Bitou Bush

Helicopter Boom

Spraying

in NSW

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Credits

Elizabeth Teakle for proofreading,

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Executive Summary

Helicopter spraying of glyphosate herbicides has achieved high levels of control of Bitou in many sites in NSW, but has achieved only 70 % or less kill of bitou at approximately one third of the total areas sprayed (see figure 2). The herbicides used in Bitou Helicopter Boom Spraying (BHBS) are predominantly glyphosate herbicides. Of the areas sprayed, 55% has been with Roundup®, 41% with Roundup Biactive®, and 5% of the area were sprayed with metsulfuron-methyl as Brush-Off ® Brushcontroller (see Figure 5).

The factors reducing the effectiveness of glyphosate were reviewed from the scientific literature. Water stress was most commonly attributed by BHBS managers to result in low kill rates of bitou by BHBS. However other environmental conditions associated with drought, such as low relative humidity and salt build-up on leaves, have been found in studies to be important in reducing kill rates of glyphosate herbicides. Low humidity at the time of spraying can result in the solidification of the herbicide on the leaf surface and reduced herbicide uptake.

The majority of native plant species monitored through BHBS with glyphosate are not considered sensitive to this procedure. A smaller number of species have been observed to be commonly killed by BHBS and are listed in this report as highly sensitive. Another set of species are heavily impacted and require an extended period to recover, and these are referred to as moderately sensitive. A list of plant species according to their observed response to BHBS has been constructed (see Table 1).

The many factors which influence herbicide injury sustained by plants by BHBS with glyphosate herbicides are reviewed from the scientific literature and from monitoring studies of BHBS. A major factor reducing the impact of herbicide on sensitive plants is the effect of sheltering of smaller plants by higher canopies of leaves. Several possible factors may increase the injury of herbicide on native plants and are speculated in the review, including increased activity of pathogenic soil fungi following spraying leading to a reduction in seedling recruitment. Coincidence of high levels of sea salt aerosols deposited on leaf surfaces after spraying may increase the damage to plants after BHBS. Plants flowering at the time of BHBS are expected to be much more susceptible to damage from BHBS.

The data collected from monitoring of BHBS operations in NSW has many deficiencies, the greatest of which is its incompleteness.

There is no data on seedling injury in BHBS possibly because very low levels of seedling recruitment have been observed in many BHBS operations.

Increased levels of sand erosion may occur after death of bitou following BHBS in some areas because of the large volumes of sand trapped by well established thickets of bitou bush.

BHBS is considered to have significant potential to create chemical drift and damage off-target native plants and plant communities. Some of the factors contributing to this situation are discussed.

Cumulative injury of native plants from exposure to low levels of glyphosate created by drift from BHBS is discussed.

The technique of BHBS has achieved satisfactory levels of effectiveness when the aims are simply to control bitou bush. BHBS has not achieved high levels of effectiveness when gauged as a biodiversity management tool due to inadequate use of precautionary measures and the inadequate monitoring of herbicide injury of native plants.

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Introduction

Bitou bush is a serious invasive species of our coast and currently infests 80% of the NSW coastline and has increased by 36% from 1982 (Thomas and Leys, 2002). Bitou bush invasion has many damaging effects on plant and animal biodiversity and hence has been listed as a key threatening process under the NSW Threatened Species Act, 1995, and as a Noxious Weed in NSW. With the finalizing of the Threat Abatement Plan (TAP) for bitou bush invasion, managing authorities will be obliged to control bitou.

Helicopter Boom Spraying (HBS) has been widely used to apply herbicides to bitou bush along the NSW coast over the last 10 years, with over 4000ha being sprayed. Phytotoxic effects have been observed in a number of native plant species, despite treatments occurring in winter when most native plants are not actively growing (Toth et. al., 2003; Toth et. al., 1997; Kholer and Whelan, 1993).

Glyphosate has been the main chemical applied by bitou helicopter boom spraying (BHBS) which applies low rates of glyphosate in small water carrier volumes compared with other methods of perennial plant control. Spraying (BHBS) is used throughout this document to indicate the standard technique of helicopter boom application of herbicide in winter with 720g.a.i./ha (grams of active ingredient/hectare) of glyphosate (most commonly in the form of 2L/ha of Roundup®) and 18g.a.i./ha metsulfuron (30g/ha of Brush-off® Brushcontroller). Both herbicides are carried in 30L of water/ha.

Figure 1 Estimates of the Area of BHBS from 1979 to 2002

This document aims to critically review the effectiveness and environmental impacts of helicopter boom spraying of bitou bush. This is timely for several reasons, including the increasing area being treated annually (see Figure 1), the problematic nature of monitoring for phytotoxic effects, and the lack of any rigorous studies into the effectiveness of bitou aerial spraying.

BHBS has given a high level of effective control of bitou bush in many cases (Vranjic, 2000), but 70% and less control rate has occurred in. approximately one third of the total area sprayed. (see Figure 2).

Based on conversations with several BHBS project managers, it appears that localised eradication of bitou bush is an achievable aim when an integrated BHBS program is applied effectively. For this to occur, BHBS needs to be highly integrated with other biologically based methods of bitou control.

In this review it is axiomatic that the maintenance and promotion of a healthy native plant community is the most effective means to reduce and eradicate bitou bush from local areas of natural ecosystems. The combined effects of a healthy dense native plant growth and a thick litter layer are expected to increase the resistance of plant communities to invasion by exotic species (Burke and Grime, 1996).

This report is divided into three sections: Section 1, a discussion of strengths and weaknesses of BHBS as a biodiversity management system and limits of current understanding of BHBS; Section 2, a review of the efficiency of HBS to control bitou bush and Section 3, a review of impacts on native plants.

1 .0 Overview of Bitou Helicopter Boom Spraying

BHBS is an efficient bitou control technique which can form part of a highly integrated weed management program. In this section we will consider the aims of treatment programs, the strengths of this technique, the major weaknesses in the use of this technique and the limits of current understanding affecting BHBS and its ramifications.

1.1 Advantages of Helicopter Boom Spraying in Controlling Bitou Bush

There are many reasons given to support the adoption of BHBS in coastal NSW and include the following:

- It is the most cost effective. The cost of treating bitou per hectare is much lower than with ground spraying.
- Most areas that are difficult to access from the ground are readily treated by HBS. This excludes cliffs which are most efficiently controlled using helicopter spot spraying.
- Chemical usage per hectare is lower than with hand spraying (for infestations greater than 20% cover, see Section 2). This reduces the magnitude of any non-target impacts.
- There is greater potential to completely cover an infestation with herbicide than with ground spraying. This means that local eradication can be achieved with fewer follow up treatments and the total application of less herbicide.
- Aerial spraying is effective because it can be applied rapidly to a large area. It can exploit the susceptibility of bitou to herbicides at a particular time, such as during peak flowering in winter.
- Helicopters produce less drift than fixed wing aircraft because helicopters produce a rotor down draft and travel at lower altitudes and slower flying speeds.
- BHBS uses a low volume of water to deliver the herbicide that may be deposited principally on upper leaf canopies with presumably very little run-off to lower leaf canopies.
- Small non-target plants are sheltered by upper plant canopies and are thus partially shielded from aerially deposited herbicide. Ground spraying directs chemical horizontally and with force and thus herbicide application is not largely restricted to the upper leaf canopy.
- Native vegetation in coastal NSW has a range of adaptations to sea salt aerosols and low nutrient soils, such as hairiness and waxy cuticles. These adaptations may provide a high level of pre-adaptive tolerance to some herbicide sprays.

1.2 The challenges of BHBS

The main challenges of BHBS are:

- The development of a reliable database of non-target plant sensitivity/tolerance to BHBS. Impacts of BHBS on sensitive native plants need to be thoroughly and consistently monitored, as the ability of native plants to survive treatments is paramount to the success of a BHBS program.
- Exclusion of areas from BHBS requires a high level of planning and demarcation. Ground spraying can exclude small areas with less planning and demarcation when applied by a well trained operator.
- Much greater potential for chemical drift exists with BHBS than with ground spraying programs. Drift is poorly understood and poorly controlled in current bitou control programs and further investigation should be undertaken.
- The use of clear and adequate precautionary measures. The potential of BHBS to treat large areas in a short period of time increases the risk and scale of unwanted side-effects. Pre-spraying surveying and planning must be thoroughly completed before BHBS.
- Helicopter flying time is very expensive and there are strong incentives to get the job done fast. This can compromise environmental safeguards.
- An agricultural perspective on chemical usage varies greatly from a nature conservation perspective. Further education of operators and monitoring of compliance with conservation objectives is crucial.

1.3 The Aims of BHBS

Can BHBS be remedial or is it, at best, part of a control and abatement program? In conversations with BHBS project managers there appeared to be some confusion over the ultimate objectives of various BHBS projects. A clear statement of objectives is necessary to avoid confusion and facilitate the achievement of the best possible environmental outcome. The effectiveness of BHBS is usually gauged as part of a control program, but within NSW NPWS it is frequently seen as part of a remediation program.

Remedial is defined as “affording remedy” and as “a cure for disease”. Local extinction of bitou bush is the most effective “cure” for bitou reinvasion.

There is no NSW legislation that requires that a remediation program for bitou infestation be implemented. The NSW Noxious Weeds Act, 1993, requires bitou to be continuously suppressed and controlled. The Threatened Species Conservation Act, 1995, Schedule 3 requires that the impact of the threatening process (bitou invasion of native plant communities) has to be abated but this does not specify removal of the threat. Neither act requires bitou to be locally eradicated nor any areas to be part of a remedial program.

BHBS is most commonly and simply used as part of an indeterminate herbicide control and abatement program for bitou invasion, and has limited effectiveness. BHBS can achieve greater effectiveness as part of a remedial bitou program as is discussed in detail in Section 2.

1.4 Where is the Uncertainty?

Where our knowledge is uncertain, greater caution is required. The major sources of uncertainty are outlined below.

1) Toxic Effects on Native Plants

There are a number of difficulties with the set of data that has been accumulated on toxic effects of BHBS on non-target plants (Toth, 2002). The largest problem is its incompleteness. There are 850 plant species recorded within bitou infested areas in NSW (NPWS Atlas), however the toxic effects of BHBS with glyphosate and metsulfuron have been monitored for only 219 (Toth, 2002) and 90 (Toth, in prep) species respectively.

Uncertainty inherent in the data collated by Toth (2002) has 3 main sources:

- Data concerning toxic impacts of HBS on native plants have not been collected under controlled experimental conditions. (see Section 3.0 Environmental Impacts of HBS for full discussion). There is a high risk of confusing environmental effects with toxic herbicide effects.
- The sole use of population-based descriptions and visual assessment of plant species after BHBS is inadequate to detect systemic damage to plants and follow individual plants over time. These methodological deficiencies are likely to have resulted in under reporting of lethal and sublethal impacts on native plants.
- Different herbicide products have been used. Some plants are more sensitive to one herbicide product than another. There has been no comprehensive comparison of the injuries created by different herbicide products used in BHBS.

Very little data has been collected on the toxic effects of HBS on seedlings (Vranjic, 2000). Discussion of the factors influencing seedling injury from BHBS is included in Section 2. Vranjic et. al. (1999) suggested that the relative impact of herbicides was particularly lacking for ephemeral annuals and geophytes.

2) How Much Chemical Drift is Occurring?

Currently no BHBS operations are systematically monitored for herbicide drift, and it is unknown how much is occurring. It is also unknown how many plant species are seriously affected at application rates below 720g.a.i./ha of glyphosate (see Section 1.5 Scope for Unknown Impacts). As a result, it is uncertain what impacts are occurring outside the application area. The impacts of low doses of glyphosate on plants (doses below 720g.a.i./ha) are discussed in Section 2. Uncertainty concerning the magnitude of chemical drift also reduces certainty that an effective dose of chemical has been applied within the target area. Minimization of the impact of drift on native plants is discussed in Section 3.

Permits for BHBS of Roundup® are issued by the Australian Pesticides and Veterinary Medicines Authority (APVMA) under the NSW Pesticide Act, 1999, which specifies that no herbicide from a spraying operation will enter a water body or sensitive area. Widths of exclusion zones surrounding waterways are not specified in the act, leaving the onus on the body conducting spraying not to pollute surface water with Roundup®. Drift can be minimized by adhering to strong drift reduction measures.

Procedures that minimize impact may be adhered to more closely if environmental auditing of HBS is introduced.

3) Native Seedling Recruitment

It is uncertain whether adequate native seedling recruitment has been occurring following BHBS for many species. It is speculated that BHBS significantly inhibits native seedling growth by creating a litter environment antagonistic to germination and establishment (see Section 2 below for discussion.). It is uncertain what long term impact poor native recruitment will have on community structure and suppression of bitou infestations. Local eradication of bitou following BHBS is much less likely if native plants have poor germination and growth rates.

4) Substrate Stability

The level of substrate instability that has been directly caused by BHBS is uncertain. It is further uncertain what management should be used in destabilized areas following BHBS. The two main locations where destabilization of substrates may occur are on headlands and along sand dunes.

Very little attention has been given in Australia to the dynamics of plant communities on sand dunes. The infestation and dominance of coastal dunes by bitou in Australia has changed their ecological character (Heyligers, 1985). Changes include increased binding of the sandy substrate, reduced habitat for native plant diversity (Weiss et. al., 1998) and decreased sand mobility and increased height and steepness of dunes (Hesp, 2002). The removal of bitou dominance from these areas by BHBS is likely to create more change in the ecology of coastal dunes. Of major concern is the increased formation of blowouts in previously bitou-dominated dunes following HBS treatment (see Section 2 for a discussion of erosion).

5) Significance and Distributional Limits

It is uncertain what level of impacts BHBS is having on species with conservation significance below that of rare and threatened. These lower levels of conservation significance are rarely accounted for when assessing the impact of BHBS.

Species with small geographic ranges and narrow habitat specificity receive a disproportionate level of conservation attention (Mokany and Adam, 2000).

The distributional limits of plant species are an important biological feature worthy of appreciation and conservation, but distributional limits of coastal plants are often unpublished. This information should be obtainable from pooled herbarium records and specimens.

1.5 Scope for Unknown Impacts

This review of the environmental impacts of BHBS would be incomplete without a discussion of how much scope there appears to be for unknown impacts, although this discussion is inescapably speculative.

There will always be some level of unknown impacts in BHBS because there is an inescapably large scope for unknown impacts on native vegetation involved with large environmental changes involved with bitou removal. Also killing of non-target plants during BHBS is not only irreversible but often the previous existence of killed plants is unrecognized and they are lost without trace.

Potential risk and scope for unknown impacts are thought to occur in the following areas and may require more detailed risk assessment in the future:

1) Locating Threatened and Significant Flora

Locating threatened species in bitou-infested areas is a difficult task. Bitou invaded vegetation is very difficult to survey for native plants because of the presence of impenetrable thickets of bitou. One can never know for certain that all sites for threatened species have been located. The scope for unknown locations for significant flora can only be gauged by the amount of effort expended and the likely size of the task.

The Threatened Species Conservation (TSC) Act, 1995, only requires that impacts on threatened species need to be considered where threatened species have been previously located. If the amount of effort used to locate threatened species is only at the level required by the Threatened Species Conservation Act, there is a high risk that injury of threatened species is occurring because thorough targeted surveys have not been performed to locate all sites before spraying. An important question is: has an appropriate amount of effort been invested in locating threatened species before BHBS is conducted?

Threatened and significant plant species occur across all land tenures infested with bitou bush, not just in conservation reserves. Poorly known and significant plant species are currently not targeted in pre-spraying surveys in BHBS projects.

Coastal landforms are subject to a large number of small-scale environmental fluctuations and have created a mosaic of habitats that may contain unique and unusual assemblages of species. These areas can be readily overlooked in flora surveys. Thus targeted flora surveys for threatened species require a thorough knowledge of the mosaic of habitats found within and surrounding the targeted area, and a sound knowledge of species ecology. Species habitat characterization is often only of a primary habitat type, yet species are often found in smaller but highly significant patches of secondary habitat eg. the primary habitat of *Acrostichum speciosum* is along brackish estuaries. A previously undescribed secondary habitat was located at Sandon, Yuraygir NP, at the base of humate sand rocks exposed along the beach (Flower and Clarke, 2002).

2) BHBS Sensitive Plants

The size of the impact of BHBS on sensitive plant populations is unknown without documentation of the size of populations of plant species before and after spraying, eg. Blue Fan Flower *Scaeveola calendulacea* is highly sensitive to glyphosate and its population is not generally documented in pre-spraying targeted flora surveys. It is unknown what impact BHBS is having on populations of this species and whether local extinctions are occurring as a result of repeated BHBS.

The range of impacts of BHBS on native plants is discussed in Section 3.

3) Herbicide Injury of Native Plants in Off-Target Areas

Drift moves chemicals unpredictably and can deposit enough chemical to produce lethal toxic effects on sensitive vegetation. The potential impact of herbicide on biodiversity outside HBS areas is large and unknown. There is a great need to determine threshold exposure levels of herbicides on a range of sensitive plant species so that acceptable levels of chemical drift can be set and appropriately wide buffer zones established.

1.6 The Need for a More Precautionary Approach

The precautionary approach is one of the key principles of sustainable development, along with conservation of biological diversity (Deville and Harding, 1997) and it is the main safeguard against detrimental environmental impacts.

This can be best achieved by the formal, transparent and consistent application of precautionary measures. Precautionary measures increase the confidence that HBS of bitou bush will not have any long-term environmental costs.

Serious Environmental Impacts

A Review of Environmental Effects (REF) process has been conducted before many BHBS programs have been approved or implemented. These reviews identify precautionary measures which mitigate the potential for serious environmental impacts. However many BHBS operations occur without a REF and have undergone much less scrutiny and implemented fewer precautions before and during spraying.

A number of precautionary measures are outlined in Section 3 which could assist in the further minimization of environmental impacts.

How Much Precaution to Use?

As the significance of an environmental factor increases, the level of precaution required to protect that factor should also increase. When significance is extremely high, BHBS may need to be excluded altogether. Species with lower levels of conservation significance may only require toxicity testing and monitoring.

The use of strict precautionary measures in BHBS may temporarily halt control programs and incur greater costs. This may not always be desirable as control programs can achieve strong environmental benefits. Bitou bush is a major threat to biodiversity and failure to control this pest with BHBS may have greater impacts than often less precautious measures, such as commonly occurs with ground spraying.

The degree of precaution employed varies with the degree of uncertainty. Less precaution is generally required in previously heavily disturbed areas, such as sand mined localities and, to a lesser extent, cleared and grazed areas. Less precaution is also required for species that have a small proportion of their local populations within bitou infested areas and aerial application areas. Strict precautions are typically implemented early in the life of a project and eased as uncertainty is reduced. Strict precautions can be avoided by increased certainty of risks. The general argument against strong precautions is weakened by the fact that most precautionary measures have a low cost, such as drift monitoring, the professional management of drift, controlled toxicity testing and threshold toxicity testing of particular species.

There is a case for strict precautions to be implemented in the following situations:

Threatened Species Sites

The uncertainty of BHBS on threatened species is too large to allow BHBS over threatened plant locations.

Wetland Habitats

Many rare species inhabit wetland areas adjoining bitou bush infested areas. These wetlands have major potential for impacts from BHBS.

Littoral rainforest

Many littoral rainforest species have not been toxicity tested or monitored.

Bitou-dominated High Dunes

Reducing vegetative binding of sand by bitou bush potentially allows for the release of large volumes of drifting sand.

Intact Hind Dune Vegetation

A high level of plant diversity occurs in hind dune vegetation and increases the risk of herbicide sensitive species being present.

Further detailed discussion of precautionary measures is outside the scope of this review.

2.0 Effectiveness of BHBS in Biodiversity Management

The effectiveness of BHBS must be judged according to its longest term and broadest scale objectives. The use of herbicides to reduce weed infestations below threshold levels can be a very effective short-term technique and is generally referred to as weed control (Norris, 1999). Most weed control is amongst short-term crops and often does not address long-term control of weeds. However, with bitou bush the aim is to protect natural ecosystems which are likely to be damaged if chemical interference continues long term. The most effective control is local eradication with minimal chemical use. Often a single BHBS application will produce greater than 90% kill of bitou bush plants (see Figure 2), but this abatement may be only very short-term. Longer term abatement requires many repeat treatments.

The commonly stated objective of BHBS project managers is of zero to low levels of bitou seed production. Herbicide treatments are triggered by the observation of flowering and seed production. Local eradication is the implied objective but this goal has been poorly formulated. I have found no explicit reference to local eradication as an objective of bitou spraying programs. The establishment of clear long term objectives for each BHBS project area has major implications for long-term effectiveness and for public support and involvement. Ongoing herbicide use can only be prevented, or at least minimized, if bitou is eradicated locally and re-infestation is suppressed by biological mechanisms.

An unavoidable and ongoing risk is distance dispersal of bitou bush seeds by ocean currents that will intermittently supply seed to beach fronts (Batianoff, 1998).

The majority of BHBS project areas in NSW are in the early stages of bitou control but lack integration with other methods of bitou suppression. If BHBS is not part of an integrated approach, it can be a highly inefficient use of herbicide and may be associated with repeated herbicide applications and higher environmental impacts, such as biodiversity loss, erosion and invasion by other exotic weeds. BHBS must be integrated in a way to maximize the long term tactical benefits from the other elements of a remedial program (Cardina et. al., 1999). Biological suppression of bitou by established native plants, eg. *Acacia sophorae*, and suppression of fire in fore-dune vegetation are the two most powerful elements of an integrated bitou control program.

