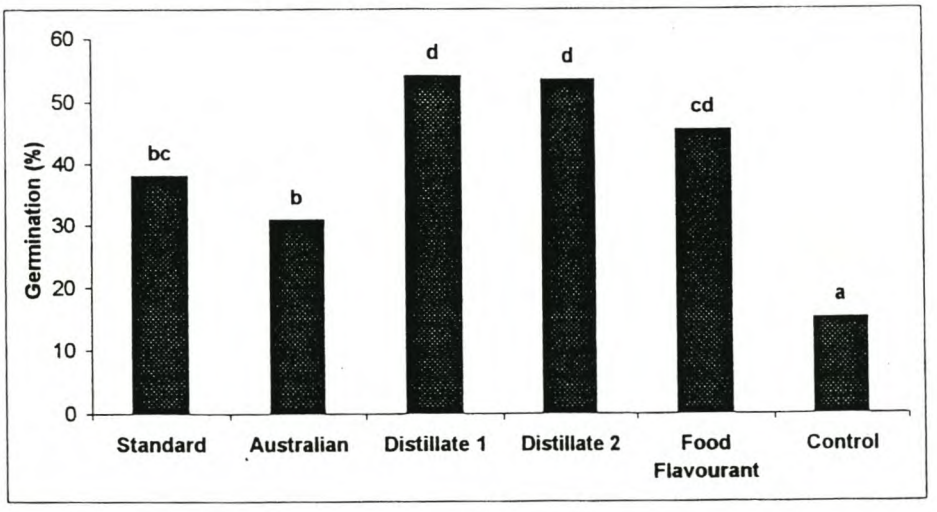

Figure 4.9 Comparison between total germination percentages of the different smoke solutions (n = 50). Bars with the same letter do not differ significantly from each other.

4.4.2 Discussion

The bioassay results suggest that the different smoke solutions differ from each other in the concentration of the active compounds (Fig. 4.1 to Fig. 4.8). All of the smoke solutions stimulate germination to a greater degree than the control (Fig. 4.9). This confirms Drewes *et al.*'s (1995) findings that smoke stimulates Grand Rapids lettuce seed to germinate by lifting the dark effect and that different smoke solutions all contain some or all of the active compounds (Jäger *et al.* 1996a).

The smoke food flavourant is most concentrated, although its total germination percentage is lower than that of the two distilled smoke solutions. The weakest smoke solution is the Australian smoke solution. It also gives the lowest germination percentage (Brown & Van Staden 1997). The amount of active compounds present in Australian plants may be less than in fynbos plants, because the intervals between fires in fynbos are shorter. More likely it may reflect the way it was made or the degree it was diluted with water.

When comparing the Standard bioassay graph to the other different smoke solution line graphs (Fig. 3.1, 4.1, 4.3, 4.5 & 4.7), it is apparent that there are minor peaks present in each one of the graphs. This consistency cancels out the possibility of contamination or incorrect dilution of the different smoke solutions. It is proposed that different active compounds present in the smoke solution cause the different peaks, acting on their own and the highest peak is caused by the most active compound(s) or that the peak is the compounded result from all the active compounds acting in unison to stimulate germination.

Chapter 5

ASSESSING THE EFFECT OF CONCENTRATED SMOKE SOLUTION ON SEED GERMINATION

5.1 Introduction

5.1.1 Pre-treatment of seed with smoke

The pre-treatment of seeds with smoke can have an enhancing effect on the germination of *Themeda triandra* seed. This effect was obtained with pre-treated seed for up to 21 days after pre-treatment (Baxter & Van Staden 1994). Pre-treatment had no effect on seedling growth. Eleven out of 18 Australian species that were subjected to smoking before sowing showed enhanced germination to an equal or greater extent than applying smoke (not smoke solution) to soil containing the same seed. Some however had a negative response to the pre-treatment, but a positive response to exposing soil to smoke (Roche *et al.* 1997).

