MEAT & LIVESTOCK AUSTRALIA

on farm

Biological Control of Paterson's curse

(Echium plantagineum)

Project number CS.209
Final Report prepared for MLA by:
CRC Weed Management Systems

Meat & Livestock Australia Limited
ABN 39 081 678 364

ISBN 1 74036 942 4

July 1998

Info Category

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INTERNATIONAL WOOL SECRETARIAT

Meat Research Corporation

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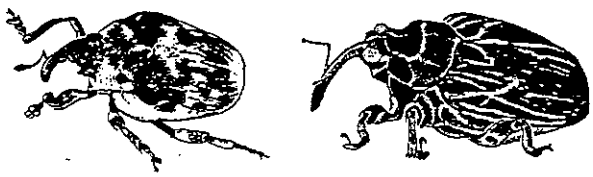
Introduction

This management package provides current technical information on the biology, rearing and recommended release protocols for biological control agents of Paterson's curse (=salvation Jane), *Echium plantagineum* L., currently available through an MRC and IWS funded project at CSIRO Division of Entomology.

This package targets organisations participating in the MRC/IWS "Redistribution of biological control agents for Paterson's curse" project, providing a basis for the development of mass-rearing and release technology for these agents. This version includes information on the most recent agent Longitarsus echii which was first released in September 1996.

General Biology

Mogulones larvatus & *M. geographicus*



4.5 mm 5.5 mm

The weevils *M. larvatus*, and *M. geographicus* are univoltine (one generation per year) and have 3 larval instars. In autumn they mate and begin to lay eggs in the leaf stalks of *E. plantagineum*, usually causing gall-like swellings. The eggs begin to hatch after about one week (at 12-20°C). The first instar larvae mine down the leaf stalk into the root crown, by which time they have reached second instar. *M. larvatus* larvae continue to mine out the crown while second instar *M. geographicus* larvae continue to mine down into the tap root. When larval development is complete, third instar larvae tunnel out of the crown or root into the soil (within a few centimetres of the plant), and form an earthen cell in which to pupate. New adults emerge in spring-early summer to feed on flowering plants before aestivating (over-summering). Decreasing autumn day-length induces the aestivating *M. larvatus* adults to feed and become reproductive. Oviposition occurs first in *M. larvatus* in February and is induced primarily by day-length, therefore, *E. plantagineum* rosettes may not yet be plentiful in Mediterranean climate parts of Australia. Adult survival prior to host plant germination is under investigation. In *M. geographicus*, oviposition is induced by shorter day lengths and starts in late March, although the influences of temperature and moisture as additional stimuli may play a role in initiating oviposition.

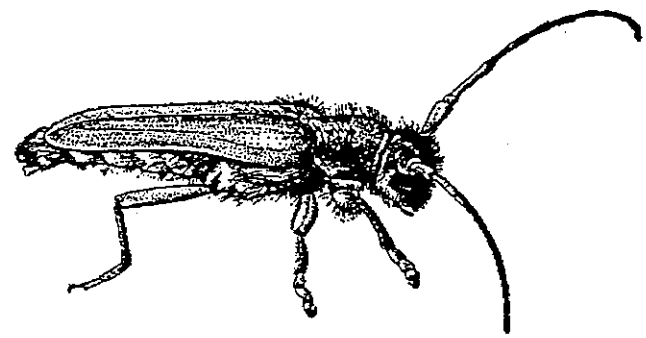
M. larvatus and *M. geographicus* females lay about 2-4 eggs per day for the first six weeks, following which egg production slows down. Individual females have been observed to lay up to 9 eggs per day. *M. larvatus* eggs are yellow and oval (0.7 mm long x 0.4 mm wide). *M. geographicus* eggs are white, oval, but narrower (0.75 mm long x 0.25 mm wide; Vayssieres 1986).

Sexing

Both male and female *M. larvatus* have a depression on the under side of the anal (last) segment of the abdomen. They differ in that the depression in the female is separated from the anus by a complete ridge. In the male a channel from the depression divides the ridge on the mid-line, resulting in two horn-like structures leading to the anus.

In *M. geographicus*, while body markings are variable, only the male has a marked depression on the underside of the anal segment which extends forward into the next abdominal segment, compared with the fully rounded abdomen of the female.

Phytoecia coerulescens



20 mm

P. coerulescens, the stem-boring longicorn beetle is also univoltine. Oviposition starts in spring, as *E. plantagineum* starts to bolt. Females typically oviposit beneath the epidermal layers on the basal third of the flowering stem, often at the base or actually in the mid rib of a cauline (stem) leaf. Eggs have also been found on cymes and in calyces. Eggs hatch within a week under spring temperatures. During the first three larval instars, larvae mine upwards and feed on the pith of the growing stem. Mature larvae have been observed within six weeks of hatching (15-25°C) in the laboratory. Once mature, larvae bore down the stem, which they girdle 4-10 cm above the ground, and continue

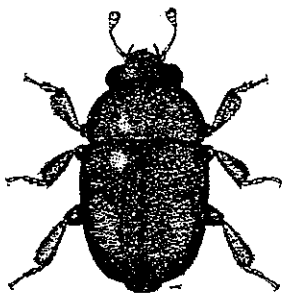
into the stem base or root stock. The mine is plugged at each end with stem material, forming a cell in which the larvae remain dormant through the winter. Pupation takes place in early spring and adults emerge from spring to early summer, mate and commence oviposition. Adult size (12-25 mm) is highly variable, resulting from both host plant size and larval competition levels within root stocks. If several larvae occur in a stem then usually only one or two survive as the largest larvae will kill the others (Kirk & Wapshere 1979).