Figure 2 Effective Kill of Bitou Bush using Helicopter Boom Spraying in NSW

Long term agricultural weed control often aims at minimizing weed seed bank size. This has greatest economic benefit in cereal crops (Panetta and James, 1999). The germination of bitou seed is poor (Weiss, et. al.1998) and dependent on disturbance such as fire (Benwell, 2002). Thus it is a mistake to primarily target bitou control at decreasing the size of the bitou seed bank to suppress bitou bush.

Integration of Native Plant Resilience with BHBS

Bitou bush is a highly invasive species and has been reported to invade undisturbed vegetation (Weiss et. al., 1989). The dune environment is naturally subject to disturbance from storm surges, salt incursion, wind and sandblasting. Disturbance increases resource availability, decreases occupation by other species and facilitates colonization by weedy species (Prieur-Richard and Lavorel, 2000) including bitou bush. It has been commonly observed that disturbance of fore-dunes by hot fires promotes the increase of bitou from isolated plants to dense thickets which have major impacts on the native vegetation. Fire interrupts the capacity of native vegetation to suppress bitou.

There are several key elements or tactics that promote resilience of native plant communities and require a much higher level of integration with BHBS than is currently practiced in NSW. They include:

1) Minimizing BHBS herbicide impacts on native plants through close monitoring of sensitive native plants.

2) Promoting biological suppression of bitou by native plants eg. *Acacia sophorae* including shading and removal of ecological opportunities for bitou re-establishment. The combined effects of dense plant growth and a thick litter layer may increase the resistance of most plant communities to invasion by exotic species (Burke and Grime, 1996). Biological suppression has contributed to prevention of bitou domination of *Banksia aemula* heath-land and sclerophyll forest (Weiss and Noble, 1984b). This is despite bird dispersal of seed into adjoining areas and the establishment of pioneer plants under bird roosts (Weiss and Noble, 1984a).

3) Suppressing frequent and hot wildfire in the fore-dune environment. It has been commonly observed that disturbance of fore-dunes by hot fires promotes the expansion of infestations of isolated bitou plants into areas with dense thickets. Fire interrupts the capacity of native vegetation to suppress bitou.

4) Developing integrated control programs for weeds that have previously established within bitou infestations. These weeds infestations are referred to here as parallel weed infestations and require the development of control programs in parallel with bitou bush control programs.

5) Using natural barriers to bitou dispersal to delimit BHBS project areas.

2.1. Factors Reducing the Effectiveness of Glyphosate

There have been some notable failures in effective kill of bitou using BHBS. A review of the known herbicidal activity of glyphosate is presented below and will hopefully increase understanding of the factors which have contributed to these failures (see Figure 2).

A better understanding of the herbicidal activity of glyphosate on bitou bush may allow us to use it more effectively and thus reduce the quantity of herbicide required to remediate bitou infestations.

BHBS seldom gives 100% kill rate of bitou, however results between 70-90% are frequently obtained (see Figure 2). Applications of 1L Roundup®/ha (360g of glyphosate/ha) can given a reasonable level of kill of bitou under ideal conditions (John Toth, pers. com.). A higher application rate of 2L/ha has been adopted to provide a safety margin, and helps avoid the cost of re-treatment when efficacy is reduced due to various factors (John Toth, pers. com.).

The ideal conditions for killing bitou using HBS-Roundup® are complex. As it is rare to treat when all conditions are optimal, the effectiveness of BHBS has frequently varied. The factors reducing effectiveness can be generalized into the following areas and are discussed in further detail below.

1) Environmental factors that create physiological stress are expected to reduce the rate of uptake and translocation of glyphosate and metsulfuron and its kill rate. The main environmental inhibitors of bitou bush growth are expected to be water deficiency, low temperatures and high levels of salt on leaves and in the soil. Reduced uptake may be compounded by morphological changes to the leaf. During drought periods water stress, low relative humidity, build up of foliar salt, soil salt and dust are all likely to reduce the effectiveness of BHBS.

Mild physiological stress often induces flowering which increases susceptibly to herbicide injury, but severe stress may inhibit flowering and physiological activity, thus reducing the susceptibility of bitou to herbicides. Conversely, the more optimal conditions are for growth, the greater the likely effect of a given herbicide treatment. The low level of growth of native coastal plants in winter is a complex phenomenon and unlikely to be a case of simple “dormancy”. During the winter, a suite of environmental stresses may occur, including low rainfall, high foliar salt loads and low temperatures. In contrast, bitou bush is growing actively during the winter, and the difference in plant growth patterns allows selective control of bitou bush with glyphosate.

2) The application rate of glyphosate used in BHBS is much closer to the minimum quantity of glyphosate that can kill bitou under ideal conditions than that used in ground spraying. Any factor which causes a decrease in the rates of absorption and translocation of glyphosate decreases the amount of glyphosate that is available to create damage to the plant, therefore the level of herbicide injury created by BHBS is due to any factors that influence the absorption and translocation of glyphosate.

3) Herbicide can be rendered unavailable to bitou plants by a number of factors. Low relative humidity results in the solidification of herbicide on the plant surface. Dust on the leaf surface and in water added to the herbicide spray tank may also bind with glyphosate. Water from rain and dew can dilute or remove the applied chemical.

4) The applied chemical may penetrate poorly through any non-target canopy, resulting in poor coverage of the target bitou bush. This is likely to be a major factor reducing bitou kill rates in hind dune plant communities. Uneven application due to under- and over-lapping swaths and drift can result in areas where inadequate herbicide has been applied to kill bitou.

The following six factors may reduce the physiological activity of bitou and hence the effective kill of bitou by aerial spraying:

1) Water Stress

John Toth (pers. com.) suggested that water stress reduces the uptake of glyphosate by bitou. However bitou has a very well developed root system and has been observed to be quite resistant to moisture stress (Cooney et. al., 1982). Several cases of low rates of kill of bitou by BHBS have been attributed to drought conditions, but there are several different factors that may be reducing the effectiveness of BHBS during drought other than water stress.

Many researchers have studied the influence of water stress on the effectiveness of glyphosate (de Ruiter and Meinen, 1998; Bouhache et. al., 1996; D'Anieri et. al., 1990; Paley and Radosevich, 1983; Dickson et. al., 1990).

The translocation of ^{14}C labeled glyphosate was observed to decrease in *Echinochloa crus-galli* as soil moisture availability decreased (Ahmadi et. al., 1980). Seedlings of *Avena fatua* were slower to die following the application of glyphosate when water stressed (Adkins et. al., 1998).

It is not clear from the literature whether water availabilities above wilting point can greatly affect glyphosate effectiveness. Although closure of stomates to conserve moisture may slightly reduce uptake of glyphosate, most glyphosate uptake is active and through cell membranes.

2) Low Temperatures and Frost

Very cold conditions effectively induce low physiological activity or "dormancy" in bitou so that growth is retarded. The minimum temperature at which bitou will grow is relatively high and has been determined at 16.8^{o}C (Weiss et. al., 1998). Many native plant species also become dormant at relatively high minimum temperatures in winter (pers. obs.; see Figure 3).

Figure 3 Mean Maximum June Temperatures in NSW

Figure 4 Mean Minimum June Temperatures in NSW

Bitou is more likely to be actively growing during winter on the north coast of NSW than on the south coast of NSW (see Figure 4).

Frost can physically damage bitou leaves and interrupt uptake of glyphosate. Frosts occur infrequently in bitou-infested areas of southern NSW and occur very rarely in bitou-infested areas of northern NSW (see Figure 4). The 30 day frost isoline has been used to circumscribe the distributional limit of *Chrysanthemoides monilifera* sp. *rotundata,* with optimal growth occurring at a much lower frost frequency (Weiss et. al., 1998). Frost data was not available from the bureau of Meteorology but minimum temperatures give an indication of colder sites.

3) Relative Humidity

The relative humidity at the time of application has been shown to strongly affect the efficacy of glyphosate in killing various plant species. Low relative humidity accelerates the process of evaporation and the crystallization of solutes onto the deposited surface. Less glyphosate is absorbed from the crystallized state than the liquid form (Hess and Falk, 1990).

The efficacy of glyphosate-based control of couch grass *Cynodon dactylon* infestations has been erratic in the USA and the response to changes in relative humidity was shown to have a major influence on the absorption process (Jordan, 1977). In this study, less than 10% of the applied glyphosate penetrated the plant at 22^{0}C and 40% relative humidity. More than 70% of the applied glyphosate penetrated the plant at 32^{0}C and 100% relative humidity. There was no influence of temperature when relative humidity was 100%. Glyphosate applied at 560g.a.i./ha was twice as toxic to *Cyperus rotundata* at 100% relative humidity (RH) than at 40% RH (Chase and Appleby, 1979). In laboratory trials the exponential movement of glyphosate salts across the cuticular membrane of an incised poplar leaf was strongly influenced by humidity (Schonherr, 2002).

Similarly the absorption of glyphosate into a legume was greatly increased when relative humidity and temperature were high at the time of spraying (Sharma and Singh, 2001). Application of 360g.a.i./ha of glyphosate also gave the greatest efficacy of control of *Echinochloa colona* at high relative humidity (Tanpipat et. al., 1997). Similar results were also found for *Avena fatua* and *Urochloa panicoides* (Adkins et. al., 1998).

The level of systemic damage to rose plants from glyphosate application was quantified by Healy (1985). A trial with roses which were cut back after the application of up to 1-3% v/v solution of Roundup® showed that damage to original shoots was greatest when applied at low relative humidity and at high temperature. Application at low RH and high temperature created the least damage to subsequent shoots (Healy, 1985).

Hallgren (1988) found that when glyphosate was applied at 1440g/ha (double BHBS rates) relative humidity did not significantly affect control of *Elymus repens*. However, there was a trend towards increased control of *Elymus repens* at high temperature and high relative humidity.

Sherrick (et. al., 1986) studied the prespraying growing conditions of *Convolulus arvensis* and found absorption of glyphosate was, on average, 9% for high light intensity with low humidity and 21% for low light intensity with high humidity (Sherrick et. al., 1986). Cuticular wax was three times thicker in the plants grown under high light and low humidity than low light and high humidity (Sherrick et. al., 1986). Similar results were obtained by Westwood et. al. (1997). The thickened wax barrier may partially explain the difficulty of killing *Convolulus arvensis* at low RH. A glyphosate susceptible biotype of *Convolvulus arvensis* was found to absorb twice as much glyphosate at low relative humidity than a herbicide-tolerant biotype (Duncan and Weller, 1985; Westwood et. al., 1997). Both biotypes absorbed similar amounts of herbicide at high relative humidity. Similarly, Sahid et. al. (1996) found that *Paspalum conjugatum* was more sensitive to glyphosate when grown in the shade than under exposed conditions. Ipor and Tawan (1995) also found that growth in the shade produced thinner leaves with less epicuticular wax which allowed herbicide droplets to spread out and be retained on the leaf.

MacIsaac et. al. (1991) found that differences in the physical characteristics of dried herbicide deposits on leaf surfaces have been used to explain the degree of absorption of glyphosate by a plant. Nalewaja and Matysiak (1991) reported that glyphosate may become entrapped in the spray deposit on the leaf. Herbicides applied as fine droplets can be observed to form dried deposits on the leaf surface within minutes of application when relative humidity is low (pers. obs.).

Contrary to the above results, low humidity may increase the effectiveness of low doses of glyphosate, with greater uptake occurring at low relative humidity. Cranmer and Linscott (1990) reported that the efficacy of low doses of glyphosate involving 1 to 3 drops of glyphosate to *Abutilon theophrasti* seedlings was increased at low humidity. This effect appeared consistent with other results that showed greater efficacy when plants were treated with fewer but more concentrated droplets for a given dose. Low humidity leads to greater evaporation and thus concentrates the solutes in the applied droplets. At higher application rates of 9 droplets per seedling, high humidity was associated with higher efficacy than low humidity.

Glyphosate is absorbed by the plant as a liquid. The use of adjuvants in Roundup® has been observed by MacIsaac et. al. (1991) to prevent or delay glyphosate crystal formation.

John Toth (pers. com.) has suggested that the presence of dew at night and early mornings is likely to re-dissolve crystallized glyphosate on the leaf surface and extend herbicidal activity. However he also suggested that heavy dew or rain that causes runoff from the leaf surface may reduce herbicidal activity.

BHBS in Moruya has had poor bitou kill rates (John Toth, pers. com.). Air from the Snowy Mountains with low relative humidity and temperatures may drain into this area. Rapid changes in relative humidity are expected to occur when the wind shifts from those originating on land to wind originating offshore. The effects of low humidity on effectiveness of BHBS can not be controlled but consideration of weather forecasts may enable better scheduling of BHBS to achieve higher bitou kill rates.

The impact of low relative humidity on HBS efficiency should be considered. Monitoring of relative humidity during BHBS will assist in the assessment of the role of relative humidity.

4) Foliar Salt

Foliar salt creates a barrier between the leaf surface and herbicide deposits and also upsets the osmotic pressure within the plant. This is important for water conservation, physiological function and translocation of herbicides including glyphosate. Bitou and many other littoral plant species produce leaves that appear thicker when exposed to salt spray (pers. obs.). It has been observed that bitou plants affected by foliar salt are difficult to kill with HBS of Roundup® (John Toth, pers. com.).

The presence of long chain hydrophobic alkane compounds in epicuticular waxes has been proposed as an adaptation to reduce uptake of foliar deposited salt aerosols (Simini and Leone, 1986; Chandrashekar and Sandhyarani, 1994). These compounds can also protect the plant from uptake of herbicides (Hess and Falk, 1990).

Bitou is less tolerant of salt spray than many other foredune species in NSW (Mort and Hewitt, 1953). Salt spray deposition is greatest on the windward leaves of a bitou plant. Partial death of bitou plants following BHBS has been observed to occur on the leeward side of plants with the windward and salt impacted sides showing poor effective kill e.g. Bundjalung NP and several sites on the south coast of NSW (Kerry Thompson, pers. com.; John Toth, pers. com.). The restriction of toxic effects in plants to a single branch may be due to the generally little lateral interconnection of vascular elements, and movement of phloem and herbicide applied to one side of a plant is restricted to that side. A similar effect is commonly observed in hand spraying of glyphosate herbicide where "poor coverage" occurs and only part of the plant is covered by herbicide. Herbicide injury is limited to the sprayed side and does not become fully systemic resulting in the death of the plant.

5) Soil Salt

Zhang and Price (1993) found that translocation of glyphosate in *Echinochloa crus-galli* was reduced when 0.4kg.a.i./ha of sodium chloride was applied to the soil. Moisture stress also reduced uptake of glyphosate from leaves in this experiment, but the combined effect of salt added to the soil and moisture stress did not further reduce glyphosate uptake.

Soil salt concentration is likely to build up during extended periods of low rainfall in bitou infested areas and may reduce the translocation of glyphosate.

6) Phenological Change in Sensitivity

Sensitivity of bitou to Roundup® peaks when bitou is flowering (during winter) and decreases as fertilized fruit develop (Vranjic et. al., 2000). Many native species in bitou-infested areas do not have flowering or peak flowering in winter. This difference in peak sensitivity creates a selective effect with otherwise nonselective broad spectrum chemicals such as glyphosate and Brush-Off®. However peak bitou flowering may vary according to seasonal variation and has been observed to come into full flowering in autumn in a post fire environment (Jeff Thomas, pers. com.).

Bouhache et. al. (1996) reported a similar effect in *Solanum elaeagnifolium* where peak flowering coincided with maximum sensitivity to glyphosate. Pereira and Crabtree (1985) reported that *Cyperus esculentus* had greatest sensitivity to glyphosate when it was applied before the initiation of tuber development. Tomatoes showed greatest sensitivity to very low rates of glyphosate in the pre-bloom stage when flower buds had just formed (Gilreath et. al., 2001).

7) Dust on Leaf Surfaces

Bouhache et. al. (1996) found that the herbicidal activity of Roundup® was greatly reduced when plant leaf surfaces were coated with soil dust immediately after herbicide application. Soil dust reduces the contact of the herbicide with bitou (Vranjic 2000). Glyphosate will be strongly sorbed by clay particles, reducing herbicidal activity. Dust frequently reduces the effectiveness of herbicides in agriculture when stirred up during spraying by machinery (Mathiassen and Kudsk, 1999).

Build up of dust on leaf surfaces is most likely to occur during prolonged dry periods. Dust or suspended particulate matter may originate from many sources and contain different particulate material.

A common source of dust in some Bitou-infested areas is unsealed roads. However sand dunes contain minimal amounts of clay or silt material, the major components of soil dust.

Bitou infested areas are often windy environments where aerosol material which may include organic material, minerals including salt, and clay particles, settles on leaf surfaces (pers. obs.). During extended dry periods, deposited particles accumulate on the leeward side of the dunes and on plants that obstruct wind flow, such as bitou. If there are sufficient dust deposits on leaves to reduce the efficacy of glyphosate, bitou plants located in the lee of wind barriers, such as dunes and other topographic features and wind breaking plants, would show reduced kill rates.

8) Hard Water Antagonism

Nalewaja etal (1992) ranked the relative antagonism of various cations in solution to the phytotoxicity of glyphosate. They found that for the same concentration of glyphosate, antagonism decreased in the following order: iron> zinc> magnesium> calcium> sodium> potassium.

Cations in the spray tank solution compete with the isopropyl amine part of the glyphosate molecule to form a conjugated cation-glyphosate salt. These cations can bind with both the carboxyl and phosphate groups of the glyphosate molecule to form a chelated structure over time (Thelen et. al., 1995). Uptake of glyphosate by plants has been found to be subsequently reduced (Thelen et. al., 1995).

Reducing the volume of water applied will reduce the quantity of dissolved cations that are available to interfere with glyphosate activity (Thelen et. al., 1995).

Another source of antagonism to glyphosate activity is soil in the spray tank water. Soil progressively reduces the effectiveness of glyphosate spray (Chorbadjian and Kogan, 2001).

9) Uneven Application of Herbicide

The nominated application rates of glyphosate in BHBS projects have been varied to a small degree from 1.8 to 2.5L/ha. But the effective deposition rate in the target area can vary widely from negligible to 4L/ha due to simple overlapping and under-lapping for a 2L/ha nominated application rate. The Australian Pesticides and Veterinary Medicines Authority (AVPMA) currently issues off-label permits for BHBS of glyphosate that are different from 2L/ha.

Several methods of GPS guidance are currently available which allow accurate placement of aerial spray runs and the documentation of chemical placement. These technologies will not be discussed in detail.

Wind swirl or turbulence can influence the pattern of deposition of herbicide spray. Variable wind speed at ground level can result in uneven wind displacement of spray aerosols as they fall to the ground, and this may result in overlapping and under lapping.

The topography and type of vegetation influence the pattern of impact of droplets, particularly when there is significant horizontal displacement of spray.

11) Penetration through Dense Foliage

Where a significant non-target canopy exists above bitou bush, the kill of bitou by BHBS has been frequently reduced to below 70% (Toth pers. com.). The retention of aerially applied spray by overhead foliage is discussed below as the "Microhabitat effect" in Section 3.

John Toth recommends that yearly treatments should be used in the hind dune areas where penetration of herbicide is the greatest problem. The lack of penetration of herbicide by BHBS can result in bitou remaining in the hind dune vegetation where it can act as a pioneer of later invasions, particularly after fire.

Bitou seedlings are frequently sheltered by overhead vegetation. Bitou seedling populations have been observed at up to 3500 /m^2 (Vranjic, 2000). The use of larger droplet size may be warranted in certain situations to allow more effective deposition of herbicide on sheltered bitou seedlings.

In theory larger spray droplets penetrate further through foliage because they move faster through the air and are more likely to drip and bounce off upper foliage (Feng et. al.2003). Nozzles that produce a high proportion of large droplet sizes of >300µm in diameter have not been trialed to achieve greater penetration of herbicide though dense foliage in BHBS operations. Microfoil® booms and Thru-valve® booms are examples of nozzles that produce a high proportion of droplets greater than 300µm in diameter.

Larger droplet sizes reduce the number of droplets per unit of area. This can reduce the effective coverage of foliage as fewer droplets are being deposited. Increasing the volume of water applied per hectare can compensate for reduced coverage, but changes in concentration of Roundup® affect uptake in plants generally (John Toth, pers. com.: see also Relative Humidity above).

John Toth (pers. com.) reported that small droplets of herbicide produced by Micronaire® nozzles had very poor penetration when applied to bitou in Eucalypt forest. The use of small droplet sizes and small water volumes in HBS may maximize the microhabitat sheltering effect. This may be an effective precautionary measure to protect native seedlings growing under overhanging plant canopies.

12) Dew

When dewy leaf surfaces are aerially sprayed, the disturbance from the helicopter and the additional liquid herbicide tend to result in dew and herbicide rolling off the leaf (John Toth, pers. com.). Uptake of glyphosate-based herbicides is mostly through the leaf surface and cannot occur if most of the applied chemical rolls off the leaf onto the soil. I found nothing in the literature to support the proposition that heavy dew promotes the loss of glyphosate from the leaf surface. In contrast to glyphosate, metsulfuron herbicides are effectively taken up through both roots and foliage, and chemical that reaches the soil has continuing herbicidal activity.

13) Rain Fastness

The length of time between glyphosate application and simulated heavy rain was investigated by John Toth applying Roundup® at the rate of 2L/ha to tomatoes *Lycopsersicon esculentum* in a controlled glasshouse study (John Toth, unpublished data). Death of the plants occurred when "rain" occurred later than 1 hour after spraying. Uptake of Roundup® was sufficient to produce major injury to the plants if "rain" fell 30 minutes after spraying (John Toth, pers. com.).

Bondcrete®, a water sealant consisting of polyvinyl acetate and resin, has been trialed as an additive to Roundup® to produce rain fastness in bitou control. Bondcrete® allowed the application of Roundup® during rain with effective control of bitou bush 75% of the time. The selectivity of Roundup® was greatly reduced by the addition of this adjuvant, and slight damage was reported to *Banksia integrifolia* and *Spinifex hirsutus* (Anderson and van Haren, 1989), species which are considered to be highly glyphosate tolerant (Toth, 2001).