The advisability of using smoke pre-treated seeds in field restoration projects is questionable as:

i. species specific dilution level requirements must be known.

ii. It is very time consuming and expensive to expose seeds to specific selected dilution levels according to species.

iii. If seed is exposed to smoke solutions then the exposure period to the liquid becomes critical as germination could be initiated before sowing or even in storage.

iv. Aqueous smoke solution is known to be very acidic e.g. pH between 3 and 4 (Prof. K. Dixon, King's Park and Botanical Garden, Perth, Australia, pers. com. 1999, Appendix 1). Theoretically a concentrated solution could be damaging to stored seed of some species.

v. In storage the seed would have to be stored under extremely dry conditions to prevent damage caused by the acid smoke. These dry conditions will probably have a negative influence on seed longevity in storage.

vi. In the case of restoration of fynbos and many other systems where topsoil is returned to a site, there is a lot of dormant seed present in the topsoil that would also need some kind of stimulus. The use of pre-treated seed eliminates the stimulation of the rest of the seed present in the seed bank and will only selectively benefit the seed that was pre-treated. Under normal field restoration procedures a smoke solution is required to stimulate as much seed as possible in the soil to enhance the species diversity of the vegetation cover and serve as anti-erosion measure.

It is clear that a lot of seed specific research still needs to be done to be able to pre-treat seed with smoke for predictable results.

5.1.2 Review on the effect of concentrated smoke solution on seed

When a smoke solution's concentration is unknown and the smoke solution applied to the soil is too concentrated it may happen, in restoration projects, that the acidity of the smoke solution damages the seeds. Some fynbos seeds are also inhibited from germinating in smoke solutions (Brown 1993a), because their hard outer seed coats may be impermeable to the solution (Brown & Van Staden 1997). Acidic smoke solutions may be beneficial in these instances because of the scarifying effect of acids.

It is clear that germination inhibition may be due to a number of factors. In the above mentioned studies selected seeds were tested and different seeds, not tested yet, might be damaged or others might benefit from a concentrated smoke solution.

Physical damage to Grand Rapids lettuce seed through acidity of concentrated smoke solutions (pH < 4) can be tested by exposing seed to a diluted smoke solution after preliminary exposure to the concentrated smoke solution, or by allowing germination to continue for more than 24 hours following exposure to the concentrated smoke solution. All seeds should germinate by 36 hours unless they are inhibited.

5.2 Methods

Grand Rapids lettuce seed were used here as a test to assess the effects of a concentrated aqueous smoke solution on seed. Seeds were exposed to a fairly concentrated solution of 30 delbs of Distillate 2 (Chapter 4) for 18 hours, 24 hours and 30 hours respectively. After exposure to the smoke solution the seeds were exposed to distilled water. This reduced the concentration of the smoke solution to nil and leached it out of the filter paper. The seeds were then exposed to 24 hours of light as a normal viability test. The control is Grand Rapids lettuce seed exposed to a dilution of 1:6 000 of Distillate 2 over 24 hours. The rest of the general methods described in Chapter 2 were applied during this experiment.

5.3 Results

None of the lettuce seeds that had been exposed to the concentrated smoke solution for 18 to 36 hours in the dark had germinated. Twenty four hours after replacement of the smoke solution with distilled water and exposure of the seeds to light, 46% of the seeds exposed to the concentrated smoke solution for 18 hours, 6% of those exposed to the concentrated smoke for 24 hours and 1.3% of those exposed to the smoke for 30 hours, germinated (Fig. 5.1). These seeds were left in the growth chamber for a further 72 hours to make sure that the germination was complete.

5.4 Discussion

Exposing Grand Rapid lettuce seed to a concentrated smoke solution for more than 18 hours, not only inhibits germination, but also damages the seed to such an extent that virtually all the seeds are killed within 30 hours. Only 1.3% of these seeds germinated after the replacement of the concentrated solution with distilled water and leaving the seeds in the growth chamber for further 72 hours. It is therefore assumed that most of these seeds were killed.

The low pH (of the smoke solution) may damage seeds that haven't got a hard seed coat. The high acidity (pH <4) is possibly responsible for the damage to the soft coated lettuce seeds. We can conclude from this experiment that the pre-treatment of

seed with smoke solution can be beneficial to scarify seeds with hard seed coats if the period of exposure is of a suitable duration and if the seed is not stored for any lengthy period after exposure. (It is presumed that the hard seed coat is an attribute that extends the longevity of the seeds). On the other hand the pre-treatment of seeds with soft seed coats can be disastrous because potential damage can occur, particularly during the drying process after pre-treatment, when the smoke solution becomes more concentrated and therefore potentially more acid. The length of exposure to acidic smoke solutions is not easily controlled during drying, because of the absorption of some appendages on seeds.

