Invasive Hakeas — Biological Control Implementations

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Chapter 1

The Hakea species

Four species of hakea were introduced to South Africa from Australia between 1835 and 1858. Three species (*Hakea sericea*, *H. gibbosa* and *H. drupacea*) have become environmental weeds while the fourth species (*H. salicifolia*) has only recently been observed invading fynbos areas. *Hakea sericea* is the most serious and widespread of the hakeas and factors thought to have contributed to its success as an invader in South Africa are its copious production of serotinous seeds (after the death of the plant the fruits split open within a couple of days and drop their seeds), the high seed longevity in the canopy, efficient seed dispersal and absence of seed predators.

1.1 Biology

1.1.1 *Hakea sericea*

The weed *Hakea sericea*, native to south-eastern Australia, is one of the most serious alien invasive trees in nearly all the coastal mountains of the Western and Eastern Cape Provinces. It is classified as a category 1 weed in South Africa and is therefore prohibited on any land or water surface and must be controlled or eradicated where possible. *H. sericea* is commonly known as silky hakea and is typically an erect single-stemmed, much-branched shrub or tree up to 5 m high. It has dark-green needle-like leaves 1mm in diameter and up to 40 mm long with sharply pointed tips. The mature fruit of *H. sericea* is a heat-resistant woody follicle (20–25 mm in diameter) which contains two winged seeds. The seeds produced annually are stored in the canopy and are only released after the death of the plant, usually by fire. There is no seed bank in the soil.

1.1.2 *Hakea gibbosa*

Hakea gibbosa is endemic to New South Wales and southern Queensland, Australia and is not as widespread as *H. sericea* in South Africa. It occurs on the Cape Peninsula, the Kleinrivierberg and the adjoining mountains near Hermanus and Stanford. It is also classified as a category 1 weed in South Africa. *Hakea gibbosa* is commonly known as rock hakea and is typically an erect branched shrub or tree up to a height of 3 m. It has stiff, hairy, grey-green needle-like leaves 1 mm in diameter and up to 80 mm long with sharply pointed

Figure 1.1: *Hakea sericea* flowers and plant

tips. The mature fruit of *H. gibbosa* is a heat-resistant woody follicle (35 mm long and 30 mm in diameter) which contains two winged seeds. The seeds produced annually are stored in the canopy and are only released after the death of the plant.

1.1.3 *Hakea drupacea*

Hakea drupacea is endemic to coastal regions of south-western Australia and some islands in the adjacent Recherché Archipelago. In South Africa it is restricted to the Cape Peninsula, Somerset West, Franschhoek, Bot River, Hawston and the Kleinrivier mountains. It occurs on the Cape Peninsula, the Kleindrakenstein berg, the Kleinrivierberg and the adjoining mountains near Hermanus and Stanford. It is also classified as a category 1 weed in South Africa. *Hakea drupacea* is commonly known as sweet hakea and is much branched, rounded shrub or tree up to a height of 6 m. The adult leaves are dark green, hairless, up to 100 mm long with sharply pointed tips. The leaves are usually divided into upright, 30–50 mm long sharp-pointed needles. The mature fruit of *H. drupacea* is a heat-resistant woody follicle (25 mm long and 20 mm in diameter) which contains two winged seeds. The seeds produced annually are stored in the canopy and are only released after the death of the plant. *Hakea drupacea* produces far fewer viable seed than *H. sericea* and *H. gibbosa* and is not as invasive as *H. sericea*. No biological control agents have been introduced for this weed.

1.1.4 Biology of *Hakea salicifolia* (willow hakea)

Hakea salicifolia is endemic to the eastern part of New South Wales and the extreme south east of Queensland, Australia and occurs in open forests. It is not widespread in South Africa and small infestations occur in Du Toits Kloof near Paarl, Kleinmond, Bot River, George and Knysna in the Western Cape. Localised infestations also occur in Stutterheim and Hogs back in the Eastern

Figure 1.2: *Hakea gibbosa* flowers

Figure 1.3: *Hakea drupacea* plant and seeds

Figure 1.4: *Hakea salicifolia*

Cape. It is not a declared invader at present. *Hakea salicifolia* is a fast-growing shrub or small tree which grows up to 5 m tall. The leaves are narrow and grow up to 10 cm long. The flowers are white, small and occur in the leaf axils. The woody fruits are much smaller than those of *H. sericea* and *H. gibbosa* and are approximately 20 mm long and 15 mm wide and contain two winged seeds. The fruits remain on the plants and the seed are only shed after the death of the plant, usually as a result of fire. No biological control agents have been introduced for this plant.

1.2 Ecology and spread

In South Africa *H. sericea* occurs in Mediterranean-type climates that have a summer drought, a summer rainfall region and an area with rainfall throughout the year. *Hakea gibbosa* and *H. drupacea* occur in the winter rainfall region.