14) Cliffs

The application of herbicide to cliffs using HBS is problematic, as helicopter boom spraying cannot deposit chemicals onto vertical surfaces without doing difficult aerial maneuvers. The Cliffs at Moruya have been a consistently difficult site to kill bitou using both Brush-off® and Roundup®. The best wind conditions to place herbicide on sea cliffs are a landward breeze, but this is likely to produce chemical drift problems on the landward side of the cliff. Sea cliffs are usually very close to the surf, often with breakers below which produce large amounts of salt spray.

The use of helicopter spot spraying reduces many maneuverability problems associated with BHBS.

2.2 Control of Bitou Bush with Metsulfuron

There is a lack of information concerning the effectiveness of BHBS with metsulfuron methyl. Detailed discussion of the different factors that reduce the effectiveness of HBS of metsulfuron methyl (MSM) is not attempted here. MSM is a much newer chemical than glyphosate despite being available for more than 15 years, and fewer scientific studies have investigated its effectiveness. The only herbicide product containing metsulfuron methyl that has been used in BHBS is Brush-off® Brushcontroller Dupont.

The four main sites in NSW of BHBS with Brush-Off® are Hawks Nest, Jervis Bay, Bouderie National Park and Coffs Harbour.

There have been several arguments used to support the integration of metsulfuron with glyphosate in BHBS operations and they are as follows:

1) Resistance of bitou to glyphosate is possible with repeated widespread use. Olsen (2000) considered that plant species with the following characteristics have the greatest risk of developing resistance: they produce large quantities of seed, are open pollinated, annual, can germinate all year round and have minimal seed dormancy. Bitou bush has many of these features, including an ability to produce seed within one year, similar to an annual. The risk of resistance developing in long-term herbicide programs is reduced by alternating with a herbicide with a different mechanism of action.

2) Metsulfuron is less toxic to grasses and hence may aid the development of grass species as alternatives to *Acacia sophorae* in suppressing bitou growth (see Section 3.6).

3) Uptake of MSM is largely by roots. This provides very good control of bitou seedlings. There is great benefit if MSM is used in heavy bitou infestations with few native species. MSM can have a 36 week half-life in alkaline soil. Bitou seedlings have been found to decrease in susceptibility to Brush-Off® as they increase in size (Fullerton, 1991; Toth et. al., 1996). Toth et. al. (1996) suggested this might be due to reduction in uptake by bitou seedling roots as roots extend deeper into the substrate.

4) Larger droplet sizes, often applied to minimize drift, can be used without reducing the effectiveness of Brush-Off®. The limited solubility of Brush-off® sets a limit on the maximum concentration that can be sprayed. BHBS has applied MSM as 30g Brush-Off®/ha, this is equivalent to18g of metsulfuron/ha and is the highest application rate that can be used when applying Brush-Off® in 30L of water/ha. Application of 9g of MSM/ha provides relatively good kill of approximately 80% of bitou bush under ideal plot conditions (John Toth, pers. com.).

Several factors require further study to allow a better understanding of the herbicidal activity of Brush-Off® in bitou-infested environments. These include:

- Studies on the persistence of herbicidal activity of MSM in bitou-infested soils.
- The fate of MSM in the dunal ecosystem, including the rate of breakdown and the extent of leaching.
- Brush-Off® is more vulnerable to the development of resistance by bitou than glyphosate herbicides and its use requires even greater caution. This is because MSM exerts a strong selection pressure at both the seedling and adult stages of plant growth (Olsen, 2000). MSM is a sulfonylurea herbicide and there are currently 14 weeds species in Australia that have developed resistance to sulfonylurea herbicides.

3.0 Susceptibility of Native Plants

It has been generally accepted that the impacts of BHBS on native plant species have been minimal (Vranjic, 2000). In this section we briefly introduce glyphosate and its various formulations, and review the available information concerning damage to non-target plants and plant communities in BHBS project areas in NSW. We then briefly discuss measures that can be taken to minimize plant injury, discuss in detail monitoring of herbicide injury and provide a listing of species that are known to be sensitive/tolerant to BHBS.

This review concentrates on the small proportion of plant species that are sensitive. It should not be implied from this that BHBS has a considerable impact on a large number of species in a target area if precautionary principles are followed. Adequate precaution includes conducting prespraying surveys, monitoring of sensitivity and recovery and excluding areas with high risk of damage.

3.1 Glyphosate Herbicides

Glyphosate is classed as a non-selective and broad spectrum herbicide. It will kill most plant species if a sufficiently high concentration is applied during periods of active growth. Glyphosate in a pure state is a crystallized solid and returns to that state when dried, such as when sprayed onto leaf surfaces.

When it comes in contact with the leaf surface as a liquid, it is actively translocated by the plant across the epidermal cell membrane (Preston, 2000), possibly through aqueous pores (Schonherr, 2002). Glyphosate is treated inside the plant in a similar way to a plant metabolite. It is actively translocated through the cytoplasm of the cell to reach the phloem where it is rapidly and actively translocated to physiological sinks. At these sinks glyphosate can disrupt an essential enzyme in the shikimic acid pathway, a pathway which produces aromatic amino acids including tyrosine, tryptophan and phenylalanine. The inhibited enzyme is 5 enolpyruvylshikimic acid 3 phosphate synthetase (or EPSPS). Tryptophan and phenylalanine are essential amino acids in the synthesis of animal proteins and cannot be synthesized by animals. EPSPS is present in the chloroplasts of most plant species and in many microbes but does not occur in animals. The development of phytotoxic symptoms of glyphosate is slow and typical yellowing of leaves may take from several days to over a week to become apparent.

Glyphosate is a negatively charged molecule and rapidly binds to cations (positively charged ions) that are electrostatically attached to soil colloids (Preston, 2000). Glyphosate is quickly adsorbed onto soil colloids where it has relatively little activity. The main route of breakdown is via soil microbial decomposition (Torstensson, 1985). The rate of breakdown varies widely depending on soil conditions.

Product Formulations

Ingredients and formulations of glyphosate-based herbicides differ between the many available products. Glyphosate concentration varies widely in different formulations and is clearly stated on the label.

The types of adjuvants included may vary between products, and may include solvents, surfactants and penetrants. These elements are not listed individually on the label of any glyphosate herbicide. The different adjuvants in different herbicide products may alter the phytotoxic response for various plant species. Therefore different observations of injury need to be made using the same product to be comparable. There has been little appreciation among BHBS project managers when collecting phytotoxicity data that varying the formulation of glyphosate applied in BHBS reduces the predictability of a species response to herbicide.

There have been several different herbicide products used in BHBS. The total area that has been sprayed with Roundup® is approximately twice the area sprayed with Roundup Biactive® (see Figure 5). The addition of data on plant responses to Roundup Biactive® to the state list of plant responses to Roundup® (Toth 2002) introduces a variable which makes interpretation difficult. When Roundup Biactive® was applied by BHBS, approximately 7 species showed significantly greater phytotoxic effects than when sprayed with Roundup ® (John Toth, pers. com. from unpublished trials).

Figure 5 Herbicides used to Helicopter Boom Spray Bitou Bush in NSW

Glyphosate has been the dominant herbicide applied in BHBS. Roundup® is one of the most widely used glyphosate products in Australia and has been the most widely used glyphosate product for aerial spraying of bitou bush. It contains glyphosate as an isopropyl amine salt, formulated at the concentration of 360g/L. Other commercial products have also been used. The concentration of glyphosate often varies between different products. The following products produced by Monsanto are provided as an example of some the different glyphosate product formulations that are available:

Roundup® contains 360g/L glyphosate as isopropylamine salt.

Roundup Biactive® contains 360g/L glyphosate as isopropylamine salt.

Roundup CT® contains 450g/L glyphosate as isopropylamine salt.

Roundup Max® contains 510g/L glyphosate as isopropylamine salt.

Roundup Power Max® contains 540g/L glyphosate as isopropylamine salt.

Roundup Dry® contains 680g/L glyphosate as monoammonium salt.

Weed Master Duo® contains 360g/L glyphosate as a mixture of isopropylamine salt and monoammonium salt.

Adjuvants

All commercial herbicide products consist of a mix of chemicals. Excluding the active ingredient, the ingredients are usually confidential.

There are two main types of adjuvants that are likely to have been added to glyphosate products: surfactants and penetrants.

Surfactants

Surfactants lower the contact angle of a droplet of herbicide on the leaf surface causing it to spread out (Chachalis et. al., 2001b). Conversely, hydrophobic leaf surfaces increase the tendency of droplets to stay isolated and this usually reduces the effective penetration of glyphosate into the plant (Chachalis et. al., 2001a).

The surfactant in Roundup® has been disclosed to be polyethoxylated tallow amine (POEA). Roundup Biactive® contains two unspecified surfactants.

Glyphosate solution applied on its own has been observed to create large crystals with little contact with the cuticle, while a blank solution of Roundup® (without glyphosate) has been observed to form a smooth and amorphous layer above the leaf surface (MacIsaac, 1991).

Penetrants

Glyphosate herbicide products may contain penetrants which damage the leaf surface directly. Feng et. al. (1999) found that leaves applied with blank formulations of glyphosate herbicide (with no glyphosate added) formed a well-demarcated zone of injury on the leaf surface. Within this zone, extensive rupturing of cell membranes in both epidermal and mesophyll cells was observed. Ruptured cell membranes facilitate herbicide entry into the leaf.

Other Effects

MacIsaac et. al. (1991) suggest that some glyphosate formulations contain adjuvants which delay or prevent crystalline deposit formation, enhancing glyphosate uptake. Once crystals form they are unlikely to be dissolved and absorbed later. Some adjuvants have hygroscopic properties and form a gel-like substance that maintains the active ingredient in solution.

3.2 Factors Affecting the Phytotoxicity of Glyphosate

This section addresses the effects of BHBS of glyphosate on vascular plants. Non-vascular plants are poorly studied in NSW (Mokany and Adam, 2000), and there has been no controlled assessment of relevant species sensitivity to the application of glyphosate.(see recommendation 6).

The main effects of glyphosate on plants can be categorized in the following ways:

- Direct toxic effects of glyphosate on the shikimic acid pathway.
- Indirect effects which include:

1 Inhibition of symbiotic relationships of plants with soil-borne microorganisms.
2 Microbe-mediated toxic effects. Glyphosate can change the micro flora of soil and allow harmful microorganisms to flourish.
3 Reduction in seed production and viability (Shuma et. al., 1995).

A number of species have exhibited high sensitivity to BHBS applications of Roundup®. The majority are littoral rainforest species, but several are dune or headland species (See Table 1).

Many native plant species experience minor injury from BHBS applications with Roundup® and can lose a significant number of leaves (pers. obs.) This leaf loss can significantly reduce the biomass of the plants in the short term but most species are able to re-grow new leaves relatively quickly. Sensitive species are slow to recover the same biomass that was present before spraying. The time to recover biomass is termed here the "recovery period" (see 3.10 Characterization of Plant Sensitivity). Long recovery periods increase the risk of cumulative impacts. Plants may not have regenerated biomass to pre-spraying levels or may not be able to reproduce before further BHBS treatments produce accumulated herbicide injury.

Winter flowering species, dependant on regeneration from seed, may suffer herbicide injury that leads to reduced setting of seed and seedling vigor (see 3.3.B Seed Vigor and Viability).

Factors Influencing Direct Toxic Effects

A number of factors influence the direct herbicidal action of glyphosate on the EPSPS enzyme. The direct effects are possibly not as long lasting as the indirect herbicide mediated effects discussed below.

1 Physiological Predisposition

Plant physiology has an obvious direct influence on the sensitivity of particular plants to herbicide application. Species with major periods of vegetative and reproductive growth in winter (June/July), when BHBS is conducted, are most likely to be sensitive to BHBS. Toth et. al. (1991) suggested that the tolerance of four native species was physiologically based, as responses to the herbicides Roundup® and Brush-Off®, which have different modes of action, were similar. The period in which a plant is likely to have highest tolerance to glyphosate is expected to be during periods of minimum growth and relatively high water stress (Paley and Radosevich, 1983).

The sensitivity of plants is also likely to change as they respond to changes in environmental conditions. The sensitivity of Norway spruce to glyphosate greatly increased if high temperatures occurred at the time of application or 3 to 4 days afterwards (Lund, 1983). Injury was particularly severe if stems were elongating at the time of spraying.

A positive correlation between EPSPS activity and glyphosate tolerance was found in an inbred line of maize *Zea mays* (Forlani and Racchi, 1995). Species and genotype differences in EPSPS levels in plant tissue may affect glyphosate sensitivity.

2 Microhabitat Effects

Overhead leaf canopies intercept a significant proportion of HBS-deposited chemical and provide shelter for smaller species and younger plants. The larger the plant biomass and diversity at a site, the more likely there is to be well developed sheltering microhabitats. Some species are likely to grow in situations where they will receive little herbicide (see 3.3.1 for discussion of microhabitat effects).

Use of smaller droplets sizes (<250µm) may result in less runoff from the leaf surface. As a result, smaller droplets stay in the upper leaf canopy where they first land, and little herbicide falls through to lower leaf canopies. Upper leaves of a particular plant are also likely to be more physiologically active than the lower leaves.

The following groups of plants are likely to receive high exposure to BHBS:

A) Sun-loving species. These have higher deposition rates of chemical than shade-loving species as the higher canopies intercept a high proportion of herbicide, e.g. the orchid *Pterostylis ophioglossa* grows in sun-exposed sites in low heath while the orchid *Acianthus amplexicaulis* grows in dense littoral rainforest. The threatened plant *Pimelea spicata*, while growing in dense heath, has a strong requirement to have its leaves exposed to direct light (Julie Matarczyk, pers. com.) and is likely to receive high exposure to BHBS applied chemical.

B) Plants on exposed headlands and incipient dunes.

Plants growing in these areas are very exposed to HBS applied herbicide. Sheltering by other plants is minimal but deposition of chemical onto plants is often reduced by irregular landforms.

C) Plants growing after hot fires.

Fire can greatly reduce overhead canopy and biomass. Many species such as vines and small plant are exposed to unusually high deposition rates and coverage of herbicide.

Plants that receive a reduced deposition of herbicide include:

A) Small, shade-tolerant plants.

These tend to be sheltered by higher leaf canopies. In rainforest and rainforest margins there is a complex layering of leaf canopies. The more complex the layering of the vegetation, the poorer the penetration of the herbicide and the greater the sheltering effect. Lower vegetation is less exposed to herbicide.

B) Vines

Vines are often difficult to kill with foliar applied herbicide. Herbicide applied to leaves must be translocated a considerable distance to reach the roots and underground storage organs. Vines are often highly branched with multiple associated bundles of roots. This increases tolerance to foliar-applied herbicide because there is generally a restricted amount of lateral movement from one side of the plant to the other and one vascular element to another. Root tissue may not connect directly with the sprayed branches and may be protected.

Vines are a diverse group and are commonly tolerant of BHBS. Vine branches that reach the upper canopy are often exposed to glyphosate herbicide but usually re-grow from lower branches of vines that are well sheltered (pers. obs.).

C) Seedlings

Although seedlings are physiologically more susceptible to glyphosate herbicides than mature plants, seedlings are often sheltered by an over story. See below for discussion.

D) Dense rainforest canopies

Upper canopy rainforest trees have a large total leaf surface and form a number of canopy layers.

E) Plants living around the edges of application areas

Application areas with irregular edges receive a much reduced rate of herbicide deposition. Incipient dunes and other bitou infested vegetation often form irregularly shaped areas that are not readily covered by the swathe pattern of a helicopter.

3 Glyphosate Soil Residue

Glyphosate generally has minimal activity in most soils due to very strong adsorption onto clay colloids, so that glyphosate effectively behaves like inorganic phosphate in the soil (Torstensson, 1985). The term glyphosate soil residue is used in this review to refer to glyphosate that is active as a herbicide, and doesn't include glyphosate that is bound within the soil. The decomposition of glyphosate is largely an indirect result of aerobic microbial activity (Kent and Preston, 2000). Decomposition is fastest where plant and microbial soil activity is greatest.

Glyphosate in soil solution is able to enter the roots of plants and seedlings along with water and dissolved ions, and is translocated in the xylem, not the phloem, as with foliar application (Preston, 2000). Activity of glyphosate in soil solution is uncommon and only occurs under particular environmental conditions.

There is significant evidence that glyphosate can have temporary residual herbicidal activity in sandy soils (Devlin et. al., 1986; Salazar and Appleby, 1982), however there have been no studies of soil residue levels in littoral sand. Cornish (1992) found glyphosate residues 15 days after spraying loamy soil with glyphosate at 1440 g.a.i./ha. This result may be due to addition of super phosphate and hence may be isolated to agricultural situations. The loss of activity of glyphosate in soil is due mostly to adsorption of the phosphate part of the glyphosate molecule on to soil colloids. Loamy sand is expected to have a low phosphate sorption capacity which, if saturated, will not be available to adsorb glyphosate residues in the soil. A very low phosphate sorption capacity may have been created in this agricultural situation by the addition of phosphorus fertilizer, which is likely to have filled the available phosphorus sorption capacity of the soil (Torstensson, 1985). Similarly Soulas (1992) found that the uptake of glyphosate labeled with ^{14}carbon by corn and soybean plants was enhanced when these plants were grown in sand with added plant nutrients that saturated the available phosphorus sorption capacity.

In an experiment reported by Devlin et. al. (1986), short term (0-2 days) residual soil activity of glyphosate was found when *Triticum vulgare* was grown in an unfertilized sandy soil after application of 2500g of glyphosate/ha. This is approximately 3.5 times the rate used in BHBS.

Extraction of glyphosate from soil in a laboratory provides little information about herbicidal activity as glyphosate that is bound and not active in the soil is extracted along with any unbound active glyphosate. This conflation of sources of glyphosate is increased as the quantity of bound glyphosate increases in the soil and is of little value in evaluating the potential for residual activity in the soil. For example, Ragab et. al. (1982) extracted glyphosate from a sandy loam soil and found high herbicidal activity after 10 days. A high proportion of bitou infestations in NSW occur on geologically recently deposited quartz sand, containing negligible amounts of clay material. The presence of glyphosate residues has the potential to reduce seedling establishment of native plants following BHBS. Phosphate sorption capacity of coastal sands from the east coast of Australia has been positively correlated with the quantity of aluminium and iron oxyhydroxides on the surface of quartz grains (Diggle and Bell, 1984), presumably due to the formation of a reversible glyphosate metal complex (Yusof and Ooi, 1992). These sands have low phosphate sorption capacities. As the age of sand increases, the degree of soil weathering and podzolisation also increases, resulting in aluminium and iron compounds moving deeper into the soil. As a result, a high phosphate sorption capacity is created deeper in the soil profile. Adsorption of glyphosate in a sandy soil in Western Australia also increased greatly as the iron, aluminum and organic carbon content of the soil increased (Gerritse et. al., 1996). This process of podzolisation is not established in beach sand and is poorly established in frontal dunes along much of the NSW coast (Jim Charley, pers. com.). Eberbach and Douglas (1983) found that nitrogen fixation by subterranean clover planted 120 days after application of up to 10 μg/g of glyphosate was reduced in sandy loam.

Organic matter provides significant additional phosphate sorption capacity in sands of coastal heaths in northern NSW (Jim Charley, pers. com.). Similarly Gerritse et. al. (1996) found organic carbon competed for phosphorus adsorption sites on clay colloids. The greatest amount of chemically active organic matter is located at the top of the soil profile and is the first soil layer to be exposed to glyphosate.

Although the phosphorus sorption capacity of coastal sands is low, saturation by the small amount of phosphorus applied by BHBS as glyphosate is unlikely. The quantity of elemental phosphorus applied as glyphosate during BHBS is 93.6g.a.i./ha per treatment, and 561g.a.i./ha is applied from six applications of glyphosate. This total quantity of glyphosate applied to the top 5cm of beach sand will raise the soil phosphorus level by less than 1 ppm. The total quantity of extractable phosphorus in beach sand is generally in the range of 10 to 50 ppm (Jim Charley, pers. com.).

It can be concluded that despite several factors reducing the residual herbicidal activity in bitou infested soils, glyphosate may persist under certain conditions in sandy soil following BHBS application of Roundup®.

Lateral Soil Movement of Glyphosate

Jacobsen et. al. (2000) found that the presence of glyphosate in subsurface drains was due to the leaching of herbicide through soil macro-pores. Soil macro-pores only occur in highly structured clay soils. Very sandy soils have a more uniform structure and have less potential for creation of macro-pores.

Long distance movement of glyphosate and its immediate breakdown products is not expected in coastal sandy soils, excluding very shallow aquifers such as on rocky headlands.

(See Suggestions for Further Research)

Glyphosate may also be transmitted in soil via roots and root exudates. Rodriques et. al. (1982) claimed to have proof of plant to plant transfer of glyphosate by root exudates from treated wheat plants to untreated maize plants.

4 Leaf Surfaces

The morphology of plant surfaces has a major influence on herbicidal activity.

Soft leaved and herbaceous species appear to uptake HBS applied Roundup® more readily than sclerophyll vegetation (Toth, 2002). Sclerophylly has been suggested as an adaptation to low nutrient availability, particularly of phosphorus (Johnson and Briggs, 1981). The following features have been used to characterize sclerophyll vegetation and are common in bitou-infested hind-dune heath and forest and foredune vegetation: broad "leathery" leaves, cutinisation and lignification of the leaves, presence of hairs and scales or waxy bloom on plant surfaces, strong development of palisade mesophyll, microphylly, needle leaves, aphylly, winged stems, spiny stems, sunken stomata, and development of tannins and resinous substances (Grieve, 1955). Several of these features may also be adaptations to foliar-deposited salt. Many of these sclerophyll features may greatly decrease penetration of foliar herbicides, however this effect does not appear to be documented for Australian sclerophyll forests. The greater the development of sclerophyll features, the greater the resistance of the leaf and other photosynthetic organs to injury from surfactants and other adjuvants in herbicide products.