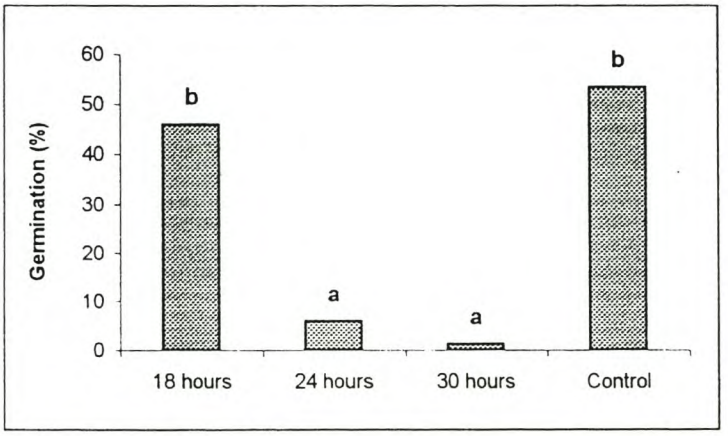

Figure 5.1 The effect of exposure to concentrated smoke solution (Distillate 2) on germination. The control is Distillate 2 (3 delbs) diluted to 1:6 000 after 24 hours. Bars with the same letter are not statistically different from each other at the 95% confidence level.

Chapter 6

GENERAL CONCLUSIONS AND FURTHER RESEARCH POSSIBILITIES

6.1 In restoration

It is reasonable to presume that general principals and techniques for ecosystem reconstruction can be designed and during the process ecological theory can be tested (Bradshaw 1982). Techniques to restore an area with the appropriate species are as yet, not adequate because of an inadequate knowledge base (Bradshaw 1983; Cairns 1988). For example, our knowledge base about seed requiring smoke to stimulate germination is grossly inadequate. Smoke plays an important role in improving the germination response of a number of taxa (Roche *et al.* 1997), from both fire prone ecosystems and those without fires (Brown & Van Staden 1997) and is therefore an important tool in the reconstruction of ecosystems.

Our knowledge base about the longevity of seed is inadequate and research is needed to be able to discover which species can be added to the topsoil to encourage the reconstruction process, because it is important to get a good plant cover as quickly as possible as a anti-erosion measure. Bakker *et al.* (1996) suggest a number of topics relating to seeds that need attention in restoration projects, which range from seed dispersal in rare and endangered plant communities, seed rain, longevity of seed, seed viability in different soils, to the improvement of techniques for catching seed rain in the field.

Industrial support for restoration research projects is essential, because the traditional resources have less money than they once had. Most of the existing funds in many of the major foundations supporting biological research goes to purely theoretical, as opposed to applied, problems (Cairns 1988). This is an opportunity for collaboration between the academic community, the environmental groups, regulatory agencies and industry to accomplish the task of restoration (Cairns 1988).

A great need has developed for set standards in restoration ecology as a means of quality control. One such a standard will be the type and dilution level of smoke solution to be used in the reconstruction of fire prone environments, as was addressed in this study.

6.2 Application of smoke solutions

The germination of Grand Rapids lettuce seed over 24 hours in the dark was stimulated through the application of aqueous smoke solution, to reach a maximum average of 38 % in contrast to 88.5% in the light. This suggests that the stimulation of this seed by smoke only partially alleviates the light requirements of this photomorphogenetic seed.

6.2.1 Smoke versus smoke solution

An aspect not addressed here, but requiring attention in future studies is to determine the differences in reactions between smoke and smoke solution for different kinds of seed. Physically smoke can only be applied to certain (relative small) areas (unless the vegetation is burnt) and the concentration of the applied smoke is very difficult to determine. Smoke solution is more practical than smoke in restoration projects because it can be manufactured in advance, the concentration can be determined, it can be transported and stored and applications can be specified precisely. The germination stimulation effect that the smoke solution will have can therefore be predicted more accurately. It is a more acceptable commercial product particularly in restoring areas without vegetation. The enhanced level of germination can be maintained by pre-treating seeds with smoke for up to 365 days while smoke-water primed seeds germinate better than smoked seeds (Brown & Van Staden 1997).

6.2.2 Measurement of smoke solutions

In the measurement of the concentration (dilution level) of a smoke solution, either the active compounds in the smoke solution can be determined (if they are known) or all the smoke solutions can be compared to a standard smoke solution. The active compounds in aqueous smoke solution are as yet unknown, and in the meanwhile the

latter technique is the only acceptable alternative. Grand Rapids lettuce seed was used in the present bioassay research, because it's reaction to smoke is known and it germinates rapidly (within 24 hours). This study has set a standard (the De Lange Standard) against which all smoke solutions can be rated. They can then be given a **delb** value for comparative purposes.