Widespread fires in the Western and Eastern Cape mountains have resulted in massive regeneration of *H. sericea*. Dense stands of hakea can alter the composition of plant and animal communities, increase fire intensities, increase evapotranspiration from important water catchment areas and restrict access to mountain areas. The rate of spread of *H. sericea* has been impressive in the south-western region of the Western Cape Province, with 9 000 ha, 111 345 ha, 279 200 ha and 360 000 ha invaded in 1939, 1969, 1974 and 1983 respectively.

1.3 Current control measures

1.3.1 Mechanical control

Present control measures include mechanical clearing, chemical and biological control. The most successful mechanical control method is the fell and burn technique. Adult plants are cut down and left 12 to 18 months before they are burnt. Shortly after the plants are cut down the fruits split open and fall to the ground. The released seeds germinate the following winter after the first rains. When the felled plants are burnt any regenerating seedlings are killed. Although this is an effective method it is costly and is not always feasible in rough mountainous terrain. The increased fire intensities using this technique can have a negative effect on sensitive ecosystems. It is not advisable to cut and leave the plants as fire is needed to kill any regenerating seedlings. Burning standing plants can be effective in some cases but may result in dense stands of seedlings and widespread dispersal. Seedlings can also be pulled out by hand but this is both time consuming and expensive.

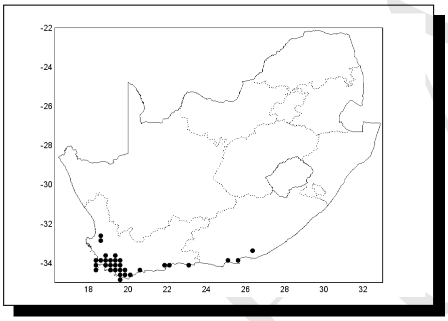
1.3.2 Chemical control

The use of herbicides to control hakea species is effective, but on the other hand it is expensive, difficult to apply in inaccessible infestations and has a detrimental effect on indigenous plant species. Tebuthiuron is the only registered herbicide against this weed.

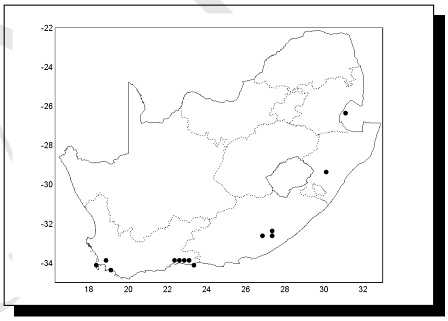
1.3.3 Biological control

Biological control is the use of the weeds natural enemies to permanently suppress the density and dispersal of the selected weed. Biological control is a long-term, environmentally sound, cost effective and often self-sustaining method to reduce the competitive advantage a weed has over the natural vegetation. Mechanical and chemical control is expensive, not always environmentally safe, and often requires repeated treatment and provides control only at or near the site of application. Biological control is a slow process and success is not always guaranteed. It is estimated that the successful implementation of biological control in general could bring about a saving of more than 50% of the total costs of controlling invasive species.

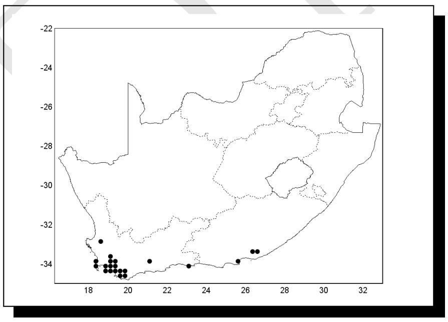
The biological control program against *H. sericea* started in 1962 and initially focused on two seed attacking insects, a seed-feeding weevil, *Erytenna consputa*, and a seed moth, *Carposina autologa*. Since then, a number of biological control agents have been established, a weevil, *Cydmaea binotata*, a bud-feeding weevil, *Dicomada rufa* and a stem-boring beetle, *Aphanasium australe* on *H.*



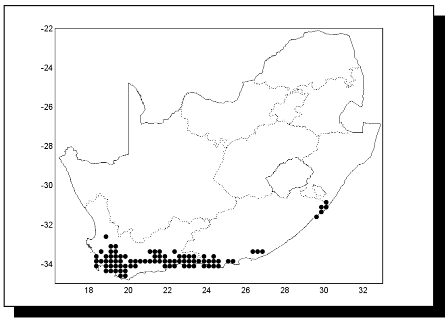
(a) *Hakea drupacea*



(b) *Hakea salicifolia*



(c) *Hakea gibbosa*



(d) *Hakea sericea*

Figure 1.5: Distribution maps of the hakea species in South Africa in (Drawn by L. Henderson; data source: SAPIA database, ARC-Plant Protection Research Institute, Pretoria.).

sericea. An indigenous fungus, *Colletotrichum acutatum*, has also been used as a mycoherbicide for the control of *H. sericea*. A strain of the seed-feeding weevil and the stem-boring beetle have been introduced for *H. gibbosa*.