Plant hairs occur abundantly on several littoral species including *Actinotus* spp., *Westringia fruticosa, Allocasuarina equisetifolia* and new growth of *Banksia integrifolia* and bitou bush.

Waxy epicuticular layers on leaf surfaces are very common in bitou-infested littoral environments. As the amount of particulate wax on the leaf surface increases, the spreading of the individual spray droplets decreases, resulting in reduced coverage of the sprayed plant (Hess and Falk, 1990; see also Section 2 Foliar Salt).

5 Parasite/Host Disassociation

Parasitic plants eg. some mistletoe and dodder are particularly susceptible to Roundup®. Aerial plant parasites receive BHBS glyphosate from both their own foliage and any glyphosate that is translocated from the host plant. Largely subterranean root parasites are likely to be affected by glyphosate only when the host absorbs and translocates glyphosate at a time when the parasite is physiologically active.

Glyphosate treatment has been observed to damage the association of both the aerial parasite *Cuscuta* (Convolulaceae) and the root parasite *Orobanche* (Scrophulariaceae) with their host plants. In an experiment using ^{14}Carbon labeled glyphosate, glyphosate was translocated from host leaves to *Orobanche* shoots where it accumulated in concentrations greater than in any part of the host plant (Foy and Jain, 1986). A histological study following application of glyphosate showed that the haustoria of *Cuscuta* did not succeed in entering the host bean plants, resulting in the death of the parasite (Zaki et. al., 1998).

Cuscuta australis and Golden dodder *C. campestris** are both expected to occur commonly in bitou-infested areas of NSW. Devils twine is another major parasite of coastal heath and includes the species *Cassytha filiformis, C. glabella, C. pubescens* (Lauraceae). The exotic plant *Orobanche minor** is parasitic on a wide range of garden and pasture plants in NSW (Barker, 1992), but has not been recorded in bitou-infested areas. *Euphrasia collina* subsp. *speciosa* (Scrophulariaceae) is a parasitic plant that has been recorded once in a bitou-infested area in northern NSW.

The major parasitic plant taxa in bitou-infested areas in NSW include the family Santalaceae and 5 recorded species which are all root parasites: *Choretrum candollei, Exocarpos cupressiformis, Exocarpos latifolius, Leptomeria acida,* and *Thesium australe*. Only *Leptomeria acida* has been monitored through BHBS with Roundup® but the results were inconclusive. *Thesium australe* is a threatened species that is parasitic on grass roots, predominantly *Themeda australis*. It seldom has above ground parts that can be directly impacted on by BHBS. Indirect impact via accumulation of glyphosate from the host plant is of concern.

Mistletoes (families Loranthaceae and Viscaceae) appear to be susceptible to low dose rates of glyphosate. A low application rate of 0.25ml of Roundup® per stem cut to the Eucalypt hosts has been reported to control mistletoe without damaging the eucalypt host (Minko and Fagg, 1989).

There are only two mistletoe species recorded in the NPWS Wildlife Atlas in bitou-infested areas in NSW. This appears to be a major under-recording for this group. The two species *Amyema congener* subsp. *congener* and *Dendrophthoe vitellina* both occur in hind dune forest habitat. Many more unrecorded mistletoe species are likely to occur in bitou-infested areas.

6 Calcium and Magnesium Deficiency

Signs of calcium and magnesium deficiencies may appear following application of glyphosate herbicides. Duke et al. (1983 abs) reported that the calcium and magnesium uptake by roots of soybean plants grown in hydroponic solutions was retarded by the addition of 0.5mM of glyphosate. Signs of calcium and magnesium deficiencies may occur following application of glyphosate herbicides when conditions favor high residual soil activity. Some signs of spraying with glyphosate may be due to induced calcium and magnesium deficiencies.

7 Flowering at time of BHBS

The reproductive state of a plant influences the effect glyphosate will have on that plant. The number of seeds set can be reduced if glyphosate is applied to plants that are flowering (Shuma et. al., 1995), (see 3.1.2 Seed Vigour and Viability Effects).

(See 3.3 Seed Vigour and Viability Effects for discussion and Table 3)

8 Location of Dormant Buds

The herbicidal action of glyphosate generally occurs at sites that are actively synthesizing amino acids, thus dormant buds, which are physiologically inactive, should be immune to the action of glyphosate and escape direct herbicide injury. Geophytes are plants that have dormant buds located underground. Regeneration of roots and shoots may occur from these buds when glyphosate activity in the plant has abated. An example is Cats Claw Creeper *Macfadyenia unguis-cati* which can produce many small underground tubers containing dormant buds which can grow after foliar treatment with glyphosate. Geophytes are most sensitive to herbicides when they are actively growing and there is less physiologically inactive tissue, eg. flowering terrestrial orchids may be generating new root systems at this time.

Plants which have few dormant buds located below ground (Phanerophytes) are less able to regenerate new roots and thereby less able to recover from severe herbicide injury.

Factors Influencing Indirect Toxic Effects

The remaining five factors influence the indirect herbicidal action of glyphosate by influencing the occurrence of injurious events such as disease, herbivory, disruption of symbiotic association and salt injury.

9 Mycorrhizal Effects

The primary mode of action of glyphosate is the inhibition of the EPSPS enzyme, an enzyme which occurs in higher plants and many species of microorganisms. However, as with vascular plants, microbial species including fungi vary in their sensitivity to glyphosate. Also glyphosate has been found to stimulate the growth of some mycorrhizal fungi in culture (Laatikainen and Heinonen, 2002).

The level of glyphosate residue in the soil is likely to determine the magnitude of the impact of glyphosate on soil mycorrhizae. Variable soil residue levels, activity and rates of microbial breakdown may explain the variable conclusions of several studies of glyphosate on mycorrhizal fungi. There have been no studies of residual soil activity of glyphosate using littoral sand (see 3.2.3 Glyphosate Soil Residue).

Glyphosate applied at the rate of 0.5 and 2.5mg/kg of very sandy soil produced a short-term, reversible, inhibitory effect on soil respiration, as well as a more persistent negative action on nitrogen mineralization (Ghinea et. al., 1998).

Nodulation of *Trifolium subteraneum* with *Rhizobium* sp. decreased when glyphosate was present in the liquid growing medium (Eberbach and Douglas, 1989). Glyphosate added to synthetic growing media is expected to have a high level of root absorption and consequently disrupt rhizosphere activity. No significant effect was observed on the establishment, growth or nodulation of seedlings of 14 pasture legumes when glyphosate was applied at either 0.54 or 1.08kg.a.i./ha, 1 to 8 days before sowing (Blowes et. al., 1985).

High application rates were found to affect nitrogen fixation of free-living nitrogen-fixing bacteria, but application rates typical of those used in the field were found to have no effect (Santos and Flores, 1995).

Mycorrhizal fungi colonization of *Ageratum houstonianum* was observed by Wu et. al. (2000) to have been significantly suppressed by the application of glyphosate to the soil. The relative impact of glyphosate on the host and symbiont was not determined. De Paula and Zambolim (1994a & 1994b) studied the two mycorrhizal fungi associated with seedlings of *Eucalyptus grandis*. At 10 ppm of glyphosate *in vitro* colonization by one species was reduced by 48% (De Paula and Zambolim, 1994b).

Chakravarty and Chatarpaul (1990) found glyphosate did not reduce mycorrhizal development on *Pinus resinosa* or seedling growth when applied at either 0.54 or 3.23kg.a.i./ha to the surface of peat and vermiculite potting mixes.

Orchids

There is little information available on the impact of herbicides on terrestrial orchids, however it has been established that glyphosate can interrupt the association of mycorrhizal fungi with orchids (Valius, 2001). Controlled testing of BHBS impacts on orchid species have not been conducted for any species (see Recommendation 1).

Orchids absorb glyphosate by several routes: foliar contact, directly from soil solution or indirectly through mycorrhizal fungi. Application of foliar sprays potentially involves all three uptake routes. Application to the growing medium involves direct root uptake and mycorrhizal uptake.

A few studies have been performed using artificial media, and have shown that in the presence of 1.0mM of glyphosate in liquid culture, mycorrhizal coils fail to initiate with orchid protocorms (Peterson et. al., 1998). The fungus multiplied through the orchid root tissue, becoming parasitic, but did not result in plant cell breakdown (Beyrle et. al., 1995).

Foliar applications of glyphosate over the Western Prairie fringed orchid (*Planthera praeclara)* in North America in autumn, when the orchid was entering dormancy, did not adversely affect orchid growth the following year (Sterling et. al., 2000).

All mycorrhizal fungi associated with terrestrial orchids in Australia are Basidiomycetes. Small terrestrial orchids associate with fungi from the order Tulasnellales and larger terrestrial orchids associate with a suite of fungal groups (Mark Clements, pers. com.). *Cheirostylis ovata* is a littoral rainforest species with chlorophyll that is obligately dependant on saprophytic fungi. *Cheirostylis ovata* is very susceptible to infection by soft rot fungi in the wild and in culture (Mark Clements, pers. com.), and may be vulnerable to changes in fungal populations following BHBS application of glyphosate.

10 Foliar Sea Salt

There is little research into the impact of foliar salt on native coastal vegetation in NSW. Maze and Whalley (1992) investigated the role of salt in the growth of *Spinifex sericeus.*

Foliar salt is a persistent element of bitou bush-infested coastal environments. Foliar salt exposure and salt induced stress is likely to be an important determining factor in the distribution and abundance of plant species in bitou-infested areas. Foliar deposited salt can interrupt plant growth and result in dieback of the canopy of native costal vegetation (Morris, 1992). Surfactants and other adjuvants in herbicides that reduce membrane integrity and damage the plant surface are expected to increase the phytotoxicity of sea spray. Surfactants in herbicide also reduce the surface tension of salt water droplets creating greater contact between the salt spray and the plant.

The dieback of Norfolk Island Pines *Araucaria hetrophylla** at seaside regions in Sydney has been strongly linked with surfactants occurring in ocean sewerage effluent (Truman and Lambert, 1978). The sodium and chloride levels approximately doubled in the sick trees (Dowden et. al., 1978, Grieve and Pitman, 1978), and suggested that surfactants facilitated salt entry. Morris (2003) found that the new leaves of *Banksia integrifolia* died in areas near ocean sewerage outfall.

The deposition characteristics of sea spray on vegetation are similar to herbicide spray deposition on foliage in several ways. Deposition or spray capture by vegetation can occur by two mechanisms: by sedimentation where horizontal upward facing foliage receives the greatest deposits, or by moving droplets impacting on plant surfaces ("inertial impaction"). Inertial impaction of droplets on vegetation depends on a complex interaction of wind speed, droplet size and leaf surface type and orientation (Marrs and Frost, 1997). The inertial impact of salt spray is greatest on the windward crest of elevated landforms where the wind buffets the vegetation.

The absence of rain to dilute and wash the surfactant and glyphosate from the leaf surface will allow the continued absorption of salt through the damaged leaf surface. The rate at which the various components of Roundup® are lost from leaf surfaces by rain is not known but could be experimentally determined.

Based on field observations, it is speculated that the interaction of sea spray and BHBS applied herbicide can increase the mortality of native plants. This speculation is based on unsystematic observations by several BHBS project managers and the author, and has not been experimentally tested. The interaction of sea spray and BHBS on toxicity to native plants may be affected by whether sea spray deposits occur before or after BHBS.

A) Sea Spray before BHBS

Plants receiving saltwater aerosols over extended periods can build up a high foliar salt cover which can reduce plant growth and depress uptake of herbicide. Crystalline salt on the leaf surface forms a layer above which glyphosate may be entrapped in a spray deposit (Nalewaja, and Matysiak 1991) and become unavailable for uptake by the plant (see 2.1.4 Foliar Salt). The quantity of foliar salt deposited before BHBS and its impact on plant growth can be ascertained before BHBS and thus influence BHBS project management. Reduced effectiveness of HBS on bitou where foliar sea salt has been deposited is discussed in Section 2.1.4.

B) Sea Spray after BHBS

Large quantities of salt-water aerosols can be generated and deposited on vegetation during sea storms. Headland sites which are often subject to high levels of salt spray, are frequently treated by BHBS. Schroder (1999) reported 100% kill of *Acacia binervia* in an exposed coastal scrub site at Tomaree National Park after a BHBS treatment that was followed by heavy seas. This was the second treatment of this area. The first treatment had minimal impact on *Acacia binervia* (Matt Clarke, pers. com.).

There have currently been no measurements of foliar salt loads on bitou or native plants before or after BHBS.

11 Soil Fungi and Glyphosate Synergism

The interaction of glyphosate and various soil microflora is complex and is not reviewed here in detail, however it has been well established that the application of glyphosate can increase the incidence of several soil borne fungal diseases but has been untested in BHBS situations (see Recommendation 2). Levesque et. al. (1992) showed that the effects of glyphosate on seedlings are synergistically exacerbated by the presence of pathogenic soil microbes. Glyphosate was less phytotoxic when seedlings were grown in sterile soil.

The fungal taxa associated with this synergism include the soil borne fungi *Pythium* and *Fusarium* spp. Both taxa include species that are not highly specialized plant pathogens but occur primarily as colonists of dying plants roots, dead organic matter and immature plants such as juveniles and seedlings. Residual soil activity of glyphosate is not required for fungal synergism to occur as uptake of glyphosate is occurring through leaves. *Pythium* spp. and *Fusarium* spp. are cosmopolitan in distribution and possibly occur in bitou-infested areas (David Teakle, pers. com.). The symptoms of pathogenic soil fungi may occur rapidly and be observed well in advance of the usual visible symptoms of glyphosate (Rahe et. al., 1990).

Build up of the pathogen *Pythium* spp. in the soil has been observed to occur following application of glyphosate herbicide in beans *Phaseolus vulgaris* (Descalzo et. al., 1996). Seedling diseases were also reported to increase following the application of glyphosate in barley (Blowes, 1987; Lynch and Penn, 1980). The larger the quantity of dying and decomposing plant material, the more likely a build up of pathogenic fungi is to occur (David Teakle, pers. com.).

Phytotoxic effects of glyphosate on sugar cane were associated with an increase in both the incidence and severity of *Pythium* root rot (Dissanayake et. al., 1998). Descalzo et. al. (1996) found that all five different soils tested contained strains of *Pythium* that operate as glyphosate synergists.

The amount of take all fungus *Gaeumannomyces graminis* in wheat crops grown in un-sterile soil was found to progressively increase following spraying with increasing concentrations of glyphosate (Mekwatanakarn and Sivasithamparam, 1987).

Glyphosate applied in glasshouse and cell culture experiments has been found to exert a mild toxic effect on Cinnamon fungus (*Phytophthora cinnamonii)* but a stimulatory effect on Cinnamon fungus sporangia and spore production (Kassaby and Hepworth, 1987).

Low levels of seedling recruitment have been observed by many BHBS project managers and may be explained by glyphosate synergistically elevating pathogenic fungi (see Section 3.12 Litter Effect, for detailed discussion). While there have been no reports of outbreaks of microbial pathogens in areas that have undergone BHBS, it is still possible that elevated pathogenic microbe populations are reducing or preventing successful germination and seedling establishment in areas after spraying.

Earl (1990) reported that many native plants germinated in plots where boneseed had been killed by the application of 1:100 dilution of Roundup®, particularly following the first good rain. This result is in contrast to the poor germination of native plant seed that follows BHBS and may be due to the smaller biomass of boneseed infestations compared with bitou bush infestations.

12 Aerial Pathogens and Glyphosate Synergism

A non-lethal but pathogenic synergism has been reported between anthracnose and glyphosate in beans. The normal delimitation of lesions on the bean infected with anthracnose was absent when glyphosate was applied to beans without storage of phenylalanine, a precursor of phytoalexins (Johal and Rahe, 1990). The resulting lesions spread and coalesced over the entire hypocotyl. These symptoms only occurred when the cotyledons were removed and seedlings were not exposed to light before spraying with glyphosate. The inhibition of phenylalanine synthesis by glyphosate is presumably only capable of blocking phytoalexin synthesis when the alternative supplies of phenylalanine from bean cotyledons and cellular storage are not available.

Banksia integrifolia appeared to be sensitive to BHBS when infected by leaf rusts (John Toth, pers. com.). Neighboring plants uninfected by rust showed no injury following BHBS.

Wallace and Bellinder (1995) found that the presence of a rust *Puccinia coronata* influenced the absorption and translocation of glyphosate.

13 Insect Herbivory

BHBS may increase insect herbivory by reducing the amount of vegetation available for consumption, concentrating herbivory on to the surviving plants, or by changing the predator defenses of surviving plants.

An elevated ratio of herbivores per quantity of remaining plant material may occur after BHBS. If herbivore mortality is initially unaffected by BHBS, and the quantity of living plant material decreases, the level of herbivory on the surviving vegetation is elevated.

The destruction of large amounts of plant biomass may change herbivore predator habitat and behaviour. A study of the predatory carabid beetle found no toxic or repellent effect of glyphosate in the field. Instead, the destruction of plant material provided a less favorable foraging habitat for the larger carabid beetles (Brust, 1990). Application of glyphosate has also been observed to inhibit the activity of entomopathogenic organisms (Vainio and Hokkanen, 1990). Reduction in the activity and incidence of predators and pathogens of herbivores following application of glyphosate may allow an increase in insect herbivore abundance.

The abundance of herbivores may be influenced by changes in plant defenses following application of glyphosate. Lydon and Duke (1989) have concluded that glyphosate caused production of increased levels of cinnamate-derived phenolic compounds in plants. These compounds can cause dramatic increases in stress associated chemicals. Elevated stress level in plants is generally associated with increased levels of herbivory.

The increased rates of growth of some plants following HBS and bitou removal may attract herbivores. Glyphosate-treated loblolly pines had greater growth rates and survival than controls but had greater tip moth damage in the first two years of glyphosate treatment (Ross et. al., 1990). The faster growing trees may have been more attractive hosts to the Pine tip moth. The destruction of bitou habitat by BHBS may result in the remaining vegetation becoming more prominent and attractive to herbivores (John Toth, pers. com.).

There are several examples of unexplained increases in the presence of herbivores that may be associated with BHBS. A 10% and 30% increase in herbivore damage of coastal wattle was noted by Fullerton (1991) following BHBS application of Roundup® and Brush-Off® respectively. A large eruption of a population of moth larvae on Tuckeroo *Cupaniopsis anacardioides* was observed after BHBS at Sandon Yuraygir National Park in 2001 (pers. obs.). The Large Coast Grasshopper *Valanga irregularis* was observed in large numbers feeding on *Acacia sophorae* in 1998 in Yuraygir National Park (Jeff Thomas pers. com.). The early instars of this insect occur in grassy habitat that is inland of bitou-infested areas (John Toth, pers. com.). BHBS is therefore unlikely to influence the initial eruption in grasshopper numbers but may influence the attractiveness of plants to herbivores.

3.3 Seedling Injury

There is a lack of data on seedling recruitment after BHBS, largely because very few seedlings have been observed (excluding post-fire environments and the constantly disturbed incipient dune). The lack of native seedling recruitment following BHBS may be influenced by a number of factors which are discussed below.

The coastal dune environment can be a difficult place for seedlings to grow. Major difficulties include nutrient deficiency, moisture stress, sand accretion, salt spray, herbivory (Maun, 1994), continued allelopathic suppression of germination and growth by bitou bush (Vranjic et. al. 2000), and litter effects (see below).

There is a "*prima facie*" case that direct glyphosate injury is greater for seedlings than for adult plants and has reduced seedling establishment for three reasons:

1. The physiological rates of seedlings are generally higher than of adult plants.
2. Seedlings have little dormant meristematic tissue from which recovery from herbicide can occur.
3. Most of the buds of seedlings are above ground and exposed to herbicide injury with little capacity for regeneration.

Despite this, it is difficult to assume that the continuing lack of seedlings after BHBS treatment is due to direct glyphosate injury. Other more persistent mechanisms, such as from indirect glyphosate injury, seem more likely to explain the apparent lack of germination and establishment.

The vulnerability of seedlings to direct glyphosate injury may actually be much less than predicted by the *prima facie* reasons given above because:

1) Seedlings are often sheltered from aerially deposited spray by various sheltering microhabitats (see Microhabitat Effect below).
2) Seedlings readily become water and nitrogen stressed, and may not be actively growing when exposed to glyphosate.
3) Cotyledons attached to seedlings can have stored reserves of amino acids that can be accessed if amino acid synthetic pathways are blocked by glyphosate.

Morash and Freedman (1989) studied the effects of glyphosate on the germination of Canadian forest species and found little evidence of any glyphosate associated injury. Only high application rates of glyphosate markedly reduced germination. Similar results were found in field trials in both sheltered and exposed microhabitats. Shipman and Prunty (1988) also found glyphosate had little effect on the germination rate or the seedling height of Red Oak Acorns. Earl (1990) reported that many native plants germinated in plots after boneseed had been sprayed with a 1:100 dilution of Roundup®, particularly following the first good rain.

Minimum tillage in agriculture is a system which applies herbicide at the same time as a crop is sown and provides a useful comparison to seedling establishment after BHBS. Torstensson (1992) concluded that damage to newly sown minimum tillage crops can be explained by the following indirect factors: 1) phytotoxic substances produced during the decomposition of large amounts of herbicide-killed plant residues, 2) colonization of herbicide-treated plant material by pathogenic fungi and 3) competition for soil nutrients between the decomposing plant material and the seedlings.