Smoke solutions can be manufactured in different ways. The most commonly used method is by bubbling smoke through water. Another approach to making smoke solution is through distillation. Smoke derived from various plant species, agar, tissue paper and even smoke food flavourant has been found to have an enhancing effect on the germination of Grand Rapids lettuce seed.

Different smoke solutions were subjected to bioassay optimum dilution level determination and related back to a Standard by means of a regression line. These were: a bubbled smoke solution received from Australia; two distilled smoke solutions from South Africa and a smoke food flavourant solution. The food flavourant (85 delbs) was the strongest in concentration, the distilled solutions were second and third (6.75 & 3 delbs) and the smoke solution from Australia had the weakest concentration (0.28 delbs). The distilled solutions gave the highest germination percentages of all the aqueous smoke treatments.

The dilution of the Standard needed for the fynbos is known (1 part smoke to 250 parts water) and after comparing the tested smoke solution, a recommendation for dilution can be made. These procedures have been used in practice to certify the quality of smoke solutions used in restoration projects (see certificate in Appendix 6).

Care must be taken not to use a too strong concentration, because a too strong concentration has a low pH value and can damage the seed. This has been proven for Grand Rapids lettuce seed in this study as very little seed germinated (1.3%) after 30 hours exposure to a concentrated smoke solution even after subsequent leaching in distilled water. Therefore the seeds were considered to be dead.

6.2.3 Smoke solutions and their applications

Catchments that have been invaded by alien plant species can yield up to 50% less water than natural fynbos vegetation (Van Wilgen *et al.* 1992; Davis *et al.* 1994; Cairns & Heckman 1996). It is very important to revegetate these sites with indigenous vegetation after they have been cleared of alien plants.

The post clearing stimulation of germination of fynbos seed *in situ* (there will be seed present in the soil seed bank (Holmes 1996)) using smoke solution will enhance the development of cover and retard exotic *Acacia* regrowth, because fire, which stimulates its seed to germinate (Pieterse 1996), need not be used. (The general practice at present is to burn debris after felling the exotic plants. In this way erosion is reduced because the soil is covered and the volume of follow-up at any treated site is reduced so that new areas can be cleared.

6.2.4 Recommended aqueous smoke solution for restoration projects

There is a marked difference in the effectiveness of smoke produced by different plant sources. Smoke solutions differ in concentration of the germination stimulating compound(s) (Baxter *et al.* 1994) and therefore require different degrees of dilution. In tests done by Brown & Van Staden (1997) different aqueous smoke solutions were compared to each other. These were Australian Smoke Water, Kirstenbosch Instant Smoke Plus Seed Primer (Brown 1994) and an extract of fynbos smoke. Their results confirm the reasoning in this study that aqueous smoke solutions will differ from each other in their concentration of active compounds. The determination of the optimum dilution level of aqueous smoke solution to be applied in fynbos restoration and other research projects was required, to provide consistent results and for quality control purposes. In this study a method to determine the optimum dilution level of smoke solutions has been developed and all smoke solutions can now be equated to each other.

The results of this study suggest that the best smoke solution to use to get a maximum total germination percentage for restoration purposes is one of the distillates. Distillate 1 is stronger than Distillate 2 but gives a similar total germination percentage and

consequently one of the distillate solutions is recommended for use in the industry where the final germination percentage and the volumes of smoke solution involved are critical. The Australian smoke solution is not as suitable for restoration in South Africa as the local product, because it is weaker in concentration.

It is much more difficult to get the dilution factor determined accurately with the concentrated aqueous food flavourant solution, because of its high concentration and the smoke food flavourant has a lower total germination percentage. This lower total germination percentage may be due to the additives mixed with the food flavourant in the manufacturing process. Jäger *et al.* (1996b) found the food flavourant smoke solution to be a good and easily obtainable commercial source of smoke solution for use in horticulture or conservation. However, the germination percentage of the food flavourant is lower than that of the distillates tested here and it is therefore not recommended for use in restoration projects. It is also not as readily available as is suggested by Jäger *et al.* (1996b) (Mr. J. Esterhuizen, Haarmann & Reimer, Johannesburg, Technical Manager – flavours, pers. com. 1999).