Chapter 2

Possible Biological control agents

2.1 The gummosis fungus *Colletotrichum acutatum*

2.1.1 Description

The indigenous fungus *Colletotrichum acutatum* has been recorded as causing a serious disease of *H. sericea* in various parts of South Africa. Colletotrichum species are commonly used as mycoherbicides and host specificity tests of this isolate of *C. acutatum* showed that it is highly specific towards *H. sericea* and it is therefore safe to be used as a mycoherbicide in South Africa. Optimum germination of *C. acutatum* spores occurs from 16 to 32°C at a high humidity (above 95%), with an optimum infection temperature of 25°C. Consequently treatment of hakea infestations must be done during the winter rain season, to ensure optimal spore germination and infection under field conditions.

2.1.2 Disease Symptoms

The most characteristic symptoms of the disease are stem and branch cankers as well as gummosis and seedling blight. The affected areas become flattened or swollen and the bark splits. Infected shoot tips of mature plants die back progressively and growing points of seedlings become necrotic which extends down the stem. These cankers girdle the stem near ground level or on branches, killing the host plant.

2.1.3 Impact on *Hakea sericea*

Seedlings as well as mature silky hakea plants are affected by the gummosis fungus. Although the effects manifest slowly over several months, the fungus is still capable of killing large stands of hakea trees, it is cost effective and it reduces the need for herbicide use and labour-intensive mechanical control procedures. If applied correctly during favorable environmental conditions, it can cause severe disease of mature *H. sericea* plants and up to 98% mortality of hakea seedlings. Rainfall favors the spread of the fungus, therefore it is most effective in high rainfall areas and becomes progressively less effective the drier the area. This

Figure 2.1: Gum oozing from mature hakea trees, after stem inoculation with the gummosis fungus

reduced spread of the fungus in drier regions needs to be compensated for by inoculating more plants, and by more frequent applications. This isolate of *C. acutatum* has been shown to be host specific, causing disease on *H. sericea* plants from all areas of South Africa, except for plants from Grahamstown (Eastern Cape Province) that are resistant.

2.2 The hakea seed weevil *Erytenna consputa*

2.2.1 Description

The weevils are mottled gray, black and red-brown, 4–5 mm long with a long snout. The adults may live up to three years. They are present on the trees throughout the year and can be found on the tree or sheltering in the dry, brown husks on the tree. The husks are undeveloped, partially split, black fruits resulting from larvae hollowing out the immature fruits. The larvae are small white grubs and can usually be found inside the green developing fruits. The adults feed on shoots, buds flowers or young fruits as these become available.

2.2.2 Life Cycle

The adults lay their eggs in excavations in the leaf-tips and buds near young fruits or in the "horns" of the fruit between July and September. During the breeding season each female lays 100–130 eggs. After about two weeks the larvae hatch and tunnel into the young growing fruits. Only one larva can live in each fruit, and may have to move between two to three fruits to complete

Figure 2.2: *Hakea sericea* trees killed by the gummosis fungus

Figure 2.3: Adult fruit weevil *Erytenna consputa*

Figure 2.4: Hakea seeds destroyed by the fruit weevil *E. consputa*

its development. When full grown in October, the larvae tunnel out through one side of the fruit and pupate in the soil. The adults emerge from the soil in January.

2.2.3 Feeding Damage

A fruit containing a weevil larva has a reddish colour at first and starts to yellow as it withers and dies. Fruits attacked by weevils remain on the tree and have a small entrance and exit hole with signs of frass (faeces) around the eaten seed. Weevils are usually present in an area if there are black, dried, partially split remains of young fruits (husks) on the tree.

The larvae destroy the green developing fruits of *H. sericea* and *H. gibbosa*. At some sites, the weevils destroyed more than 80% of the annual *H. sericea* seed crop. A strain of *E. consputa* was collected on *H. gibbosa* in Australia and introduced to South Africa but it has not been as successful. Adults are present on the trees throughout the year and shelter in the husks when not feeding or laying eggs.

2.3 The hakea seed moth *Carposina autologa*

2.3.1 Description

The adult moths are grey-brown, about 10 mm long and not easy to find in the field. Adult moths are present between February and June and live about 30 days. They are active during the twilight hours and feed on nectar.

Figure 2.5: The seed moth *Carposina autologa* on mature hakea fruit

2.3.2 Life cycle

The females lay their eggs in crevices on the surface of mature *H. sericea* fruit or between touching fruit in autumn. More than 100 eggs can be laid per female. After about 45 days, the larva hatches and tunnels into the fruit through a tiny hole which it excavates at a point along the suture of the fruit. The larva develops in the fruit by feeding on both seed. Only one larva can develop in a fruit. The larval stage last about 135 days and the mature larva emerges from its exit tunnel, falls to the ground and pupates in the soil. The adult moth emerges in the autumn. *Carposina autologa* has only one generation a year.

2.3.3 Feeding damage

The hakea seed moth destroys the seeds in mature, woody fruits. It complements the hakea fruit weevil because it destroys the seeds in fruits that escape attack by the seed weevil. The moth has reduced the accumulated seeds by more than 64% at some sites and has shown a surprising ability to disperse and establish new colonies.