Although seedlings may be less vulnerable to herbicide injury than is presented in the “prima facie” case, it does not follow that seedlings are tolerant to glyphosate. In some species seedlings are very sensitive to herbicide injury and herbicide application can kill large numbers eg. *Acacia soporae*.

A) Direct Injury of Seedlings by Glyphosate

A number of factors alter the exposure of plants to glyphosate. These include the following:

1 Microhabitat Effect

Individual species respond differently to glyphosate application depending on the structure of the plant community in which they are growing (Marrs et. al., 1991). Seedlings are often sheltered from aerially applied herbicide by taller plants that intercept the applied herbicide and effectively reduce the rate of deposition on the underlying seedlings.

Spraying can be timed to coincide with the presence of a sheltering canopy. Oak seedlings in North America are protected by competing vegetation at a particular time of the year and spraying at this time has shifted the succession towards an oak dominated community (Thompson et. al., 1995). Deferring BHBS till bitou seedlings provide good spray shelter for native seedlings could minimize the impact of herbicide on native seedlings.

2 Water Stress

Plants experiencing water stress cannot maintain high rates of metabolic activity nor synthesize amino acids. Plants conserve water through stomate closure and by increasing the production of waxy cuticular layers. These factors may reduce injury from herbicide exposure (see Water Stress Section 3).

Water-stressed seedlings of red maple and loblolly pine showed less injury when sprayed with glyphosate than seedlings grown with non-limiting supplies of water (D’Anieri et. al., 1990).

Low water availability commonly occurs in bitou-infested areas in NSW and may reduce seedling sensitivity to BHBS. This may occur for several reasons: a) seedlings are shallow rooted and have small volumes of water available to roots, b) many bitou-infested substrates are very sandy and have low water holding capacity, and c) the winter period when BHBS is conducted is often dry, especially on the North Coast of NSW.

3 Nitrogen Stress

Low levels of soil nitrogen have been observed to reduce injury by glyphosate. Dickinson et. al. (1990) reported that oats *Avena sativa* were less sensitive to glyphosate when grown in soil with low available nitrogen than when soil nitrogen was adequate.

Available soil nitrogen levels may be lowered by microbial activity in the heavy litter layer following BHBS (see Litter Effect below). Seedlings that manage to establish in soils with heavy litter layers may be partially protected from direct herbicide injury by nitrogen stress. Sand dunes are very low in all available nutrients including nitrogen.

4 Seed Size and Reserves

Larger seeded species may be less susceptible to injury from glyphosate herbicides because they have larger stores of the amino acids phenylalanine, tryptophan and tyrosine.

Santos et. al. (2002) concluded that smaller starch reserves in the leaves of one species of *Commelina* resulted in greater susceptibility to glyphosate injury. Johal and Rahe (1990) suggested that bean seedling cotyledons (*Phaseolus vulgaris)* were an alternative source of phenylalanine. Seedlings were able to use this store of phenylalanine when synthesis was blocked by glyphosate.

B) Indirect Injury of Seedlings by Glyphosate

Seedling injury may be caused or enhanced by indirect effects of glyphosate. These effects include the following:

1 Litter Effects

Vranjic et. al. (2000) reported that the presence of bitou bush litter suppressed germination and growth of *Acacia sophorae* seedlings. This effect is likely to gradually diminish after BHBS. This has not been experimentally shown to be due to allelochemicals associated with bitou litter but may be occurring by several different mechanisms.

1) Allelopathy

Nevertheless large quantities of chemical are released from plants that are killed by the initial BHBS treatments. This creates a large flux of potential allellochemicals into the soil. It is speculated that the chemical environment of the litter is unlikely to be conducive to the establishment of seedlings.

2) Phytotoxic microbial products

It is well known that numerous microbial products act synergistically to reduce germination and growth. Build up of microbes antagonistic to the establishment of seedlings is expected in this litter and dying plant environment.

3) Nutrient deficiency

The microbial decomposition of great quantities of plant material following BHBS treatments creates a large microbial demand for plant macro- and micro-nutrients. The resulting high carbon to nitrogen ratio is particularly important. The coastal sand dune environment is very low in available nutrients, particularly for seedlings which have small rooting volumes (Jim Charley, pers. com.). Torstensson (1992) has suggested that decomposition of dense weeds following pre-sowing application of glyphosate can reduce the availability of nitrogen and result in crop failure even in fertile soils.

Kachi and Hirose (1983) performed a series of experiments growing test plants in coastal sand dune soils and suggested that nitrogen was more limiting than phosphorus or potassium in pot experiments despite total phosphorus being small. They suggested this was due to a lack of microbial activity which would otherwise release inorganic nitrogen from organic matter.

4) Physical suppression

Plant litter covering the ground shades the mineral soil surface and reduces the soil temperature. This creates an unfavorable environment for seedlings of many species.

The large litter layer of dead bitou plants prevents exposure of mineral soil after the first successful BHBS. Many native plant species in coastal heath and sclerophyll forest require bare soil for seedling establishment.

5) Glyphosate suppression of decomposition

Glyphosate may have variable effects on the microbes involved in the decomposition of above ground litter. Cellulose decomposing fungi have variable sensitivity to glyphosate (Wardle and Parkinson, 1990). The rate of cellulose decomposition varies according to the soil type, environmental factors and the soil microflora (Ismail et. al. 1995). Wardle et. al. (1994) studied the above ground decomposition of four glyphosate-treated pasture species. Decomposition of three species was delayed in the short term but accelerated for one species. It was considered unlikely that glyphosate would delay litter decomposition in the longer term.

Despite this potential effect of glyphosate on litter, it is unlikely BHBS would inhibit litter decomposition enough to elevate the litter layer to an extent which impacted on seedling establishment.

2 Seed Vigour and Viability

Shuma et. al. (1995) reported that Roundup® applied after anthesis of *Avena fatua* resulted in significant reduction in the number of seeds set, the viability of seeds and the vigour of seedlings. Similar application rates were used to those used in BHBS. The viability of *Avena fatua* seeds decreased more the closer the time of spraying was to anthesis.

Inhibition of pollen tube growth of *Nicotania sylvestris* by glyphosate and metsulfuron was observed *in vitro*. This only occurred if amino acids released from the pollen were first leached from the suspension (Grabe and Kisten, 1997).

BHBS application of glyphosate could reduce populations of winter flowering species by decreasing seed production and viability and seedling vigour. Native winter flowering plants that have been exposed to BHBS may not be suitable for seed collection because of reduced seed vigour and viability.

The following winter flowering species occur in foredune and incipient dune plant communities and have a high potential exposure to BHBS: *Banksia integrifolia* subsp. *integrifolia, Acianthus exiguus, Pterostylis nutans, Cyperus stradbrokensis, Ischaemum triticeum, Kennedia rubicunda, Vigna marina* and *Sophora tomentosa* subsp. *australis. Banksia integrifolia* is a prominent species of foredune plant communities in NSW (see Table 3). The possibility of reduced seed production, viability and vigour in this species following BHBS may have implications for the regeneration of coastal foredune communities.

3 Soil Fungi and Glyphosate Synergism

Pathogenic soil fungi can act synergistically with glyphosate to increase plant injury. (see above adult plant injury discussion.)

3.4 Cumulative Phytotoxic Effects

Cumulative herbicide injury can be caused to sensitive taxa when successive applications are made to plants or populations of plants before they have recovered from the previous herbicide injury. BHBS is expected to be repeated six times to remediate many established bitou infestations. Treatments occur every 12 to 24 months for the first few years, extending to every second or third year, after which ground spraying may be required in patches.

Cumulative herbicide plant injury caused by successive BHBS is most likely when treatment intervals are very short. An assessment of the extent that herbicide injury has accumulated in BHBS projects cannot be made at this early stage because of problems with the reliability of information obtained from monitoring of herbicide injury (see 3.9 Monitoring of Plant Injury), and the lack of monitoring of recovery periods for sensitive species. As a result only a general discussion of this important topic can be conducted.

The half-life of glyphosate within plants from a single application is typically from 10.4 to 26.6 days in foliage and litter (Newton et. al., 1984). Accumulation of glyphosate within the plant may occur following successive treatments (Foy and Jain, 1986). Accumulation of glyphosate within sprayed plants may occur when ground spraying closely follows BHBS as does sometimes occur when there is a poor kill from BHBS. Elevated sensitivity and direct herbicide injury is expected when the level of herbicide in the plant is accumulated.

Re-treatment Interval

Motooka and Nagai (1990) found that zucchinis could only tolerate half the application rate of glyphosate when retreated within a week than when they were only given a single treatment.

A short re-treatment interval can increase native plant impacts by two different mechanisms:

a). minimal time is allowed for recovery from herbicide impacts and

b). less compensatory growth has occurred and reduces the development of herbicide sheltered microhabitat (see Microhabitat Effect, above).

Deferment of repeat treatments (either ground spraying or by HBS) should be considered when native plants have not recovered to pre-spraying levels of health.

Monitoring Recovery Periods

Monitoring plant injury and recovery periods allows the identification of potential for loss of species from a site and allows the use of abatement measures (see 3.9.4 for Abatement Options). The loss of species from a site due to accumulated herbicide injury is difficult to ascertain without adequate monitoring which is aimed at following injury through various treatments.

Few BHBS project areas have been treated with six or more HBS treatments, and monitoring of plant injury has not been adequate to measure the magnitude of herbicide injury nor the period of time required for the plant to fully recover. If the period to recover is greater than the re-treatment interval, accumulated impacts will occur.

3.5 Seasonal Variations in Plant Sensitivity

The ability of BHBS to selectively kill bitou and minimally damage native plants is due to the coincidence of low native plant sensitivity and peak sensitivity of bitou bush to BHBS in winter (Toth, etal, 1991, 1993 and 1996). The seasonal change in sensitivity of most native species in bitou infested areas has not been documented. Development of a functional classification according to maximum tolerance of herbicide application may help to identify a number of different application windows (see 3.9.4 Shifts in the BHBS "Application Window").

Bitou-infested sites containing winter active ground orchids should undergo BHBS when the above ground orchid parts have died off in early spring. Also peak bitou flowering can be changeable, and appears to be brought forward after fire to early autumn (pers. obs). A simple model based on temperature and day length may be applicable.

Several different patterns of seasonal variation in glyphosate sensitivity are expected among native species in bitou infested areas. Neal and Skroch (1985) determined the seasonal change in sensitivity of 13 woody ornamental species to glyphosate. Plants were then classified according to when their maximum sensitivity occurred. No application was made during their very cold winter. Paley and Radosevich (1983) tested seven species of 5-year-old trees and a shrub. Both studies found most species exhibited high tolerance to glyphosate in autumn, a time when plants have minimum growth and relatively high water stress. Pieterse and McDermott (1994) found that *Acacia longifolia* was more tolerant of glyphosate in late summer than in other seasons.

Bell et. al. (2000) found that the summer growing blue-joint grass was most sensitive to glyphosate in autumn, when flowering had ending and aboveground senescence was beginning. Regehr and Frey (1988) reported that autumn treatment of Japanese honeysuckle *Lonicera japonica* minimizes off-target damage of hardwood species that have completed their autumn defoliation while the Japanese honeysuckle still retains its leaves.

The geography of the bitou-infested landscape may influence the seasonal changes in sensitivity of native plants to glyphosate. The following factors may be important:

1) Northerly aspects

Higher temperatures are experienced on north facing slopes. The sensitivity of spring growing species may be earlier at sites with higher solar radiation.

2) Low lying areas

Native plants in low lying areas will experience lower temperatures, which may delay spring growth and result in cold injury during winter. The result may be reduced sensitivity to herbicide in lower lying areas.

Many geophytic orchids in bitou infested areas are active in winter but are not physiologically active above ground during the remainder of the year. Aerial shoots of many ground orchids wither and die in the heat of spring. Thus BHBS would have less impact on ground orchids in spring than in winter. BHBS in spring may be feasible if the sensitivity of native plants which co-occur with ground orchids was also low at this time, however these seasonal changes in sensitivity have yet to be determined (see Further Research).

Latitudinal Variation

It is possible that latitudinal variations in phytotoxic effects are due to variations in the depth of winter "dormancy" in NSW. Relative humidity and temperature vary significantly with latitude along the NSW coast (see 2.1 Factors Reducing Effectiveness). The northern coast of NSW has milder and shorter winters than the south coast of NSW. Depth of winter dormancy for a particular species is expected to be greater and extend for a longer period of time in southern regions. A possible example of latitudinal variation in phytotoxic injury after BHBS is blue fan flower *Scaveola calendulacea*. Kohler and Whelan (1993) reported that *S. calendulacea* was unaffected by BHBS on the south coast, while Schroder (1999) observed some death of plants at Myall Lakes National Park, and in Yuraygir National Park, *S. calendulacea* has been killed (pers. obs).

Intraspecific variation in response to temperature is possible and further complicates this possible effect. This can be tested using the same genotype under controlled conditions.

If this latitudinal effect is present and widespread, it should influence the planning of BHBS operations. There may be less flexibility in the timing of BHBS for North Coast regions than areas further south. Suitable application dates in the south may not be acceptable dates in the north of the state.

3.6 Post BHBS Plant Communities

The impact of bitou removal by BHBS on the composition of native plant communities is poorly understood.

At least six BHBS treatments in an area may be required in established bitou infestations. This can strongly determine the composition of the post BHBS plant community through responses to herbicide injury. Despite this, BHBS also creates a major change in the environment of native species simply by removing bitou and the subsequent growth of different plants. Environmental changes associated with post bitou habitats will benefit some species and be unfavorable to others. The long term loss of species from a BHBS area may result from these changes and not be directly related to the various forms of herbicide injury.

The major plant response observed after BHBS treatments has been the growth of previously established individual plants by expansion in size and vegetative reproduction (pers. obs.). Thus, the composition of the plant community before BHBS had a major influence on the post BHBS community (pers. obs.). Seed germination and seedling growth has commonly been poor after BHBS treatments (see Seedling Injury, above) although there may be some significant exceptions, such as after fire and in incipient dune communities.

The incidence of fire before or after BHBS is likely to change the composition of post BHBS plant communities significantly. Disturbance by hot fires of bitou infested vegetation kills many individual plants and reduces the ability of many frontal dune species to reproduce vegetatively, eg. *Banksia integrifolia* (Benwell 2002), leading to increased domination by bitou thickets in the burnt areas. The interaction of fire with BHBS is not discussed further in this review.

The greatest threat of weed reinvasion after BHBS is where weed species are well established before treatment. This is commonly observed in previously disturbed sites such as areas that have been sand mined.

The composition of the plant community after herbicide treatment is determined by both the character of the pre-spraying plant community and pattern of herbicide impacts. Ralphs (1995) reported that long-term use of different herbicides in rangelands in the USA created different weed assemblages. Glyphosate plots became dominated by weedy annuals and rhizomatous perennial forbs such as *Chenopodium album, Senecio crasulus* and *Taraxicum oficinale*. Metsulfuron plots became dominated by grasses. Similarly Lund and Andersen (1993) found that glyphosate treatment in a Norwegian forest caused a temporary shift from the original perennial flora towards a flora dominated by annual species. Glyphosate selected strongly for certain germinating species and inhibited others.

As the magnitude of the herbicide impact on the sprayed vegetation increases, there is an increased opportunity for shorter lived weeds to grow and create seed banks before any further herbicide treatments occur. The promotion of annual weed assemblages after BHBS has been observed by several BHBS managers eg. M. Clarke at North Sandon.

This appears to have occurred in sites with few other perennial species besides bitou. BHBS can create a large unoccupied ecological niche for annual weedy species. This effect may be limited to highly disturbed sites and appears to be of minor importance as most serious weeds in bitou infested areas are perennials.

Of greater significance is where perennial weeds have established at a site before BHBS. Weeds that are minimally injured from the herbicide or have short recovery periods can rapidly use resources that become available after death and injury of other plants. Prior and Armstrong (2001) observed that ground spraying of glyphosate on madeira vine *Anredera cordifolia* killed most of the surrounding non-target plants and may have favored re-invasion of madeira vine from glyphosate tolerant subterranean tubers.

Parallel Weed Infestations

BHBS cannot be considered effective if the removal of bitou bush by BHBS allows other weeds to thrive or invade. Bitou infested environments can contain a large number of exotic species. Batianoff and Franks (1998) found that 59% of the vascular flora found in the foredune in SE Queensland were exotic.

Invasion by kikuyu *Pennisetum clandestinum** was considered a greater threat to the persistence of the endangered species *Pimelea spicata* than bitou bush on the south coast of NSW (Matarcyzk, pers. com). Controlling bitou in this situation may accellerate the impact of kikuyu on this species.

The worst weed species which grow in association with bitou include *Gloriosa superba*, Asparagus aethiopicus*, Ipomoea cairica*, Lantana camara*, Acacia saligna*, *Senna pendula**, *Euphorbia cyathophora*, Hydrocotyle bonariensis** and *Coprosma repens**. Many of these species appear to be able to persist in dense bitou thickets often under dense shade, sometimes with seedling recruitment occurring in the patches between the bitou. Post sand mining areas are often the most difficult to remediate due to the large number of previously established weedy species and few established natives.

Glory lily *Gloriosa superba* is uninjured by BHBS as it has died back to subterranean rhizomes during BHBS application period. Glory lily has shown rapid vegetative growth in the 12 months following initial aerial spraying at several sites on the North Coast of NSW (pers.obs).

Lantana has been identified as a major problem at Wyong (Darren Williams pers. com.) and Shoalhaven (Kerry Thompson, pers. com.) following BHBS.

Acacia saligna has been observed to be highly sensitive to BHBS with glyphosate particularly in the seedling stage (pers.obs. North Sandon). BHBS is a highly effective treatment for parallel infestation of *Acacia saligna* and bitou.

There are a potentially large number of exotic grass weeds that may be encouraged by BHBS application of MSM. The greatest risk of parallel infestations of exotic weedy grasses occurs along roads and in ex-sand mining areas.

The following exotic grasses have been recorded in bitou-infested areas:

Ammophila arenaria, Andropogon virginicus*, Axonopus fissifolius*, Briza maxima*, Briza minor*, Cenchrus echinatus*, Chloris gayana*, Digitaria sanguinalis*, Ephrata erecta*, Eleusine indica*, Eragrostis brownii*, Melinis repens*, Paspalum urvillei*, Pennisetum clandestinum*, Rostraria cristata*, Sacciolepis indica*, Sporobolus africanus*, Sporobolus fertilis*, Sporobolus indicus.*

There have been no general exotic plant or weed surveys conducted prior to BHBS projects in NSW.

Holt et. al. (1984) found that, 8 years after the application of a number of herbicides, only glyphosate had continued to suppress a number of exotic weed species.

Coastal Wattle Dominance

The establishment of dense stands of coastal wattle *Acacia sophorae* is a principle remediation tool that is integrated with BHBS to control and eliminate bitou. Barron and Dalton (1996) observed that significantly more *Acacia sophorae* seedlings established when weed control occurred during summer.

The coastal wattle is very successful in a post BHBS environment, despite seedlings being highly sensitive to glyphosate, because established plants are highly tolerant to BHBS and have an enormous capacity to increase in size. Growth of coastal wattle can take a number of forms including tall thickets on the swales, low canopies on the windward edge of the foredune and low creeping bushes on the incipient dune which often form lateral stems that run and root along the ground. Also coastal wattle has similar adaptive strategies to bitou bush and can readily utilize the areas formerly occupied by bitou. Both species have dense shrubby habits and deep root systems that can colonize and trap mobile sand.

Coastal wattle is capable of forming dense mono-specific stands. These can reduce biodiversity of plant communities, especially grassland (Carr, 2001). *Acacia sophorae* is estimated to have invaded 10 000ha of native vegetation in southwestern Victoria (McMahon et. al., 1994). *Acacia sophorae* reduces the growth of surrounding plants by several different mechanisms, and this facilitates the expansion of *Acacia* thickets. The area of *Acacia sophorae* greatly expanded at Wilson's Promontory NP following the restriction of cattle that had previously grazed coastal wattle (Bennett, 1994). Cessation of burning and grazing were similarly associated with the invasion of *Acacia sophorae* into native grasslands at Eurobodalla National Park (Costello et. al., 2000). Floristic diversity continued to reduce after coastal wattle became established. Anecdotal evidence supports the possibility that thickets of coastal wattle established after removal of bitou by BHBS may be long lived and limit biodiversity levels to below that found in pre-bitou-infested communities (see Recommendation 3).

Models of Community Succession after BHBS

There are various opinions on the significance of the impact *Acacia sophorae* colonization has on the composition of plant communities following BHBS. This appears to be based on different perceptions of the mechanisms of plant community succession following BHBS. Two different models of plant succession are presented here to assist in understanding these different viewpoints.

A) Initial dominance by coastal wattle after BHBS delays the establishment of key native species. Continued regeneration of coastal wattle thickets after BHBS is limited and the death of coastal wattle trees due to old age and disturbance creates adequate opportunity for many native species to establish after BHBS operations.

B) Initial dominance by coastal wattle after BHBS effectively removes the opportunity for many species to establish at a site eg. *Banksia integrifolia*. Coastal wattle persists by seedling recruitment without the need for major disturbance. This ability may allow the continued dominance by coastal wattle thickets and the exclusion of many significant native plant species and vegetation formations.

Individuals of *Acacia sophorae* may be long lived (this has not been documented) however longevity depends on the conditions under which individual coastal wattles grow.

Coastal wattle continually produces large seed crops that have little dormancy and do not require fire for germination (McMahon et. al., 1994). Seeds can readily germinate and are present in large numbers throughout the year to occupy suitable sites. Under suitable conditions *Acacia sophorae* dominance may persist after BHBS, excluding other native species. To date, there is little data on the persistence of coastal wattle along the NSW coast.