A more concentrated smoke solution is an advantage in restoration where large volumes of solution are used. Storage is easier in concentrated form (in respect of space). Also the handling of a small container is far easier than large containers.

Smoke solution has been used in the industry to rehabilitate disturbed areas in fynbos by stimulating germination of seed sown and seed in the soil seed bank e. g. in Du Toits Kloof (Boucher *et al.* 1996). Seeds will differ in the amount of smoke solution (and concentration) they need (this is evident comparing lettuce seed (*Lactuca sativa*) with *Syncarpha vestita*). Care must be taken to apply the right dilution of smoke solution to the soil to get the desired response. In theory a strong concentration is more practical than a weak concentration, because the germination-enhancing substances in the smoke solution will dilute through natural processes over the course of time (Ward *et al.* 1997). Care must be taken in this regard as a high concentration may damage the seed.

Damage of seeds can cause serious problems in restoration projects. Soft coated seeds may be damaged if the solution is not sufficiently diluted in time, either by applying water or by rain. A concentrated smoke solution can inhibit seeds to germinate, which can be beneficial if the smoke solution is applied just before the rain season (this was shown for *Syncarpha vestita* by Brown (pers. com. 1998, see p. 26)). There is however still much research needed to determine what smoke solution dilution is needed for specific seeds.

Application of the correct dilution level has important ramifications both aesthetically and economically. Standardisation of the expression of smoke solution dilutions is seen to be of considerable importance in seed germination experiments and in practical applications such as in restoration projects and the horticultural industry.

In restoration it is very important to apply the smoke (or smoke solution) at the right time of year. In the dry season the seeds can be stimulated to germinate, but because of the lack of water the seedlings cannot grow and will die off. If the active compound is volatile it may be lost before the seed is sufficiently moist. In the wet season the smoke solution can become diluted too quickly through the rain before the smoke can stimulate the seed to germinate (De Lange & Boucher 1993). The best time to apply smoke appears to be in the late summer (late dry season) or in the autumn (early wet season) in the Fynbos Biome (De Lange & Boucher 1993; Brown & Van Staden 1997).

Studying the response of germination to fire, the use of smoke can overcome the practical problems of burning small experimental patches that are usually difficult to control in burn seasons (De Lange & Boucher 1993). The use of smoke to promote seed germination may find a wide application in the conservation of endangered difficult-to-germinate species and for commercial use (De Lange & Boucher 1990; Van Staden *et al.* 1995b), in the revegetation of disturbed sites in fire prone floral communities (Baxter & Van Staden 1994), in nurseries, the wild flower industry and different restoration projects.

6.2.5 Application in the industry

The above results can have great implications in further research:

6.2.5.1 Categorising of seed

The characteristics of seed stimulated by smoke may differ from seed stimulated by heat shock. Smoke stimulated seed are distinctly different from those that are not stimulated by smoke in the Californian chaparral (Keeley & Fotheringham 1998) thus all the seeds tested that gave a germination response to smoke are distinctive in that:

- Their outer seed coats are highly textured;
- they have a poorly developed outer cuticle;
- dense tissue in the coat of the seed is missing; and
- they have a membrane, which allows water but not larger particles to pass through (Keeley & Fotheringham 1998).

The variations in response of different species to smoke may be related to the differential sensitivity to the active compound(s). Fynbos species tested, indicate that the species responding to smoke are mainly serotinous. Myrmecochorus species in the Proteaceae and in the Restionaceae did not show a significant response to smoke (Brown & Van Staden 1997). *Helichrysum areonitens* was not stimulated by smoke to germinate (Afolayan *et al.* 1997), but inhibition is known to be concentration dependent and it may germinate with another concentration applied.

Systematic screening of economically and aesthetically important seed should be given a high priority in this country because of obvious benefits in smoke treatments.

6.2.5.2 Potential differences between pioneer and climax species

The first plants to inhabit disturbed areas after a burn are pioneers while the climax species only appear later. In Fynbos the pioneer species may need a relatively strong smoke concentration for germination in contrast to the climax species. They will therefore germinate when there is a great deal of smoke in the region. The climax species may be stimulated to germinate by a combination of smoke and some other

factor like soil storage (Roche *et al.* 1997; Keeley & Fotheringham 1998) or the climax species may need a lower concentration of smoke to stimulate germination. Further investigation is needed to determine if differences in pioneer and climax species could be smoke related.