2.4 The stem-boring beetle *Aphanasium australe*

2.4.1 Description

The adult of *A. australe* are blackish brown and about 20 mm in length.

2.4.2 Life cycle

Clusters of 10–20 eggs are deposited on the base of the stem. The neonate larvae enter the stems directly from the eggs and reddish brown mucilage (gum)

Figure 2.6: Larva of Carposina autologa feeding on seed of mature fruit

Figure 2.7: The stem-boring beetle *Aphanasium australe*

Figure 2.8: The bud-weevil *Dicomada rufa*

is exuded by the plant tissue at the entry points of the larvae. The larvae of *A. australe* tunnel gregariously within the base of stems and in the sub-surface roots of the plants. Nearly mature larvae construct large tunnels, pushing frass to the outside, thus weakening the trunk. Plants structurally weakened by larval attack are easily blown over. Summer winds are expected to cause the most damage to the plant. Once mature, larvae move to just beneath the bark surface, where they pupate. Most larvae take two years to reach maturity although some can complete their life cycle in one year.

2.4.3 Feeding damage

This is the first insect agent introduced that can kill the plant. Extensive tunnelling weakens the plants structurally and may cause them to fall over, particularly when subjected to the strong winds typical of the Western Cape.

2.5 The bud-weevil *Dicomada rufa*

2.5.1 Description

The adults are dull-brown and 2–3 mm in length.

2.5.2 Life cycle

The adults feed predominantly on the dormant auxiliary buds. The eggs are laid singly in the reproductive buds of the plant. The larvae feed on the inflorescences and young succulent vegetative growth in protective capsules formed by "gluing" the flowers of the inflorescences or individual young leaves together. The larva is

Figure 2.9: The leaf weevil *Cydmaea binotata*

a cream-coloured, legless grub with limited mobility. The mature larva pupates just under the soil surface. Dicomada rufa has one generation a year.

2.5.3 Feeding damage

The adults feed on the buds and the larvae feed on the young succulent leaves and flowers.

2.6 The leaf weevil *Cydmaea binotata*

2.6.1 Description

The adult weevils are small, black with a prominent pair of white spots on their elytra (wing covers). They are 2–3 mm long and feed on the young succulent leaves, stems and buds of the plant. They shelter in the husks of the tree and are present on the trees throughout the year.

2.6.2 Life cycle

The adults lay their oval-shaped eggs singly, throughout the year, in excavations in the succulent leaves or on sprouting buds when these are available. The larva tunnels down the leaves or distal sections of soft stems, this being the most damaging stage, especially when succulent shoots are attacked. Pupation occurs in a flimsy cocoon just below the surface of the soil. There are at least four generations per year.

2.6.3 Feeding damage

The larvae tunnel down the succulent leaves or the soft stems of the plant causing die-back of the leaves or soft stems. The adults make feeding cavities in succulent leaves and axillary buds. Although this agent is fairly widespread it is not abundant and the damage to the plant is negligible. It was initially thought that their feeding damage may enhance the infection rate of the fungus, especially on seedings.

Target species	Biocontrol agent	First release	Damage
Hakea sericea	*Erytenna consputa*	1970	Destroys seed in green developing fruits
(silky hakea)	*Carposina autologa*	1970	Destroys seed in mature fruits
	Cydmaea binotata	1979	Feeds in the leaves and shoots
	Aphanasium australe	2001	Larvae feed in the roots and stem at base of the plant
	Dicomada rufa	2006	Adult destroys flower buds and larvae feed on flowers
	Colletotrichum acutatum	Indigenous	Stem canker fungus on seedlings and mature plants
Hakea gibbosa	*Erytenna consputa*	1975	Destroys seed in green developing fruits
(rock hakea)	*Aphanasium australe*	2001	Larvae feed in the stems at base of the plant

Table 2.1: Summary table of the biological control agents introduced against hakea species in South Africa and their properties relevant for their usage

Chapter 3

Guidelines for biological control implementation

It is important to note that the use of biological control against hakea species must not be seen as the only method of control, but rather as an additional tool to ensure the sustainability of other clearing efforts. Depending on the specific site, mechanical clearing, burning and chemical control could provide adequate results, but biological control must be incorporated into control programs to achieve sustainable control in the long run.

3.1 Information needed and initial considerations

To be able to make an informed decision about the best strategy to use biological control agents in hakea control, it it essential to have information about the target area, the hakea stands in the target area and the presence or absence of biological control agents. The information in detail is:

1. What are the spatial characteristics of the area targeted?
   (a) How large is the target area?
   (b) Is the target area one hakea stand?
   (c) If the target area consists of several hakea stands, how far are these apart?
2. How old are the stands?
3. How dense are the stands?
4. Are biological control agents already present in the area, if yes,
   (a) which ones?
   (b) in the whole area?
   (c) are they abundant?