A key species in bitou-infested sand dune environments is *Banksia integrifolia*. The recruitment of large numbers of *Banksia integrifolia* seedlings after BHBS has not been reported, and the restoration of gallery forests of coastal Banksia in foredunes has not been initiated. On the Mornington Peninsula, Victoria, extremely high *Banksia integrifolia* seedling mortality was reported to be due to browsing and summer soil desiccation. Stand replacement was concluded to be unlikely (Price and Morgan, 2003).

Poor establishment of species such as *Banksia integrifolia* and *Cupaniopsis anacardioides* in well-established Coastal wattle thickets (pers. obs.) may be due to suppression of seedlings by low light conditions. Coastal banksia seedlings require low, open habitat with exposed mineral soil (Alex Floyd, pers. com.). *Banksia integrifolia* growing in rainforest areas were originally recruited in open forest prior to invasion of rainforest and do not recruit seedlings at these sites (Alex Floyd, pers. com.). Well established *Banksia integrifolia* seedlings have been observed in Yuraygir and Bundjalung national parks (pers. obs.) in coastal sand dune communities with stable low vegetation dominated by *Zoysia macrantha* and *Carex pumila*. Similar densities of seedlings were observed in lightly vegetated open grassy woodland, often under extensive cattle grazing on the NSW North Coast. Plant communities with sparse grassy vegetation may be more conducive to *Banksia integrifolia* seedling establishment (pers. obs.).

Species that are adapted to constantly changing environments often do not have highly specialized adaptations to particular environmental stresses (Fenner, 1987). *Banksia integrifolia* may be regarded as not highly specialized, as seed release does not require fire (non-bradysporous). This lack of adaptation to catastrophic disturbance such as fire, may also apply after BHBS. The small seed of *Banksia integrifolia* is not stored in cones but is released over time as the fruit becomes ripe (Price and Morgan, 2003).

The presence of live parent trees of *Banksia integrifolia* enables continual seed dispersal which facilitates the species persistence in an area. Large banksia trees are also frequently killed by fire. A common feature of bitou-infested areas is the poor health of mature coastal banksia trees, which have low seed output. Poor health of coastal banksia is likely to contribute to the poor establishment of *Banksia integrifolia* seedlings after BHBS.

Alternatives to coastal wattle suppression of bitou bush

There appears to have been little discussion concerning alternatives to coastal wattle suppression of bitou bush after BHBS.

Minimizing the impacts of BHBS on remnant native vegetation will assist, but some major changes to the BHBS technique may be required to favour the growth and dominance of other suitable native species or plant taxa which are capable of suppression of bitou bush.

Promotion of native grasses rather that coastal wattle may be of assistance for key species such as *Banksia integrifolia*. This may be achievable using seeding techniques or by the use of metsulfuron herbicides in BHBS.

3.7 Erosion after BHBS

BHBS reduces vegetative binding of substrate by killing large numbers of bitou plants. This can increase the rate of erosion, causing damage to the coastal landscape and decreasing ecological integrity. Two main issues concerning erosion and BHBS are apparent. These are 1) natural sand dune dynamics have been altered by bitou invasion and subsequently by removal of bitou by BHBS, and 2) mass wasting or slumping occurs after bitou removal.

Wind Erosion and Natural Sand Dune Dynamics

Invasion of areas by aggressive weeds such as bitou bush and marram grass cause extreme states of sand binding, possibly well above the pre-weed invasion level. Bitou bush removal in this situation has the potential to significantly increase wind and water erosion of sand dunes.

In New Zealand, the domination of sand dunes in may areas by marram grass *Ammophila arenaria* has resulted in fixed dune structures with little freely moving sand. Actively moving sand is important for the maintenance of populations of several plant species, including golden sand sedge *Desmoshoenus spiralis* (NZ DOC, 2003). This species dominates in low, undulating and hummocky dune fields which loosely retain sand and form depressions close to the water table that are sheltered moist sites containing a diversity of plants, including golden sand sedge. Golden sand sedge was once widespread in New Zealand but is now rare and very restricted geographically. A similar situation has been described at Monterey Bay in California, where marram grass infestations have produced a steep, unstable foredune face (National Oceanic Service 2003). Dunes dominated by native New Zealand plants are lower in height and contain larger quantities of actively moving wind-blown sand (Brown and McLachlan, 1993). Dune stabilization using marram grass in Australia results in lower plant species diversity and significant differences in species composition (Webb et. al., 2000).

A similar situation exists in subtropical coasts of eastern Australia. For example, Australian Spinifex produces less cover and allows much more sand movement (Sauer, 1985). As a result the dunes that are created are much more complex in shape and vegetation than comparable coasts on other continents. Two significant species in bitou-infested areas of NSW, *Stackhousia spathulata* and *Chamaesyce psammogeton*, may also require a loose sand environment similar to Golden sand sedge and are absent from areas that are well vegetated (pers. obs). According to herbarium records since 1946, both species are now much rarer (Heyligers, 1998). *Stackhousia spathulata* has virtually disappeared from the coast between Coffs Harbour and Forster and has become rare in the Sydney area. *Chamaesyce psammogeton* is locally extinct in the Sydney area (Heyligers1998). Stabilization of sand by bitou bush on sand dunes has possibly contributed to the decline of these species. Both species have been recently located in BHBS project areas at Yuraygir and Bundjalung National Parks. Increased sand movement following BHBS and the death of bitou plants may have either assisted their survival in local populations or facilitated re-establishment of populations of these species. The growth of the key sand dune species, *Spinifex sericeus,* is vigorously promoted by sand burial. This only occurs along dynamic sections of foredune (Maze and Whalley, 1992).

Blowouts are common in coastal dune environments and are expected to increase in occurrence and size following BHBS, especially where 1) beaches are receding and foredunes have eroded, 2) wave and wind energy is high or 3) where vegetation is sparse, particularly after long dry periods (Hesp, 2002).

Slumping or Mass Wasting

A common concern among local residents has been that removal of bitou bush by aerial spraying causes unacceptable levels of erosion, particularly when seas are heavy. Mass wasting or slumping of the seaward edge of the foredune or headland area is a natural process and is often facilitated by water, which lubricates blocks of sand or soil to slip down a slope, or by erosion at the foot of the slope.

The seaward slope of foredunes is a site for large-scale erosion and release of sand onto the beach. The frequency of slumping may be increased after BHBS because bitou bush no longer traps large amounts of sand in the foredune, often on very steep slopes.

Bitou bush infestations on the landward side of a sandy beach can very effectively trap sand blown from the beach by the wind, causing the dune to grow and advance towards the sea. Increasingly large volumes of sand can be stored in the frontal dunes in this way.

During periods of heavy wave action, beach sand is moved to offshore areas and the beach moves progressively landward. Eventually this landward movement of the beach reaches the frontal dune. Sand is then supplied to the beach by mass wasting of the frontal dunes, and slowing down the rate at which the beach moves landward. The larger the frontal dune, the greater is its ability to continue replacement of sand to the beach during periods of heavy wave erosion.

Destroying a large proportion of the vegetation in sand dunes by BHBS increases the chance that the wind can blow the sand away, thus increasing the vulnerability of the coast to erosion. Minimizing the herbicide injury to native plants by BHBS can decrease the loss of vegetative binding of sand and soil.

Headlands are another site for accelerated erosion following BHBS; for example, in Crowdy Bay NP a large area of headland that was aerially sprayed for bitou bush has slumped into the sea (Mike Dodson, pers. com.).

3.8 Herbicide Drift

Drift occurs with all herbicide spraying. Herbicide drift, or the application of the equivalent low dose of glyphosate, have been shown to adversely affect herbicide sensitive plant species. Drift therefore has the potential to influence the composition and structure of certain vegetation communities. These adverse impacts can be minimized using drift minimization approaches and by developing a greater understanding of the phenomenon of spray drift.

The level of spray drift that is occurring in BHBS is undetermined. There have been no reliable monitoring experiments attempting to quantify drift from BHBS operations. Nor has any study been conducted into the impacts of low dosage rates of glyphosate on any plant species which occur in the vicinity of bitou infested areas. It has been assumed that species that have been observed to be sensitive to glyphosate at nominal BHBS rates are likely to be effected by low dosage drift situations. There has been no attempt to test or monitor likely herbicide sensitive plant species that do not occur outside bitou infested sites for tolerance /sensitivity to application of low doses of herbicide (see Recommendations 4 & 7).

There are a number of factors that suggest herbicide drift should be quantified in current BHBS operations in NSW, and includes the following:

1) Bitou-infested coastal environments are windy locations. Excessive wind conditions are frequent on the coast, and this constraint may represent a major restriction on when operations are permitted.
2) Significant areas of herbaceous and wetland habitats adjoin bitou infested areas in NSW. These are likely to contain plant species that are sensitive to low doses of herbicide.
3) BHBS application areas are often very narrow. The large perimeter to application area ratios means that displaced spray droplets can readily move into exclusion and buffer zones.
4) Major temperature inversions occur along coastal NSW in winter. Calm mornings in winter generally result in the development of thermal inversions. Thermal inversions capture glyphosate in the still layer above the ground and allow it to be blown elsewhere. Approximately 10% of mornings were recorded as "calm" at 9am for a representative sample of NSW coast (see Figure 5 and 6). There is a common misconception among BHBS participants that calm mornings are ideal for HBS.
5) No routine monitoring for plant injury is conducted outside the application areas.
6) No deposition studies have been conducted to characterize the on-ground distribution of HBS herbicide.
7) Drift minimization nozzles are currently not being used in BHBS operations.
8) Currently no NPWS staff or contractors have been trained in drift minimization. Drift minimization is routinely left up to the contractor. Relevant training is available.
9) There is little awareness among BHBS managers and operators of the scope for impacts of drift on native plants.

The standard application rate of glyphosate as Roundup® by BHBS is 2L/ha or 720g of glyphosate/ha. A low dosage of glyphosate is considered in this review to be below 720g.a.i./ha.

The deposition of glyphosate involved in drift from BHBS is expected to decrease as some function of distance from the target area.

Lethal impacts

Glyphosate is lethal to many herbaceous plant species at levels below 720g.a.i./ha. Roundup® is routinely used to selectively control herbaceous plants at 500g glyphosate/ha (Whitson and Koch, 1998). The minimum application rate of herbicide that is lethal for a species is of prime importance and varies widely. Application rates that create non-lethal forms of herbicide injury may have less ecological significance as the plant may subsequently recover. However data on the recovery periods of plants injured in BHBS has not been currently collected (see Plant Sensitivity below). Cumulative injury could occur at lower than expected dose rates in species which have not recovered fully before re-spraying.

Sub-Lethal Impacts

Glyphosate-sensitive plants have been reported to be non-lethally injured at very low deposition rates of glyphosate. Low dosage effects are capable of changing the vegetation structure when both glyphosate sensitive and tolerant plant species are present. Stasiak etal (1991) reported a sub-lethal reduction in growth and stem length of pin cherry *Prunus pensylvanica* and trembling aspen *Populus tremuloides* occurred when glyphosate was applied at 40g.a.i./ha. Growth was still depressed 2 years after application. Stasiak et. al. (1992) reported sublethal impacts of glyphosate applied at 10.5 to 250g.a.i./ha on birch seedlings *Betula papyrifera*. They also found major metabolic changes at very low rates of glyphosate application. Photosynthetic activity was depressed. Similarly a 10-fold increase in shikimic acid levels occurred when 25g glyphosate/ha was applied to birch seedlings. Levels remained elevated for many months.

Shikimic acid and other secondary compounds can build up within a plant exposed to glyphosate without visible signs of plant damage (Lydon and Duke, 1989). Glyphosate directly affects specific biosynthetic steps of certain secondary compounds, creating a build up of high levels of shikimic acid, benzoic acid, benzoic acid derivatives and phenolic compounds. Secondary plant compounds are involved in metabolic roles and various exogenous interactions, such as allelopathy between plants, plant/insect interactions and plant disease resistance.

Other examples of known sub-lethal impacts from low doses of glyphosate include the following:

- Tomato plants sprayed with 60 and 100g.a.i./ha of glyphosate in the pre-bloom stage developed moderate to severe foliar injury (Gilreath, 2001). This is 8.3 and 13.8% of the standard application rate used in BHBS. Tomatoes are known to have a high level of sensitivity to herbicide (Gilreath, 2001)
- Two woodland species *Lamiastrum galaeobdoblon* and *Primula vulgaris* were sub-lethally impacted by the application of 108g of glyphosate/ha (Dixon et. al., 2002).
- The highest application rate at which no visible injury occurred from a single application of glyphosate to zucchinis was calculated at 200g.a.i./ha (Mootooka and Nagai, 1990).

Dual Liability of Consequences of Drift

Everyone involved in decision-making for the use of a pesticide is responsible for ensuring proper use, and they may also share the liability if the pesticide is misused under the NSW Pesticide Act, 1999; thus HBS contractors and project organizers are both legally responsible for the minimization of drift. Section 11 of the Pesticides Act, 1999, determines that the use of a pesticide should not harm non-target plants or animals.

The common law principle of vicarious liability also applies. This means that in cases where the person applying the pesticide is an employee of another person, charges can be laid against the employer, as well as, or instead of, the employee.

NSW EPA website advises the following for proper use of pesticides:

Carefully follow the NRA instructions on the label or permit for the correct use, storage and disposal of the pesticide. Preparation for each application should be thorough and completed before using the pesticide. All reasonable actions should be taken to ensure that non-target impacts are avoided. These include the following:

1. Make sure that the right chemical for the job has been selected.
2. **Spray in suitable weather conditions so that spray does not drift outside the target area**.
3. Ensure that spraying does not take place if people are likely to be downwind of an application and exposed to the spray.
4. Provide adequate buffer areas between the application area and dwellings or **sensitive areas**.
5. **Provide adequate instructions and training to employees before application is carried out**.

6. **Assess potential risks for harm before application and take steps to minimize risks**.
7. **Use equipment for the job that minimizes or prevents non-target impacts**.
8. Ensure that the equipment used is well maintained and calibrated.
9. **Obtain all relevant information from the landowner about surrounding sensitive or susceptible areas**.

The level of spray drift can be reduced by a greater understanding in the three following areas:
1. preplanning of exclusion areas,
2. suitable environmental conditions for spraying and
3. aircraft operations and equipment settings.

1) What is an Appropriate Buffer Width?

Exclusion areas can reduce the impacts of BHBS on sensitive areas. There are many reasons why areas may need to be excluded, such as being near houses or areas of BHBS sensitive or significant plants.

An extensive review of the literature of aerial spraying exclusion distances has limited value for this review. Many drift deposition studies have little applicability to BHBS because they use different equipment and are often conducted at different heights above the ground than used in BHBS (Payne et. al., 1990).

The width of a buffer required to protect non-target seedlings from glyphosate applied by a tractor mounted spray unit was determined for a range of sensitive species in Britain by Marrs et. al.(1993). BHBS releases herbicide spray at heights much higher than tractor mounted spray units and is therefore expected to produce significantly more drift.

The drift produced by fixed wing and helicopter aircraft, ground applicators, concentrated air-blast machines and high and low pressure boom sprayers was investigated by Franks et. al. (1994). All except low-pressure boom spraying equipment resulted in measurable spray drift to 80m off-target with appreciable deposits up to 30-40m away. Serious drift was confined to 9m off-target.

The buffer width for most exclusion areas surrounding sensitive vegetation in BHBS is 20 to 30m. This level of precaution may not be appropriate for all situations. Study of the deposition characteristics of HBS in a range of situations would allow appropriate buffer widths to be determined.

2) Drift Minimizing Environmental Conditions

Wind Speed

Wind speed and direction are major factors in determining the size and eventual deposition of chemical drift. Average wind direction and speed were determined for mornings (9am) and afternoons (3pm) in June over a number of years in coastal NSW for a representative set of sites (Bureau of Meteorology). Wind data from the following sites were collated: Murwillumbah, Ballina, Coffs Harbour, Port Macquarie, Forster, Nora Head, Sydney Harbour, Kiama and Narooma. Wind speed and direction were unsuitable for BHBS 57% of mornings and 66% of afternoons if drift was to be minimized (see Figure 6 and Figure 7). There was no data available on the periods of time helicopters have been stood down in BHBS operations but the above data illustrate-that wind speed should be a major constraint.

Figure 6 Average Wind Speed and Direction at 9am along NSW Coast

Figure 7 Average Wind Speed and Direction at 3pm along NSW Coast

Suitable low drift wind conditions are likely when wind speed is not calm but less than 10km/h. This occurs along the NSW coast on approximately 40% of mornings and 37% of afternoons (see Figures 6 and 7). Higher wind speeds may not impede low drift spraying but for this discussion desirable HBS conditions are restricted to the above wind speed range. Nevertheless this data show that wind speed is often unfavourable to BHBS and if wind speed is not considered a constraint on BHBS, chemical drift is likely to result.

Undesirable spraying conditions are created during thermal inversion and are likely in winter on calm mornings (i.e. no wind). This situation occurs 9% of the time at 9am and 3% of the time at 3pm. No record of the duration of calm or other wind conditions was available from the Bureau of Meteorology, NSW. In discussions with BHBS managers, calm conditions were frequently mistakenly considered as ideal conditions for aerial spraying. No tests for the presence of thermal inversion have been undertaken during BHBS operations to my knowledge.

Wind direction

Many areas that are sensitive to herbicide drift from BHBS operations are located on the landward side of the bitou treatment area. Spraying when winds have a westerly component minimizes drift in these areas. Winds from the NW, W and SW and under 10km/h occur 36% of mornings and 14% of afternoons (see Figures 3 and 4 above).

3) Helicopter Operations and Equipment

Droplet size manipulation is a major tool in optimizing weed kill and can be manipulated to minimize chemical drift (Knoche, 1994). The influence of droplet size on the uptake of herbicide is greatest when low application rates are used as in BHBS (Prasad and Cadogan, 1992). Droplet size of spray has an important influence on herbicidal action. Small droplets of 150-450µm are more phytotoxic because of the greater number of droplets affecting each unit area and greater translocation rates. The routine manipulation of droplet size to minimize drift has not been conducted in BHBS as emphasis has been solely on increasing herbicide uptake.

Manipulation of droplet size can be used to minimize native plant impacts in two different ways.

1) Drift reduction can be achieved by using larger droplets that fall faster to the ground and reduce drift displacement, and

2) A particular canopy layer can be targeted by changing droplet size. Small droplets stay on the leaf surface they first fall on, while larger droplets are capable of penetrating through overhead canopies to the plants below (Feng et. al., 2003).

BHBS has traditionally used D6-46 nozzles, which produce relatively fine droplets compared to droplets produced by drift minimization nozzles. Wallens (1984) recommend that droplet sizes of 250-350µm be used to minimize Roundup ® drift in Australia. Further they recommend using water volumes greater than the 20L/ha that is currently used in BHBS. This would allow a greater number of larger droplets to be applied, creating comparable coverage to a smaller carrier volume but with smaller droplets. Droplet size is controlled by nozzle aperture and type and hydrostatic pressure. Spray droplets smaller than 300µm are slow to fall to the ground and are readily drifted from the intended application area by wind. Droplet size can be varied by:

1) changing nozzles: there are approximately 30 nozzles on a helicopter boom, and
2) changing hydrostatic pressure at which the herbicide is pumped.

The size of droplets emitted in BHBS operations has not been measured for the standard BHBS application equipment.

Payne et. al. (1990) compared glyphosate deposition by D8-46 'Thru-Valve Boom' and 'Microfoil boom' and found off-target deposits were highest from the D8-46 applicator and lowest from the 'Microfoil' applicator. A mathematical model constructed from the data suggested that a buffer width of 25m around shallow water bodies would greatly limit mortality of fish and aquatic invertebrates.

Low humidity and high temperature can cause excessive droplet evaporation and a shift to smaller droplet sizes that are prone to drift. This results in an increase in off-target deposition (Clarke and Blowes, 1984; see Low humidity in Section 3.)

3.9 Environmental Activity of Metsulfuron-methyl

The sulfonylurea herbicides have become popular partly because of their low application rates (10-40g.a.i./ha), and low mammalian toxicity (Sarmah et. al., 1998).

Brush-Off® is produced by Dupont and contains 60% of the active ingredient metsulfuron methyl (MSM or metsulfuron) as a soluble powder.

The herbicidal activity of MSM is due to the inhibition of the enzyme acetolactate synthase (ALS) that is involved in the synthesis of the 3 amino acids, leucine, isoleucine and valine and effectively stops cell division and growth. The ALS pathway does not occur in animals. Metsulfuron belongs to the sulfonylurea set of chemicals and the B group of herbicides. The chemicals in the B group all have a mechanism of herbicidal action which inhibits the ALS enzyme system and includes four sets of chemicals: sulfonylurea, imidazolinone, triazolopyrimidine and pyridinylthiobenzoate (Preston, 2000).

Resistance to the B group of herbicides has developed very quickly (Preston, 2000). MSM has a higher risk of developing resistance because it acts on both the recruitmen of seedlings as well as the established plant population (Preston, 2000).

Uptake of metsulfuron is through both leaves and roots. Uptake from the leaves is by unassisted diffusion and is reliant on a concentration gradient of the herbicide to transfer chemical into the leaf. Metsulfuron is translocated very rapidly from leaves to roots and vice versa. Rapid translocation to anabolic sites, particularly in the roots, allows effective control of bulbs and tubers.

All group B chemicals have herbicidal actions at low concentrations and can be effective at rates as low as 2g.a.i. /ha. MSM is applied typically to control broad-leaved weed species and some grasses in cereal crops at 4.5-8g/ha and in non-crop areas from 5.5-180g/ha. Brush-Off® has been applied to bitou bush at the rate of 30g Brush-Off®/ha (18g of Metsulfuron /ha) (Hayes and Blackmore, 1999).