6.2.6 Reaction of different veld types to smoke solution

Plants of fire prone environments with fires every 2–5 years like grasslands etc., may react differently to smoke induced germination than would plants in fire prone environments with fires every 8–30 years like the fynbos. The fuel load of a frequent fire environment will not be very big and the resultant smoke will be less. These plants may then require a lower concentration of smoke to induce germination. Smoke derived from burning grass (*Themeda triandra*) has more biological active material than smoke derived from burning fynbos (Van Staden *et al.* 1995b). It is postulated that the grasslands have less fuel than the fynbos and each plant needs to produce more of the active compound to stimulate germination. Plants in non-fire prone environments may react completely differently to smoke than those in fire prone environments, but smoke may play a role in inducing germination in both types (Pierce *et al.* 1995). This hypothesis requires further testing now that the role of smoke concentration effect is more clearly understood.

6.2.7 Potential economic developments

As a result of this study, smoke solution can now be marketed in specified dilutions. This will enhance its commercialisation, as the quality will be controllable. Mr. J. D. van Eeden (see p. 7), the University of Stellenbosch, South Africa and the Agricultural Research Council of South Africa are currently developing fixed concentration smoke solution as a commercial product for use on a small scale by gardeners and nurseries. A lot of refinement still needs to be done towards the development of this product.

6.3 Roles for smoke solution other than as a stimulant for seed germination

6.3.1 As a root stimulant

The question as to whether the stimulant(s) for germination and root growth are the same is important. Aqueous smoke solution has been observed to have a positive effect on radicle emergence and lateral root development. Taylor & Van Staden (1996) have tested smoke water as a root stimulant on *Vigna radiata* (L.) Wilczek (mungbean) and the conclusion they reached is that the same cue that stimulates germination also stimulates root growth. This leads one to speculate that smoke is a root growth stimulator, rather than a root initiator. Studies are currently underway to determine if it may have a better effect than commercially available products such as Terrahume.

6.3.2 As flowering stimulant

Smoke solution also enhances the flowering of some bulbous plants and orchids and these plants show better growth (Holmes 1986; Tompsett 1985). This may have great implications in the nursery and floriculture industry.

Smoke (and smoke solution) is a very interesting product of fire. The stimulating effect of smoke on many aspects of plant growth (germination, flowering etc.) has captured the interest of scientists worldwide. Much research in the different aspects and benefits of smoke needs to be done. There is a lot of potential in applying smoke solution as an organic plant stimulant for various reasons. This is a natural product that can be utilized for our benefit instead of polluting the air!

Chapter 7

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APPENDICES

Appendix 1

Smoke solution	pH
Standard	3.15
Australian	3.68
Distillate 1	2.94
Distillate 2	3.24
Food Flavourant	3.8

Figure 1 This table show that all the smoke solutions tested had a low pH.

Appendix 2

Smoke solution	Resistance (ohms)
Standard	3153
Australian	1361
Distillate 1	1701
Distillate 2	1569
Food Flavourant	2438

Figure 2 This table show that the resistance of the different smoke solutions were not constant and cannot be used as an indication of concentration.