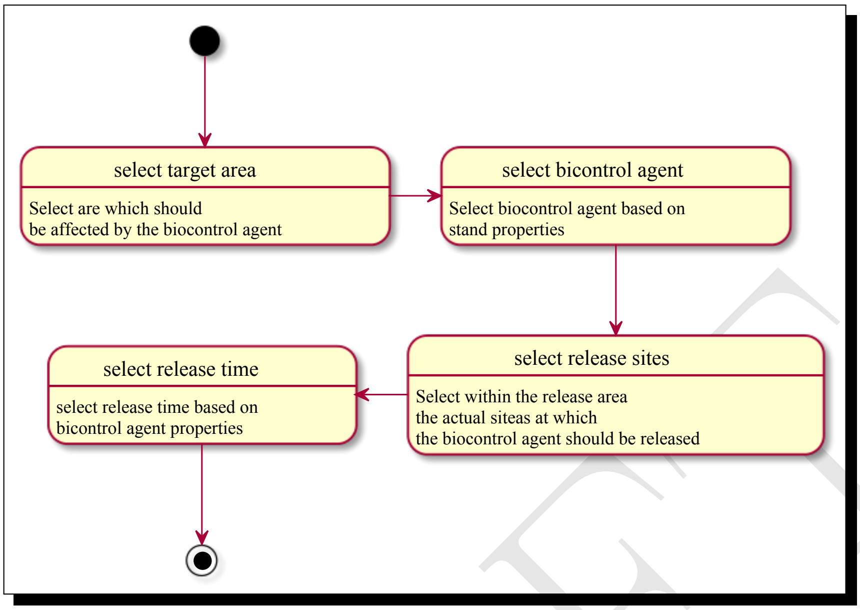


Figure 3.1: Flowchart of the steps needed to be taken to improve the effectiveness of biological control agent usage

The "target area" is the area which should be affected by the biological control agent over time, in contrast to the release sites, which are the locations at which the biological control agent will be released (see details about release site selection see 3.3).

3.2 Biological control strategy

If a biological control agent is already present in the target area, a release of a second one needs to be carefully considered, as there are likely interactions between the different biological control agents. These interactions could have detrimental effects on the performance of the biocontrol agent present. Therefore the release of a second biological control agent should only be considered if the effect of the already present biocontrol agent is not satisfactory. If this is the case, the new biological control agent should not attack the same state, e.g. if the existing bilogocal control agent attacks seed, the new one should e.g. be the gummosis fungus or the stem boring *Aphanasium australe*.

The general planning process for biological control usage for *H. sericea* should be as follows:

1. After fires:

    (a) After a fire, young seedlings that emerge after the first rain should be targeted with the gummosis fungus. This will reduce the hakea population especially if there was a high seed load present before the fire. The ideal time to collect and release the agents is prior to flower bud formation in the case of *D. rufa* and at flowering time in the case of *E. consputa*.

(b) Three years after a fire, release the biological control insects *Erytenna consputa* and *Dicomada rufa*. This is critically important as the plants start setting fruits after three years and the agents need to be in place to prevent an accumulation of fruits of the plants.

(c) If any fruits escape *E. consputa* attack introduce the hakea seed moth, *C. autologa*. The seed moth attacks the seed in the mature woody fruits.

(d) Release the stem-boring beetle *Aphanasium australe* when the hakea stems are "pencil" thickness or bigger.

2. Mature stands (if agents are not present)

   (a) Identify appropriate release sites (see 3.3)

   (b) Wound inoculated mature hakea trees at the beginning of the rainy season with the gummosis fungus. Monitor the spread throughout the population and do follow-up treatments if necessary, to ensure that there is a high inoculum pressure. It is important to remember that the gummosis fungus is intended to be used as a mycoherbicide and it will only be successful through constant and proper implementation.

3. Determine the best time for the collection and distribution of the selected biological control agents and release them into the selected release sites.

4. Monitor the presence and spread of the biological control agents, by looking out for gummosis disease symptoms and the presence of insect damage for instance fruit husks.

5. Perform follow-up treatments or re-introduce biological control agents where necessary.

Biological control "reserves" must be established in areas to be mechanically cleared to prevent local extinction of the agents during clearing operations. In addition these "reserves" will serve as foci from which recolonisation of resurging hakea populations can occur and collections of agents for redistribution can be made. Ideally hakea reserves should be around 1–5 ha in size and 5–10 km apart and should consist of plants that have healthy populations of the biological control agents on them.

3.3 Release Site selection

Selecting the correct sites for release of the biological control agent will determine if and to which degree the biological control agent is effective. The selection process should consider:

1. suitability of the site for the release: Is the release site a "safe" site i.e. what are the chances of the site being cleared or likely to burnt in the foreseeable future? There is no point releasing the agents if the site is going to be cleared within five years. If at all possible the release site must be in an area that is unlikely to burn if there is a wild fire.