MSM is described as rain fast for four hours (Dupont, 2003). The solubility of MSM in water is 1g/L (Dupont, 1992). The low water carrier volume of 30L used by BHBS and the low solubility of Brush-Off® restrict the application rate of Brush-Off ® to 30g.a.i./ha. Higher application rates can only be achieved by increasing the water carrier volume.

Metsulfuron has a much faster direct herbicidal action than glyphosate and begins to produce discoloration of bitou after 7 days. There is very little understanding of the indirect plant injury caused by MSM (see 3.3B Indirect Glyphosate Seedling Injury).

There are significant concerns about the effects of drift with these herbicides as they can cause damage to many plants at very low, concentrations.

1 Selectivity of MSM

Several factors that influence the selectivity of HBS application of herbicides have been discussed above for Roundup® and are common to Brush-Off®. They include the role of winter "dormancy", sclerophylly, microhabitat sheltering and the influence of droplet size in herbicide injury. Despite these factors many native sclerophyll species are unacceptably sensitive to HBS with several other herbicide products (see Table 2).

Brush-Off® has proven to have a suitably low level of injury to native plants in bitou infested areas (Toth, 1989; Mc Millan, 1989).

Brush-Off® is a broad-spectrum herbicide that is active against a lot of herbaceous species when applied at rates of 5–15g Brush-Off®/ha and is significantly lower than the rate of 30g /ha used in BHBS. Weed species controlled at this rate include Paterson's curse *Echium plantagineum*, Cape tulip *Homeria* spp., and dock *Rumex* spp. Woody species require higher rates for control eg. Bracken *Pteridium esculentum* 60g /ha, and blackberry *Rubus* spp. 160g /ha (Ken Bell, Dupont, Proserpine, pers. com.).

2 Low Dosage Effects

Information concerning low dosage impacts by MSM on non-target plants is not as well developed as for glyphosate and Roundup®. The application rate used in BHBS of 18g of MSM/ha is moderately high, and many herbaceous species are sensitive to MSM at very low application rates. The potential for damage to non-target plants inside and outside of the target area is much higher than with BHBS of glyphosate.

Sulfonylurea herbicides can cause increased levels of cinnamate-derived phenolic compounds (Lydon and Duke, 1989). These compounds may have important roles in plant defense and allelopathic effects. Monitoring of plants sprayed with MSM should include indirect plant injury (see discussion of Indirect Plant Injury for Roundup® above).

Some examples of sub-lethal impacts for low dosage of metsulfuron included the following:

- Application of 0.45g of MSM /ha onto foliage created foliar injury in several herbaceous wetland and terrestrial plant species (Boutin et. al., 2000). This application rate is 1.5% of the usual BHBS application rate. The seedling stage was found to be the most sensitive.
- Metsulfuron applied at the rate of 8g.a.i./ha was found to seriously damage *Lolium perenne* and *Trifolium repens* in New Zealand (Popay et. al., 1985).
- Pea plants can sustain some level of plant injury from MSM without a large reduction in yield (Al Khatib and Tamhane, 1999).

3 Root Uptake

The B group of herbicides are all potent inhibitors of root growth and are frequently used in pre- and post-emergence applications. A major difference between the herbicidal activity of glyphosate and MSM is the greater soil activity of MSM and its ready uptake by plant roots from soil solution.

MSM has been observed to cause obvious injuries to the root tips of maize *Zea mays* (Flaburiari and Kristen, 1996). Scanning electron microscopic observations of the root tip surfaces of maize indicated that the inhibition of slime secretions surrounding the roots had occurred at a MSM concentration of 1.5mg/L. It was suggested that growth retardation of seedlings is a consequence of early root tip injuries caused by herbicide residues in the soil.

Root injury caused by the application of MSM to soil containing maize *Zea mays* roots was investigated by Anderson (1985). Increasing soil water increased metsulfuron degradation in the sand at 24°C but not at 16°C, while in the loam, metsulfuron degradation was not affected by soil water level at either temperature (Anderson, 1985).

Metsulfuron retained on surface straw residue was washed off by simulated rainfall. The duration of metsulfuron bioactivity was increased in the retained washed straw residue (Anderson, 1985).

4 Residual Soil Activity

Metsulfuron is not adsorbed by cations and clay colloids and is only significantly adsorbed by soil organic matter. Soil organic matter, apart from living material, is concentrated in the upper few centimeters of the soil. Once metsulfuron has moved past the organic top layer of soil it remains in soil solution and is available for uptake by plant roots. The time period metsulfuron is active in the soil is dependent on:
1) amount of rain leaching to levels below the root zone,
2) level of soil microbial activity and
3) level of acid hydrolysis, which is rapid in acid soils (Sarmah et. al., 1998).

Residual soil activity of MSM is determined by the quantity that is adsorbed by organic matter and the rate at which MSM breaks down (mostly by acid hydrolysis) and is leached down the soil profile. MSM is not degraded by sunlight.

In one New Zealand study the duration of the phytotoxic effect varied from 1 to 9 weeks, depending on species and soil type (Rahman et. al., 1991).

Broad-leafed crop plants can be particularly susceptible to MSM herbicide injury. The recommended interval between spraying of MSM and crop planting is recommended at 22 months for sunflower, flax and corn, and 10 months for sorghum. It is not recommended for use in perennial pastures that contain lucerne or clover species as they are sensitive to MSM (DuPont, 1992).

The rate of breakdown of metsulfuron decreases as it moves deeper into the soil (Walker et. al., 1989). MSM can be mobile in soils when rates of decomposition and adsorption are low and the soil structure is porous and infiltrating water is available to move MSM through the soil profile.

a) Soil pH

Adsorption of MSM has been shown to increase at low soil pH (Samah et. al., 1998). Sorption of sulfonylureas is pH-dependent and has a strong negative correlation with pH (Sarmah et. al., 1998). Hydrolysis of the sulfonylureas is more rapid under acidic conditions (pH 4-7) (Pons and Barrisuo, 1998). The half-life of metsulfuron applied to an acidic soil with high organic matter content in one study ranged from 8 to 36 days (James et. al., 1995). In another study the half-life varied from five days in acidic soil to 69 days in alkaline soil (Pons and Barrisuo, 1998).

The pH of bitou-infested soils is expected to vary according to soil type. Seawater is alkaline. Siliceous soils are highly permeable and easily leached and become acidic relatively quickly. Coastal heath on the north coast of NSW is normally highly acidic, around pH 4.5 (David Morand, pers. com.). There are a few situations where soil pH is expected to be alkaline. Calcareous sand deposits, such as at Jervis Bay, are alkaline (John Toth, pers. com.). Humic gley soil, which is common around coastal estuaries, has a humic or peat based topsoil and gleyed clay based subsoil that is highly alkaline. Undisturbed gleyed soil is unlikely to be highly infested with bitou, as bitou prefers free draining soil (Vranjic, 2000). Incipient dunes contain recently deposited sand material and flotsam, including a large proportion of dead plant and animal material. This organic matter is expected to contribute humic acid and be slightly acidic.

b) Soil Texture

The presence of clay influences the persistence of MSM by changing the soil texture. Soils with high clay content have lower rates of leaching and retain MSM in the upper layers. In a Finnish field study using an onion bioassay, MSM persisted longer and leached to a greater depth in sandy soil than clay or organic soil (Junnila et. al., 1994). This study also found that the phytotoxicity of the dose of 12g.a.i./ha persisted for at least

1 month and usually for 1 year. Stimulation of plant growth in bioassays was common one to two years after application in the 5-15 and 15-25cm layers of the sandy soils.

MSM injury of sugar beet was found to be greater in plants growing in soils with impermeable or intermittently waterlogged subsoil (Nicholls and Evans, 1987). It was concluded that at drainage impeded sites, two possible mechanisms of MSM injury may occur: 1) by sugar beet tapping into MSM residues in the subsoil and 2) by capillary action moving MSM contaminated water from the waterlogged subsoil into the aerated soil above.

Organic matter content and soil pH largely influence movement of the sulfonylurea in soil. The reviewed data show that sulfonylureas have substantial leaching potential in the sandy alkaline soils of Australia. This is likely to result in increased persistence in alkaline subsoil lacking in organic matter and biological activity (Sarmah et. al., 1998).

c) Soil Organic Matter

Adsorption of MSM is largely by organic matter, hence soils with high organic matter content have lower levels of residual MSM (Walker et. al., 1989). The level of organic matter strongly influences the level of microbiological decomposition of MSM. The rate of degradation of MSM increases with soil microbial activity (Walker et. al., 1989).

The organic matter content of bitou-infested soils varies widely.

d) Season of Application

Phytotoxic soil residue levels of MSM were observed to persist slightly longer in autumn/winter than in spring/summer in New Zealand (Rahman et. al., 1991abs).

Winter BHBS applications of Brush-Off® are likely to have longer soil activity and possibly extend influence through winter, particularly on the southern coast of NSW.

5 Seedling Injury

Cumulative injury from annual treatments with MSM in alkaline soils is a strong possibility. The rate of leaching and the depth that MSM moves to in the soil profile influences the likelihood of seedling injury by MSM. In alkaline beach sands, MSM will be highly residual

3.10 Monitoring of Plant Injury

Monitoring for non-target plant injury makes two significant contributions to BHBS projects. Firstly, it increases our understanding of the current impacts of BHBS on native plants and builds our level of understanding, and secondly future impacts can be avoided using information from previous monitoring. Monitoring becomes a precautionary measure only in this second way where it is used to change future BHBS operations.

Monitoring programs are not a standard practice in current BHBS operations. Monitoring and precaution should be applied at higher levels than is currently practiced, given the current level of understanding of native plant responses to BHBS. The necessity for monitoring and precaution will lessen as the level of knowledge increases.

3. 8. 1 Methods of Monitoring

The use of plant monitoring techniques that are highly sensitive to the impacts of BHBS on native species is vital for the minimization of off-target impacts and the success of bitou remediation projects.

Current BHBS Monitoring

Toth (2002) has collated tolerance /susceptibility data for a large number of plant species following BHBS from various sources. The information gathered on plant responses to glyphosate has been considerable, with results for more than 200 species, and has provided very valuable instruction in the cautious development of BHBS (see Recommendation 5).

Despite this, there are several deficiencies with the data presented by Toth (2002). As a result there is a diminished confidence in the stated herbicide sensitivity for most species monitored during BHBS projects.

Deficiencies in BHBS monitoring to date include the following:

1) Visual assessment of plant burn has been used as the only indicator of herbicide injury (eg. Toth, 2001). There are other systemic symptoms of glyphosate injury that may be more important to plant health.
2) Results from various BHBS programs are not directly comparable when different herbicide formulations are used. Toth (2002) combined plant injury data from programs that have used different glyphosate formulations i.e. Roundup® and Roundup Biactive®. There have been few studies where herbicide injury resulting from spraying with Roundup® and Roundup Biactive® can be systematically compared. A limited set of photographs of plant injury comparing Roundup® and Roundup Biactive® was conducted by John Toth in 1997 at Conjola Beach. Seven native species were observed to have greater injury when sprayed with Roundup Biactive® compared with Roundup® This information has not been analyzed and described in any detail (see Recommendation 5).
3) Monitoring of BHBS is frequently only short term. Important information should be gleaned by monitoring until an effect is lost or the plant is dead. This can only be done with identified or labeled plants, and preferably where particular cohorts of plants are monitored over time. Data collated by Toth (2002) was collected at 2 and 6 months after spraying only.
4) Varying numbers of individuals have been observed in reported herbicide injury observations. Monitoring reports should include the number of individuals that were monitored as it is often difficult to get reasonable sample sizes, and results may not be statistically significant. It can be difficult to locate more than three independent sites for many species in bitou-infested habitats.
5) Results collated from different operators, who may use different methods of assessment of plant injury, are hard to compare.
6) Classification of herbicide injury should be differentiated enough to allow scrutiny of the different types of injury. For example, Toth (2002) divided foliage burn into only 3 classes: no effect, 0-25% foliage burn and >25% foliage burn.
7) Many projects have not conducted any monitoring of plant health prior to BHBS. Interpretation of the data from these studies is problematic (Kholer et. al., 1995, 1996 and 1998; Floyd, 1998). The impact of conflating factors, such as salt damage, can not be reasonably excluded if prespraying monitoring has not been performed.
8) Observations of plant sensitivity to BHBS have combined data from both single applications and repeat applications of herbicide. Individuals are expected to be more susceptible to repeat treatments due to accumulated injury.

Canopy Estimates

Canopy estimates are a useful method of assessing the photosynthetic area of different species in a plant community. Estimating canopy cover is problematic when there are a number of overlapping levels of leaf canopies. The use of plant labeling in combination with visual estimates of plant canopy cover may be of use.

Visual estimates of plants canopy cover before and after spraying, without identification of individual plants, is an inaccurate method of assessing herbicide injury because dead individuals will be readily overlooked, particularly when the plant takes a prolonged period to die.

Plant labeling enables the monitoring of individual plants. This allows a closer identification of lethal herbicide effects. Without labeling the fate of an individual is readily lost among the changing vegetation.

Leaf Counts

An important chronic response of plants to systemic damage, such as is caused by glyphosate, is to drop (abscise) leaves. The degree of burn on leaves does not appear to accurately indicate the magnitude of glyphosate herbicide injury when translocation of glyphosate is a significant element in herbicide injury. Plants with severe foliage burn are often less likely to be lethally effected than a plant that has low leaf burn but high absorption and translocation of glyphosate.

Leaf counts of a small sample of a plant is a labor intensive method which allows a close monitoring of the general health status of individual plants. Other methods of estimating leaf areas photographically are also time consuming.

Exposure to Herbicide

Monitoring the level of exposure of an individual plant is difficult and can be most readily achieved in an experimental situation (see Recommendation 7). Estimation of the level of deposition onto a particular leaf canopy can be achieved in BHBS operations by the visual estimation of canopy cover above an observed plant. This may allow a more reliable comparison of herbicide injury responses under different levels of exposure. Monitoring of BHBS plant injury has never included the rates of herbicide deposited on non-target plants. This could be conducted in only the most systematic of monitoring but would provide invaluable quantification of plant injury within and outside the target area.

Recovery Periods

A valuable operational measurement is the monitoring of individual plants of sensitive species till they have recovered to pre-spraying levels of health. This technique was effectively employed at South Sandon, Yuraygir NP, for a population of *Persoonia stradbrokensis*. Many plants had not recovered by the time a repeat treatment was scheduled 12 months later. The area containing those species was then excluded from the subsequent treatment. When plants have recovered to pre-spraying levels, repeat BHBS treatments can be applied without risk of accumulated impacts on recovering individuals.

Toxicity Thresholds

The lowest quantity of herbicide to cause a pre-defined level of toxic effect, or "toxicity threshold," can be obtained by increasing the amount of applied herbicide to that at which toxic effects are observable.

The scientific justification of no spray buffer zones includes the consideration of toxicity thresholds of the most sensitive species for which herbicide impacts are to be minimized (Wilson et. al., 2001).

Controlled Exposure Testing

Controlled exposure testing is used to construct a species-specific index of relative sensitivity to BHBS herbicide application. This index is most important for species with potential for serious impacts as well as species with known sensitivity and tolerance. The inclusion of species previously monitored in BHBS operations in controlled exposure testing will provide an improved level of certainty to the data collated by John Toth.

These trials may involve pot trials and simulated HBS, where conflating injury factors are controlled or eliminated. Nevertheless, glasshouse trials can produce results that are difficult to extrapolate to the field. The controlled testing of native plants can only be achieved initially for a limited number of species. Species for toxicity testing must firstly be prioritized. There are 850 plant species recorded within bitou-infested areas in NSW (NPWS Atlas) and only 219 and 90 species have been monitored for toxic effects after BHBS with Roundup® (Toth, 2002) and Brush-Off® (Toth pers. comm..) respectively.

The potential for serious impacts from BHBS can be estimated objectively and species can be ranked according to risk using various computer programs e.g. Expert Choice®. Each species is scored for each of the weighted features that contribute to the seriousness of a potential impact. These assessments can be revised and fine-tuned to provide a transparent justification for the inclusion of each species in toxicity testing (see Recommendation 6).

Species for which phytotoxicity data is lacking should be ranked on the basis of potential for serious impacts. Weighted features may include:

1. Level of Plant Significance. There is a generally accepted hierarchy of species according to the form of rarity and geographical distribution of significance eg. decreasing significance from endangered, vulnerable, nationally rare and rare in NSW.

2. Species primary habitat. Species that have large proportions of local populations within bitou-infested areas are more vulnerable to species-wide impacts from BHBS. The majority of native plants recorded in bitou-infested areas (NPWS Wildlife Atlas) are likely to have the majority of their local habitats outside bitou-infested areas, and their whole population cannot be as seriously threatened with impacts from BHBS as species whose population is largely within bitou infested areas.

3. Species that have major growth periods or flower during June & July. These species are more vulnerable to the herbicidal injury.

4 Herbaceous species. These are generally expected to have higher sensitivity to herbicide than woody plants.

5 Slow maturing and slow growing species. These are less likely to set seed and recover between herbicide applications, and will tend to accumulate injury from successive treatments.

6 Species growing in exposed microhabitats. Species living in exposed environments such as sun loving plants have a high risk of damage to the entire population, as few individuals are likely to be sheltered from deposition of herbicide.

7 Phanerophytes with dormant buds above the ground. These may be less capable of re-growing following herbicide injury than geophytes which have dormant buds located underground.

3.8.2 Conflation of Different Sources of Plant Injury

Field monitoring of bitou control programs is prone to conflate plant injury from other independent causes with herbicide effects. True controls, which can establish the incidence of certain forms of plant injury independent of BHBS, are difficult to establish.

Kholer et. al. (1995) did not detect any differences in plant mortality, die back and vigor between the two HBS applied herbicides. Plant health status was very variable and this may have been inflated by plant injury not connected with BHBS. Indicators that provide true measures of systemic plant health may be hard to find.

A major problem with field monitoring of herbicide injury is that the actual quantity of herbicide delivered to leaf surfaces is highly variable. Some of the factors affecting the amount of herbicide that reaches the leaf surface include:

a) Degree of microhabitat shelter which provides protection from aerially applied spray. This factor may be a consistent feature for particular species eg. shade tolerant species, and should be used to assess the likelihood of serious impacts of BHBS. See above for full discussion.
b) Over and under application of herbicide due to over- and under-lapping of spray runs. Double application and missed strips result in variation of application rate from 0 to 1440 g.a.i./ha of glyphosate.
c) High wind resulting in loss of chemical as drift.

The following sources of plant injury can occur independently of BHBS yet may compound and synergistically increase BHBS herbicide injury.

1 Salt Exposure

Storms and high onshore winds can deliver toxic loads of salt onto vegetation which complicates patterns of plant die back and death along the coast. High levels of salt can result in over-reporting of herbicide effects if not identified. Many species in bitou infested areas are susceptible to salt spray or salt laden air despite various levels of adaptation to minimize salt injury.

2 Water Stress

Plants that are in decline due to water stress may be difficult to distinguish from those suffering from chronic herbicide effects.

3 Microbe Pathology

Pathogenic diseases commonly create systemic effects such as leaf loss and leaf necrosis that are similar to the symptoms of glyphosate injury discussed below. A clear knowledge of the pathogenic diseases present in coastal plant communities minimizes this confusion.

3.8.3 Signs of BHBS Roundup® Injury

Monitoring of plant injury from BHBS requires some knowledge of the various signs of injury that may be associated with Roundup® application. The first four signs discussed are a result of direct injury from the application of glyphosate. The remainder are a result of indirect effects and involve the interaction of pathogenic agents with the herbicide. All of the following signs, except necrotic leaf lesions, are systemic in nature and occur distant to the initial deposition of the herbicide.

1) Leaf Chlorosis or Yellowing

Chlorosis is the loss of green coloration of the leaf due to the loss of chlorophyll. It takes various forms including yellowing and spotting. The target enzyme EPSPS is located in the chloroplasts of plants as well as other sites. The leaf of *Carprobrotus glaucescens* turns a bright crimson due to lack of chlorophyll when exhibiting advanced toxicity to glyphosate (pers. obs.). This sign may require several weeks to become fully apparent.

2) Leaf Drop

Abscission of leaves is a common feature of herbicide damage and has been observed in the majority of cases of herbicide toxicity to Roundup® at Yuraygir National Park (2001, 2002, 2003, pers. obs.). Leaf loss is often not visually apparent but recently dropped leaves may be noticed under the plant.

3) Leaf Necrosis

Damage may occur to the upper surface of the leaf after exposure to the surfactants in Roundup® (Feng et. al., 1999) and many other herbicides. Necrotic leaf burn is a non-systemic sign of herbicide injury.

4) Epinasty or Leaf Curling

Epinasty is created by the more rapid elongation of the leaf on one side, causing it to curl to the other side. It is commonly seen in leaves following exposure to Roundup® (Marrs et. al., 1989).

5) Herbivory

Outbreaks of herbivorous insects have been associated with the application of glyphosate (see Section 3.2 above). The incidence of insect pest outbreaks should be noted and collated.

6) Synergistic Fungal Diseases

The sudden death of plants may be due to synergistic pathogenic fungi.

7) Parasite-Host Dissociation

The presence of dead parasitic plants is an important indicator of injury. Many above ground parasites are easily identified as parasites however many root parasite species occur in bitou infested areas in NSW, eg. family Santalaceae, and may be easily overlooked as parasites.

3. 8.4 Abatement Options

There are a number of measures that can be taken to reduce the impacts of BHBS on native plants. These measures provide flexibility in the BHBS treatment program so that bitou remediation can be achieved with minimum herbicide injury to native plants.