Appendix 3

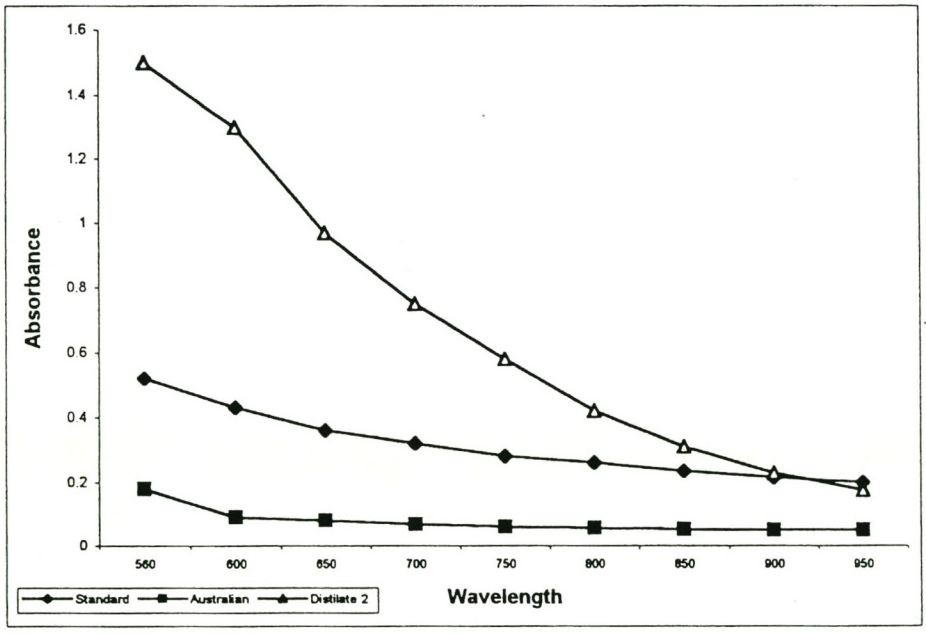


Figure 3 The graph show that there is no correlation between colour and concentration.

Appendix 4

				Ave.	SE
Germ. Box	94	90	84	89.33	1.676667
Petri dish	94	80	78	84	2.906667

Figure 4 Looking at the different germination figures of the different repetitions of the germination boxes compared to the petri dishes, it can be observed that the germination boxes have more consistency in germination.

Appendix 5

Replicate 1	100
Replicate 2	100
Replicate 3	98

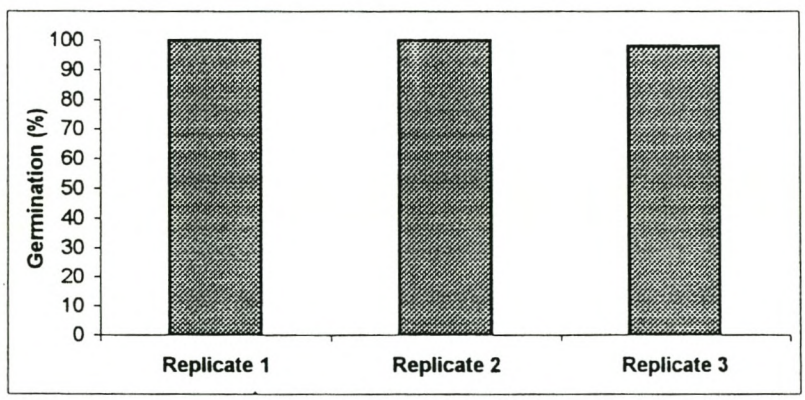


Figure 5 After 48 hours the germination of the Grand Rapids lettuce seed was 99.3%.

Appendix 6

unistel®
Registration Number:-98/21073/07

Unistel Group Holdings (Pty) Ltd
A Company Wholly Owned by the University of Stellenbosch

Aqueous Smoke Solution Concentration Certification

Client: A

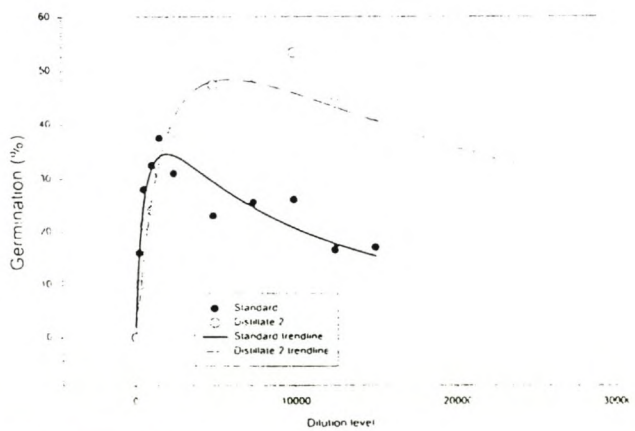
Our Batch nr. : Y

Client Batch nr.: X

Date received: June 1998

Comparison made to the De Lange standard (De Lange & Boucher, 1990) by bioassay (Meets, in prep.)

Results



Optimum germination concentration

De Lange Standard: 1:2 000

Batch assay: 1:6 000

Batch concentration compared to standard [neg.(-) = weaker; pos.(+)= stronger concentration]

De Lange Standard: +1 delb

Batch assay: +3 delbs

Recommended usage concentration for fynbos: De Lange Standard = 1:250

Batch assay = 1:750

(i.e. De Lange standard diluted 1*l* smoke to 250*l* water)

Certified by: _____ on _____

Date

Figure 6 Example of a certificate to be awarded to clients after certification of a smoke solution.