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seeds</td><td colspan="4">mature fruit</td></tr>
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larvae</td><td>Pupae</td></tr>
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<tr><td colspan="2">Colletotrichum acutatum</td><td></td><td colspan="6">Natural dispersal of fungal spores by rain splash and establishment of new infections. Optimal conditions for spores to germinate, penetrate through wounds or stomata, and colonise host tissue</td><td colspan="5">Die-back of shoot tips, growing points of seedlings become necrotic, cankers develop on the stems with the distinctive oozing of gum and cankers develop on the stems with the distinctive oozing of gum and</td></tr>
<tr><td colspan="2">Western & Eastern Cape environmental conditions</td><td></td><td colspan="6">Winter rainfall season</td><td colspan="2"></td><td colspan="3">Natural and accidental wildfires</td></tr>
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<tr><td colspan="5">mature trees - Stem wound inoculation</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
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Table 3.1: Life cycles of biological control agents and timing of biological control implementation

2. the size of the hakea infestation: If it is only a couple of hectares large then only one release needs to be made. If it is a large infestation as many release as possible should be made if insects are available.

3. if hakea target area consists of separate hakea stands. In this case, the biocontrol agents should be release in several stands, to make sure that the biological control agents can reach all stands. As a rule of thumb, biological control agents should be released in stands which are more then 1 km away from the next stand.

4. if the plants are in a reproductive phase i.e. if they do have fruits or flowers. If the seed weevil is being released onto three-year old hakeas the plants should have flowerbuds or should be flowering. The hakea seed moth needs mature fruits. The bud-feeding weevil needs flower-buds to complete its development.

5. if the hakea fungus is present and killing the plants. If the fungus is present and causing considerable damage to the hakea infestation it is not necessary to release the stem-boring beetle as the beetles, which have a two-year life cycle, may not be able to complete their development if the plant dies. Similarly the seed-moth should not be released as it will not be able to complete its development in the fruits. The seed-weevil, flowerbud-feeding weevil and the leaf-feeding weevil can be released as they can disperse to adjacent stands of hakea if the plants die.

3.4 Biological Control Agent selection

It is recommended that all the agents, if available and not present in the infestation, be released. If biological control agents are already present and effective, it is recommendet to select a complementary agent, i.e. if a seed attacking agent is present, release one which attacks the plant itself and not it's reproduction and vice versa.

3.5 Specific guidelines

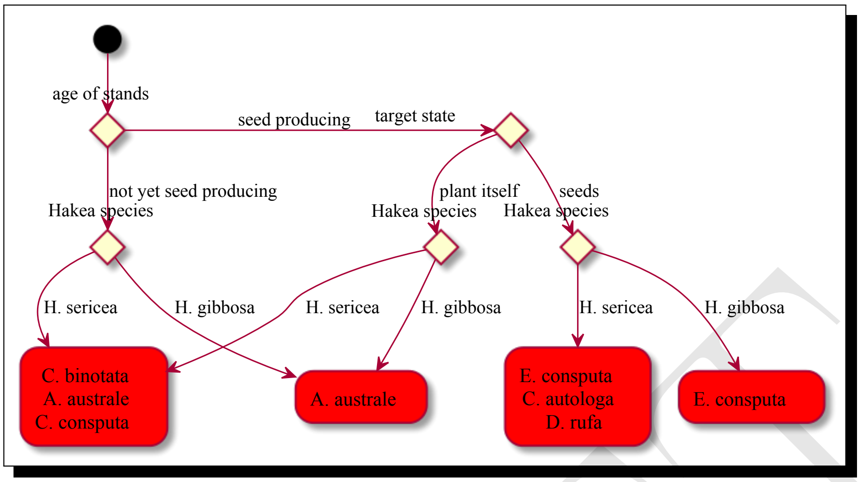

Figure 3.2: Flowchart of the steps needed to be taken to select the biological control agent

3.5.1 Gummosis fungus Colletotrichum acutatum

3.5.1.1 Implementation procedure

A number of application methods were developed for the use of *Colletotrichum* as a mycoherbicide against hakea. These include using a spore suspension of *C. acutatum* to wound inoculate the stems of larger hakea trees, full-cover spraying of younger plants, a soil drench, as well as coating shotgun cartridges with the spore suspension and firing them into a stand of *H. sericea*. All inoculation methods proved to be effective, but the fungus is the most effective when targeting young emerging seedlings or succulent new growth of hakea trees.

3.5.1.1.1 Mature hakea trees

- Stems of accessible hakea stands can be wound inoculated with *C. acutatum* spore suspensions.
- Packets of dried spores of the fungus are obtainable from ARC-PPRI, Stellenbosch.
- Suspend each spore packet in 1 litre of water.
- Inoculate the base of stems using a hand-held jabbing device (design available from ARC-PPRI) which lightly wounds and inoculates the plants. If jabbed higher, the top parts of the plant will die but the plant will regrow from the base.
- One litre of fungal spore suspension is more or less enough to wound inoculate 1 hectare of hakea trees.
- In high rainfall areas use a zigzag pattern of inoculation throughout the stand and allow the fungus to spread to neighbouring plants. In drier areas it is more feasible to inoculate every second or each tree if possible.

- Monitor disease spread and re-introduce the fungus in areas where necessary, for instance after fire or hot dry summers.
- Follow-up with seedling treatment if fires occur.