1 BHBS Exclusion Areas

The most effective way to minimize herbicide injury of particular non-target plants is often to exclude particular areas from BHBS treatment. These areas can then undergo careful ground based spraying or other methods. The impacts of BHBS need to be assessed relative to other bitou treatment techniques.

BHBS exclusion zones that are inappropriately buffered are in effect reduced spray application areas. Despite the lack of herbicide exclusion in these areas these herbicide-reduced areas may play an important role in abating BHBS impacts on native plants. Newmaster and Bell (2002) observed that strips of aerially treated forest that missed direct application assisted the rehabilitation of heavily impacted fern populations in Canada.

2 Reduction of Application Rate

Effective kill of bitou has been achieved with the application of 1.5L/ha using otherwise standard BHBS techniques (Toth, pers. com.). Reduced application rates are expected to reduce the severity of native plant impacts. This option is seldom used and requires a permit from the APVMA, the authority that regulates BHBS in NSW (see Appendix 1).

3 Covering of Sensitive Plant Species

Sykes and Wilson (1990) have suggested that the well developed tolerance of darkness that they observed experimentally in sand dune plants was a pre-adaptation to burial in a mobile sand dune environment. The ecological feature has been exploited in the use of hessian or sand to cover glyphosate sensitive plants in the incipient dune to abate the impact of BHBS. It has been successfully used for several years at Sandon, Yuraygir National Park and Bundjalung National Park in northern NSW.

Hessian Covers

Hessian can be used to cover small patches of sensitive plants to prevent herbicide contact. Hessian covers can be left over plants for several weeks prior to spraying but should be removed as soon as possible after to avoid rain leaching the herbicide onto the covered plant. This flexibility is valuable especially in larger BHBS projects. Sand is excavated to pin down the edges of the hessian and efficiently avoids disruption by the wind.

Sand Covering

Moving sand is an important part of the habitat of many coastal sand dune plant species. Mobile sand that covers plant parts commonly promotes rooting at nodes and the creation of elongated shoots e.g. *Spinifex sericeus* (pers. obs.). Covering *Carprobrotus glaucescens* and *Scaveola calendulacea* with sand was trialed at Bundjalung in 2002. The results are not currently available.

4 Droplet size manipulation

Droplet size strongly influences the final deposition site of droplets when moving through layers of plant canopies (see Section 3.8 Drift Minimization).

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5 "Safeners" and Injury Reversal

A valuable abatement measure is to assist the recovery of sensitive plants that have been BHBS, either because they can not be protected in any other way or because they have been sprayed accidentally.

Aromatic acid reversal of herbicide injury

Glyphosate suppresses plant growth by inhibiting the shikimic acid cycle. This effect can be alleviated by the external application of aromatic amino acids. Applying 312 and 468g/ha tryptophan and phenylalanine at 500ppm reversed glyphosate injury of the faba bean *Vicia faba*. A 0.4% solution of cytokinin added to faba beans reversed glyphosate injury and the beans subsequently produced yields higher than that yielded from unsprayed plants (Shaban et. al., 1987).

Activated charcoal

Activated carbon or charcoal can destroy herbicidal activity by strong adsorption of the herbicide.

Seed pelleting with activated charcoal has been frequently used to protect crop seedlings from the activity of pre-emergence herbicides. Bermuda grass seed pelleted with activated charcoal effectively protected seedlings from metsulfuron in the soil (McCalla et. al., 2001).

Foliar application of activated carbon dust to protect susceptible plants from herbicide sprays may prevent some off-target damage.

Clay coating

The coating of plants with soil dust has greatly reduced glyphosate herbicidal activity (Bouhache et. al., 1996). It is possible that a liquid suspension of clay sprayed onto non-target plants could provide protection from glyphosate injury.

Naphthalic anhydride

Tolerance of maize to MSM was increased by dressing seed with 0.5% 1,8-naphthalic anhydride (Mersie and Foy, 1984). This may be a useful technique when aerial seeding occurs in conjunction with BHBS.

6 Split Treatments

Split treatments involve the use of two treatments of lower application rates to replace a single treatment using the standard application rate. This approach attempts to reduce the impact of each treatment on the non-target species while having similar or greater cumulative impact on the targeted weed species.

Split application of several herbicides was trialed for the control of St. John's Wort *Hypericum perforatum* L. while minimizing damage to annual clovers and perennial grasses. A split treatment of a glyphosate based herbicide applied at1.35kg.a.i./ha still impacted on perennial grasses (Campbell and Nichol, 2000).

Abatement of Impacts on Significant Plants

BHBS treatment areas have generally excluded threatened plants because of the seriousness of any herbicide impacts. The sensitivity of most threatened and significant plant species to injury from BHBS is unknown for either glyphosate or metsulfuron. As a group, threatened plants are not expected to be more sensitive to herbicide injury than any other collection of plant species. Some plant groups which may be particularly sensitive such as orchids have not been toxicity tested or monitored at all during BHBS.

A notable exception is *Pimelea spicata,* studied by Matarczyk et. al. (2002). This species was found to have a high sensitivity to ground spraying of Roundup® at a dilution of 1:200. Matarczyk et. al. (2002) state that "winter applications of glyphosate to manage infestations of bitou bush will impact adversely on populations of *P. spicata* and may also affect the other rare and endangered species whose survival is threatened by this species". This experiment was used to predict the sensitivity of this species to application rates and methods of application used in BHBS.

Potential impacts from aerial spraying are best avoided by excluding populations of rare and threatened plants from BHBS unless there is evidence of tolerance to the applied herbicide.

Monitoring individual plant responses, such as from tagged plants will provide the detailed information of plant responses required to safeguard significant plant species.

3.11 Categorisation of Plant Sensitivity

This sub-section categorizes the known sensitivity of plant species to BHBS of Roundup® & Roundup Biactive® and Brush-off® Brushcontroller. While these lists are incomplete, understanding is continuing to accumulate from monitoring of BHBS.

Three categories are used to describe plant species sensitivity to BHBS of herbicide. These are highly sensitive, moderately sensitive and tolerant. These categories are crude and may be further divided when the need arises or when more information is accumulated.

Many native plant species are expected to show a low and temporary level of injury from BHBS under normal conditions.

1) Highly sensitive is used here to describe plant species which have been observed to be repeatedly killed following BHBS.

2) Moderately sensitive have been observed to have a high level of injury following BHBS and which may lead to death in some cases. Also included in this group are species which have been occasionally observed to die in significant numbers following BHBS, but most reports have noted that the species has recovered. Moderately sensitive species are particularly susceptible to accumulated injury resulting from successive BHBS treatments, as a large proportion of the sprayed population may have not fully recovered from initial herbicide injury before further BHBS.

3) Tolerant is used here to describe species where there has been no reported impact on sprayed plants or where the injury is small and plants have commonly recovered quickly eg. *Hibbertia scandens*. Tolerant plants species often benefit following BHBS in the medium to long term.

The characterization of the sensitivity/tolerance of species has been observed from BHBS projects using the same application methodology and approach as outlined in Section 1 above. These characterisations should not be generalised to include different methods of applying herbicide eg. ground spraying, different application rates eg. rates higher than 720g.a.i./ha of glyphosate or for different seasons of application eg. spraying in late spring or early autumn.

Bryophytes and Lichens

Newmaster et. al. (1999) categorised responses of bryophytes and lichens to application of glyphosate ≥ 710g /ha. They found responses corresponded with three ecological classes of herbicide tolerant colonisers: semi-tolerant, long-term stayers from dry open forest and sensitive forest mesophytes. Ipor and Tawan (1991) concluded that less dense leaf canopy of *Imperata cylindrica* plants grown in sand compared to plants grown in sandy loam were more sensitive to glyphosate because glyphosate was retained and translocated within the more open leaf canopy.

Grasses

Grasses are often sensitive to glyphosate. Lym and Kirby (1991) found that the greater the spring precipitation following a fall application of glyphosate, the less effect glyphosate had on biomass. *Danthonia* spp were only affected by glyphosate at levels above 360g/ha. (Lodge and McMillan, 1994).

Breeze et. al. (1992) reported half of 14 species tested had 10% reduction in dry weight with the addition of < 1 μg/ plant, but it was concluded even at these levels of sensitivity drift was only likely to be unsafe when glyphosate was applied at very high rates.

Table 1 Sensitivity/Tolerance of Plant Species to BHBS of Glyphosate

Highly Sensitive to BHBS of Roundup® & Roundup Biactive®

Native Species

Acacia sophorae bipinnate seedlings *6*
Bracteantha bracteata 3
Carprobrotus glaucescens 2, 4, 19, 8, 16, 14, 17, 15, 9
Clerodendrum tomentosum 2
Gonocarpus teucrioides 10-1, 14
Scaveola calendulaceae 1, 2, 4, 6, 16
Stackhousia spathlulata 2
Velleia procumbens 3
Eragrostis interruptus 3
Ischaemum australe 3

Exotic Species

Acacia saligna 2*
Conyza spp.** 1, 2*
Cuscuta campestris 16*
Osteospermum eklonis 1*
Ehrhrata erecta 1*
Sonchus oleraceous 1, 2*

Moderately Sensitive BHBS of Roundup® & Roundup Biactive®

Natives Species

Acacia suaveolens 8
Alphitonia excelsa 21
Breynia longifolia 2
Cayratia clematidea 2, 5, 21, 22
Dodonaea triquetra 21, 22
Duboisia myoporoides 4, 21, 22
Casuarina glauca 30
Einadia hastata 1
Ficus fraseri 6
Glycine clandestina 8
Hardenbergia violacea 2, 8
Imperata cylindrica 30
Maclura cochinchinensis 2
Muehlenbeckia gracillima 3
Myoporum boninense3
Pandorea pandorana 21
Pelargonium australe 1, 14
Phragmites australis 20, 14
Pimelea linifolia 8
Pimelea spicata 23, 24
Stephania japonica 21, 2
Themeda australis 3, 16

Exotic species
Adenophorum riparium 9*
Brassica spp.* *1*
Conyza bonariensis 1*
Cortadiera selloana 1*
Hydrocotyle bonariensis 2, 16*
Lagurus ovatus 1*
Lantana camara 1, 2, 8*
Pennisetum clandestinum 1, 9*
Plantago lanceolata 1*
Stenotaphrum secundatum 9*
Trifolium spp.* *1*
Verbena officianalis 1*

***Tolerant to BHBS of* Roundup® & Roundup Biactive®**
Native Species
Acronychia imperforata 2
Actinotus helianthi 10-13, 8, 14
Actinotus minor 14
Allocasuarina distyla 10-13, 14
Allocasuarina littoralis 10-13, 14
Allocasuarina nana 14
Angophora costata 14
Astroloma pinifolium 10-13, 14
Baeckea brevifolia 14
Baeckea imbricata 14
Banksia aemula 3
Banksia ericifolia 3, 14
Banksia integrifolia subsp. *integrifolia 2, 4, 6, 8, 14, 19*
Banksia oblongifolia 16
Banksia serrata 5, 10-13, 8, 14
Brachyloma daphnoides subsp. *daphnoides 5, 8*
Callistemon citrinus 10-13
Casuarina equisetifolia 2, 4
Conospermum taxifolium 10-13
Corymbia gummifera 8, 10
Crinum pedunculatum 2, 4
Cynodon dactylon 2, 3
Dianella caerulea 10-13, 14, 21
Dianella congesta 2, 4
Dianella revoluta 10-13, 8
Epacris microphylla var. *microphylla 14*
Epacris obtusifolia 14
Eucalptus spp. (part) *25*
Eucalyptus botryoides 8, 10, 18
Eucalyptus globoidea 14
Eucalyptus gummifera 2, 8
Eucalyptus pilularis 8, 10-13

Eucalyptus punctata 8
Eucalyptus robusta 8
Eucalyptus signata 10-13
Ficus rubiginosa 8
Hakea spp. *26*
Hibbertia scandens 2, 4, 10-13, 16, 17, 15
Ipomoea pes-caprae subsp. *brasiliensis 2, 5, 27*
Leptospermum juniperinum 8
Leptospermum laevigatum 8, 10-13, 14, 19
Leptospermum liversidgei 10-13
Leptospermum polygalifolium 16
Leucopogon ericoides 10-13, 14
Leucopogon parviflorus 6, 10-13, 19
Leucopogon virgatus 10-13
Lomandra longifolia 19, 8, 14, 22
Lomandra multiflora subsp. *multiflora 10-13, 14*
Melaleuca armillaris subsp. *armillaris 8, 14*
Melaleuca ericifolia 8
Melaleuca nodosa 16
Melaleuca quinquenervia 4, 28
Monotoca elliptica 19, 10-13, 8, 21
Monotoca scoparia 10-13, 14
Pittosporum revolutum 4, 8
Pittosporum undulatum 10-13, 18
Rhodomyrtus psidioides 6
Sesuvium portulacastrum 2, 4, 6, 27
Sprengelia incarnate 14
Styphelia viridis 4
Zoysia macrantha 2, 3

Exotic Species

Anredera cordifolia 8*
Cakile edentula (C. americana*) 5*
*Cakile maritima * 5, 6, 8*
Gloriosa superba 5*
Opuntia sp.* *5, 8*
Pinus sp.* *8*
Protasparagus aethiopicus 5*
Protasparagus plumosus 8*
Sporobolus indicus 1*

Table 2 Tolerance of Plant Species to BHBS of Brush-off® Brushcontroller

Zoysia macrantha: Dernoeden (1995) reported that metsulfuron at 35g.a.i./ha application induced unacceptable levels of chlorosis in the lawn grass *Zoysia japonica*.
Cynodon dactylon was tolerant of metsulfuron 140g.a.i./ha (Dernoeden, 1995).

Anon (1992) has suggested that the following species encountered in bitou-infested areas have tolerance of Brush-off® Brushcontroller:

Acacia spp. most species
Maclura cochinchinensis Cockspur
Senecio madagascariensis Fireweed
Cordyline spp. Flax Lily
Most established grasses
Eucalyptus spp. Ironbark
Solanum spp. Nightshades
Sida rhombifolia Paddy's Lucerne
Juncus spp. Rushes
Urtica spp. * Stinging Nettles
Melaleuca spp. Tea Tree
Rubus spp. Wild Raspberry

Footnotes

* indicates exotic species.

Table 3 Susceptibility to Reproductive Impacts from BHBS

Fore-Dune Species

Scientific Name	Flowering Period [29]	Ranking of Susceptibility#
Banksia integrifolia subsp. *integrifolia*	Mainly Winter	1
Acianthus exiguus	June-July	1
Cyperus stradbrokensis	Winter	1
Ischaemum triticeum	Winter	1
Kennedia rubicunda	late winter to Spring	1
Pterostylis nutans	June-July	1
Sophora tomentosa subsp. *australis*	Winter	1
Agrostis billardieri	Spring	2
Alectryon coriaceous	Spring-Summer	2
Billardiera scandens	Spring	2
Dianella congesta	Spring	2
Dianella crinoides	Spring-Summer	2
Geitonoplesium cymosum	Spring-Summer	2
Hardenbergia violacea	Mostly Spring	2
Hibbertia scandens	Spring-Summer	2
Isolepis cernua	Spring-Summer	2
Isolepis nodosa	Spring-Summer	2
Juncus kraussii subsp. *australiensis*	Spring-Summer	2
Leptospermum laevigatum	Spring ?	2
Macaranga tanarius	Spring	2
Mucuna gigantea	Spring-early Summer	2
Persoonia stradbrokensis	Spring ?	2
Pomax umbellata	Spring-early Summer	2
Strangea linearis	Spring	2
Tetragonia tetragonioides	Spring-Summer	2
Viola hederacea forma D	Spring-Summer	2
Cynodon dactylon	Throughout Year	3
Cyperus polystachyos	Throughout Year	3
Desmodium varians	Throughout Year	3
Eragrostis interrupta	Throughout Year	3
Leucopogon parviflorus	Throughout Year	3
Oxalis rubens	February-November	3

Table 3 (cont'd)

Scientific Name	Flowering Period [29]	Ranking of Susceptibility#
Pandanus tectorius var. *australianus*	Throughout Year	3
Pimelea linifolia	Throughout Year	3
Senecio lautus subsp. *maritimus*	Throughout Year	3
Acronychia imperforata	February-March	4
Casuarina equisetifolia subsp. *incana*	Summer	4
Cenchrus sp *A*	Summer	4
Crinum pedunculatum	Summer	4
Digitaria sanguinalis	Summer	4
Elyonurus citreus	Summer	4
Gleichenia dicarpa	Summer	4
Gleichenia mendellii	Summer	4
Glycine tabacina	October-November	4
Glycine tomentella	November-March	4
Hibiscus tiliaceus	Summer	4
Imperata cylindrica var. *major*	Summer	4
Lomandra longifolia	September-November	4
Oxalis exilis	October-May	4
Pandorea jasminoides	September-March	4
Pelargonium australe	October-March	4
Stenotaphrum secundatum	Summer	4
Velleia spathulata	Throughout Year	4
Incipient Dune Species		
Vigna marina	Autumn - Early Winter	1
Acacia longifolia subsp. *sophorae*	June-October	2
Carex pumila	Spring - Summer	2
Schoenus nitens	Spring - Summer	2
Sporobolus virginicus	Spring - Summer	2
Stackhousia spathulata	Early Spring - Summer	2
Zoysia macrantha	Spring - Summer	2
Cakile edentula	Throughout Year	3
Carpobrotus glaucescens	Throughout Year	3
Ipomoea pes-caprae subsp. *brasiliensis*	Throughout Year	3
Scaevola calendulacea	Throughout Year	3
Sesuvium portulacastrum	Throughout Year	3
Chamaesyce psammogeton	Summer	4
Melanthera biflora	Summer	4
Spinifex sericeus	Summer	4

Footnote

Throughout Year indicates flowering most if not all the year

Ranking of susceptibility to herbicide injury to reproduction

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Table 1-4 Susceptibility/Tolerance of plant species to BHBS of glyphosate

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4.0 Suggestions for Further Research

1. Experimental investigation of adsorption and movement of glyphosate and metsulfuron in the various sand substrates of bitou-infested areas.
2. Investigation of the impact of glyphosate on seed production and seed viability of winter flowering native plant species.
3. Controlled monitoring of seasonally dependant changes in sensitivity and tolerance to glyphosate herbicides for key coastal plants species in NSW.
4. Investigation and documentation of the ecology of seedling recruitment for key native species following BHBS using glyphosate and metsulfuron.
5. Study of the deposition characteristics of herbicide spray in bitou using HBS equipment to test the following claims:

   Smaller droplet size is appropriate for treatment of dense adult bitou which shelters suppressed native species.

   Larger droplet size is appropriate where there are canopies of native plants above bitou plants and reduces herbicide deposition onto upper plant canopies.
6. Archival research of the northern and southern distributional limits of species with conservation significance which occur in bitou infested areas in NSW.
7. Experimental investigation of the role of relative humidity on effectiveness of glyphosate herbicides in killing bitou bush under common BHBS environmental conditions.
8. Experimental investigation of the effects of relative humidity and sodium chloride exposure on the surface structure of bitou leaves.

5.0 Recommendations

Recommendation 1

That controlled trials be conducted with glyphosate and metsulfuron based herbicides on terrestrial orchids.

Recommendation 2

That the incidence and abundance of plant pathogenic soil organisms should be investigated in areas before and after BHBS and include monitoring of signs of pathogenic diseases in seedlings.

Recommendation 3

That techniques be investigated and developed that promote dominance by native species other than *Acacia sophorae*, so that bitou re-establishment is suppressed and recruitment of key native species such *Banksia integrifolia* is encouraged.

Recommendation 4

That on ground managers of BHBS be appropriately trained in drift minimization techniques.

Recommendation 5

That the results published in Toth (2002) be separated according to the herbicide product used (eg. Roundup® or Roundup Biactive®) and published as separate lists.

Recommendation 6

That native plant species be ranked for seriousness of potential effects from aerial spraying of glyphosate. The highest ranked species should be tested for sensitivity in controlled herbicide exposure experiments using Roundup®, Roundup Biactive® and Brush-Off® Brushcontroller.

Recommendation 7

That deposition studies be conducted to quantify the degree and pattern of herbicide placement in BHBS operations at several different sites.

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7.0 Appendices

Aims of This Report

This review aims to critically review data on the effectiveness and environmental impacts of aerial boom spraying of bitou bush. Specifically the project will review available data and consider the personal experience of operators of the effectiveness and impacts of aerial boom spraying on mature and seedling stages of:

- bitou bush,
- native plant species and native vegetation communities in which bitou bush commonly occurs,
- plant species reported to be susceptible to aerial boom spraying,
- rare and threatened flora such as orchids, and
- other threatened species identified in the Draft Threat Abatement Plan for Bitou Bush.

Consideration should be given to:

- Regional and site variation of plant responses to aerial spraying, especially the differences in responses between the north and south coasts.
- Adverse changes in the composition, structure and/or function of native vegetation communities that may be induced by aerial spraying.
- The frequency and timing of applications as they relate to efficacy on bitou Bush.

Information available from ground spraying may be used to support the conclusions from aerial boom spraying data/experience.

The tasks of the review are:

- Liaise with employees of the NPWS, NSW Agriculture, local council and elsewhere to obtain information relevant to the aims of the project.
- Conduct literature searches for literature relevant to the aims of the project.
- Tabulate all information for ease of analyses /interpretation.
- Interpret trends and, make conclusions.
- Identify species which clearly show tolerance to glyphosate.
- Identify where information is inadequate.
- Develop best practice guidelines for aerial spraying of bitou bush which complement the Bitou Bush Best Practice Management Guide prepared by the CRC for Weed Management Systems.
- present the findings in a written report.