3.5.1.1.2 Hakea seedlings Young hakea seedlings that emerge following fire must preferably be targeted, since they are most susceptible to *Colletotrichum* disease. Successful establishment of disease is however dependant on having ideal environmental conditions for spore survival and germination. Seedlings can be treated with colonised bran inoculum or sprayed with an oil-based formulation of *C. acutatum* spores.

Wheat bran colonised by *C. acutatum*

- The first method of application is by hand distributing a dried formulation of *C. acutatum* spores on wheat bran, between young emerging seedlings. The bran facilitates rapid colonisation by *Colletotrichum*, from which it sporulates abundantly when remoistened and conidia are adequately dispersed to ensure infection of seedling tips.
- Treatment is the most effective during early winter at the beginning of the raining season, when seedlings are in the cotyledonary to 20-leaf stage, in comparison to later applications. Infected seedlings which are no more than 10 cm in height will die, but older plants need to be inoculated by physically damaging or wounding the plants.
- Distribute 1 gram per square meter bran inoculum by hand, between young emerging seedlings. The bran inoculum can be stored at room temperature for at least four weeks before it loses viability. The only disadvantage with the bran inoculum is that it is bulky, requiring approximately 10 kg of bran inoculum per hectare.
- The bran-fungal inoculum is obtainable from the ARC-PPRI in Stellenbosch. Place orders a month before the intended application, to allow sufficient inoculum to be produced.

Ultra low volume application of an oil-based formulation of C. acutatum

- A more practical method of introducing the fungus over a large area of emerging hakea seedlings is ultra-low volume (ULV) application of an oil-based formulation of *C. acutatum* spores. The oil protects the fungal spores from desiccation and allows for application under non-optimal environmental conditions, while still providing adequate infection. Hakea plants can therefore be sprayed at times that is more practical for labour to cover large areas and which is safer in mountainous areas.
- The fungal spores are protected by the oil from dry periods up to 96 hours after application, and only require a minimum dew period of 2 hours for germination.

Wounding and inoculation of mature hakea with the gummosis fungus

Dissolve dried fungal spores in water

Lightly wound mature hakea trees at the base of the stem and apply the fungal spore suspension with a brush or sponge

Monitor disease symptoms and spread and reintroduce the fungus where necessary

Figure 3.3: Inocculation procedure

Figure 3.4: Hand-distributing *Colletotrichum* bran inoculum amongst young emerging hakea seedlings following fire

- The best time for application is nonetheless the best during the wet periods of the year, to allow for successful spore germination and infection.
- Suspend each spore packet in 1 litre of cooking oil.
- Spray seedlings with a hand-held ULV applicator (e.g. HERBI-4) at a rate of 10 l/ha.
- Aerial application of the oil formulation of the gummosis fungus can be done with helicopters or small fixed-wing aircraft over large areas of emerging hakea seedlings.

3.5.1.2 Indication of presence

The most characteristic symptoms of the disease are stem and branch cankers as well as gummosis and seedling blight. The affected areas become flattened or swollen and the bark splits. Infected shoot tips of mature plants die back progressively and growing points of seedlings become necrotic which extends down the stem. These cankers girdle the stem near ground level or on branches, killing the host plant.

Figure 3.5: ULV-aerial application of an oil-based formulation of *Colletotrichum acutatum*

3.5.2 Seed weevil *Erytenna consputa* Collection procedure

3.5.2.1 Collection procedure

Weevils are most active and easiest to collect between May and July before they start laying their eggs. The weevils can be collected by shaking the plant or branch over a large plastic sheet which has been spread over the ground under the tree. The weevils then fall to the ground and can be sucked up using a pooter. Once a number of weevils have been collected (approx. 20) they are transferred into plastic containers, with mesh lids, containing crumbled up paper towelling. This method is more successful during hot days when the weevils are active on the plants. Once collected the weevils must be kept cool and should be released within 2 days.

3.5.2.2 Implementation procedure

A new hakea fruit weevil colony can be started with as little as 20 adults per release site but ideally the weevils should be spread throughout the infestation. The weevils are fairly hardy and the containers containing the weevils should be gently tapped so that the weevils can fall out onto the plants. The weevil is widespread throughout the South African range of *H. sericea*.

3.5.2.3 Indication of presence

Look for the black, dried, partially split remains of immature fruits (husks) resulting from larval attack

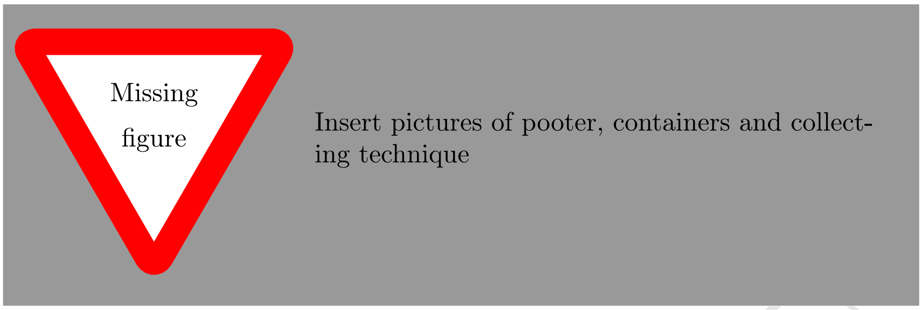

Figure 3.6: Pooter, containers and collecting technique

3.5.3 Seed moth *Carposina autologa*

3.5.3.1 Collection procedure

The best method to redistribute *C. autologa* is to attach egg-bearing follicles to healthy fruits in the field. Hakea sericea fruits are harvested during the egg-laying period in May / June. The fruits are then inspected for eggs using a magnifier. The eggs containing lavae are reddish in colour with spikes on the top and resemble pineapples. The eggs are generally laid in crevices on the surface of the fruit or between two touching fruit. The egg-bearing fruits are split in half along the suture of the fruit and the follicle with eggs kept. The egg-bearing follicles are then taken to the field and attached to healthy fruits. Although splitting the fruits involve additional work, follicles are used instead of whole fruits because, (a) the follicles are easier to attach than whole fruits, and (b) follicles eliminate the possibility of the larvae entering the attached fruit rather than the healthy fruit.

3.5.3.2 Implementation procedure

The follicles can be attached to healthy fruits using a silicon sealer. Care must be taken not to attach the follicle to, or near, the suture of the fruit as the larva enters the fruit at a point along the suture. The moth has been released into a number of sites in the Western Cape Province but is not widespread as *E. consputa* due to slow population growth. It is therefore necessary to release the moth into as many sites as possible.

3.5.3.3 Indication of presence

An exit hole (approx. 2 mm in diameter) in the side of a mature fruit is a clear indication that the seeds in the fruit have been destroyed by the seed moth. Besides the exit hole an attacked fruit is indistinguishable from healthy fruit.

Missing figure

Insert pictures of releasing technique and of eggs on fruit

Figure 3.7: Releasing technique

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Insert pictures of collecting technique

Figure 3.8: Collection technique

3.5.4 Stem-boring beetle *Aphanasium australe*

3.5.4.1 Collection procedure

The technique used for collecting *A. australe* is fairly simple but labour intensive. Plants infected with the beetle are easily recognisable due to the presence of sawdust (frass) at the base of the plant. This sawdust is ejected from the tunnels by the mature larvae. Plants infested with beetles are cut down approximately 8 cm above the ground in October / November when the larvae are mature. The stump and roots are dug out using a small pick and placed individually in plastic packets to prevent them drying out. The stumps should then be kept in insect cages until the adults emerge. The cages should be inspected daily for emerging adults. The adults should be collected daily and placed in small plastic containers and released on *H. sericea* plants in the field. The adults should be kept separately because they are cannibalistic when kept together in a confined space. Adult emergence is from the end of November to the end of February.

3.5.4.2 Implementation procedure

Between 20 and 50 adults can be released at each site. Every little is known about the dispersal ability of the beetles and it is recommended that the beetle be released in to as many sites as possible.

3.5.4.3 Indication of presence

Plants infected with the beetle are easily recognisable due to the presence of frass (sawdust) at the base of the plant. The bases of the stems also have characteristic thickening due to the formation of scar tissue.

Figure 3.9: Frass at the base of the plant indicating larval activity

3.5.5 Bud-weevil *Dicomada rufa*

3.5.5.1 Collection procedure

The method used to collect this agent is similar to that used to collect *E. consputa* (see 3.5.2.1). The best time to collect *D. rufa* is in May before the adults lay their eggs.

3.5.5.2 Implementation procedure

The release technique is similar to that of *E. consput*a (see 3.5.2.2). This insect is not yet available for implementation.

3.5.5.3 Indication of presence

The damage caused by the weevils is not that noticeable. The best indicator is to feel the flower buds of the plant. If they are soft and pliable the weevil is present. The larvae also "glue" together the flowers of the inflorescence which do not open properly.

3.5.6 Leaf weevil *Cydmaea binotata*

3.5.6.1 Collection procedure

The collecting method is the same as for *E. consputa* (see 3.5.2.1) and *D. rufa*.

3.5.6.2 Implementation procedure

The releasing technique is the same as for *E. consputa* (see 3.5.2.2).

3.5.6.3 Indication of presence

Look for hollowed-out leaves with tip die-back and scarred, wilted growth tips.

Chapter 4

Monitoring after release

To be able to asses the establishment and spread of the biological control agents, it is essential to monitor the plants for signs of the biological control agents in the years following the releases. The signs to look on the plant can be found in the “Indication of presence” section under each agent.

Chapter 5

Additional information

5.1 Literature

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5.3 Contact information

The website of this handbook can be found at http://www.No_Idea_Yet.org.za. This website will always contain the newest version of the handbook available in different formats (pdf, eBook, and as html). The book is also available, in limited quantities, in print. For ordering details see http://www.No_Idea_Yet.org.za/hardcopyorderdetails.

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