P. coerulescens eggs are 2-3 mm long, yellow and cylindrical in shape. Adult female fecundity is high with egg production of 2-4 eggs per day.

Sexing

The abdomen in the male protrudes further beyond the elytra (wing cases) than the female, when viewed from above. The female abdomen (beyond the elytra) is also rounder. The safest way to check this, is to cage supposed pairs and check for mating

Meligethes planiusculus



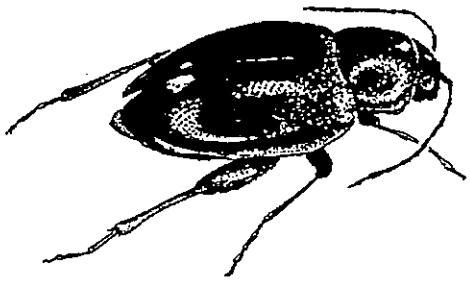
2 mm

Meligethes has 1-2 generations a year. Adults over-winter becoming active in spring on the developing flower stalks of *E. plantagineum*. Here they feed, mate and oviposit. At 15-25°C *Meligethes* produce on average 130 eggs per female (range 5-342) over an average oviposition period of 44 days (range 9-92) before dying. The white to opaque eggs (0.6 x 0.25 mm) are laid in the terminal buds of the flowering cymes and can be easily seen by separating unopened buds from each other. Eggs take 4-6 days to hatch at 20°C and the first instar larvae bore through the calyx and petal to feed on anthers, pollen and ovules within the unopened flower bud. Here they moult into final instar larvae. Final instar larvae are mobile and move between flowers on a cyme feeding on the developing seed. Final instar larvae complete development in 14-20 days at 20-25°C. They then drop from the flowers to pupate in the litter/soil. At 20°C, fresh adults emerge 10 days later return to the host plant and feed on pollen, developing ovules and maturing seed in open flowers of *Echium* spp. Adults that emerge before the longest day can oviposit leading to a partial second generation (Swirepik et al. 1996). At the end of the flowering period adults pass the remainder of the summer and following winter fairly inactive around their host (in the litter/soil).

Sexing

No morphological characteristics have been clearly identified so far to distinguish male and female adults due to the small size of the adults.

Longitarus echii



3-4 mm (female)

The flea beetle (so-named because its hind legs are adapted for jumping) *L. echii* is univoltine. Over-summering adults emerge from pupal cells in the soil in autumn to winter. Emergence is linked to decreasing soil temperature, daylength and most importantly rainfall. The uniform dark coloured adults immediately feed on rosettes forming small shot holes (smaller than *Mogulones spp*) in the leaves. Oviposition begins 2-3 weeks after emergence and eggs are laid on and around the taproot. Eggs (1.5 x 0.5 mm) are orange to red-brown in colour. At 12-20°C eggs take 2-3 weeks to hatch. Females produce 3-5 eggs/week and oviposition continues until late spring. Exact fecundity is not known but given the rate and period of oviposition, 100-150 eggs/female could be expected. Larvae mine into the cortex of the taproot and the finer secondary roots and can occasionally be found in leaf stalks if they are touching the soil. When feeding is complete the larvae leave the root and form an earthen cell in which they pupate. Adults remain in the ground until autumn rains trigger emergence. The over-summering biology of *L. echii* should make it an effective insect in Mediterranean climates as adult activity occurs with *E. plantagineum* germination.

L. echii may be confused with a native *Longitarsus* sp. that also feeds on Boraginaceae. The native is smaller and has two lighter bronze patches, one at the rear of each of the wing cases.

Sexing

No morphological characteristics have been clearly identified so far to distinguish male and female adults due to the small size of the adults. Though females tend to be larger than the males.

Phase 1: Insect Rearing

Plant propagation

Only *E. plantagineum* is a suitable host for *M. planiusculus*, while *Echium vulgare* L. is also suitable for *Mogulones* spp. and *P. coerulescens*. *E. vulgare* can be grown larger and may support, therefore, a greater number of insects.

Echium spp. rosettes should be grown in a light well-drained soil mixture. For example, a mixture of 1 part peat; 4 parts combined pearlite, vermiculite, and river sand; and 5 parts of light compost potted in a 20 cm pot has been successful. Fresh soil should be used each season to prevent the build up of soil borne pathogens that can increase insect mortality.

In order that plants can tolerate insect damage and produce many agents, they need to be as large as possible. This means allowing at least 2 months of rosette growth in a warm glasshouse before exposing them to ovipositing weevils (ie. start growing plants for weevils in early January). Plants need at least 3 months growth before they can be used to culture *P. coerulescens* and *M. planiusculus* (start growing by July). At that age, flowering stems should start to develop after 3 weeks in long day conditions.

If time and space limit culturing large plants, it is possible to transplant 20 cm rosettes with large tap roots from the field during autumn. In this instance, cut off the old rosette foliage, leaving a few young leaves around the crown, before re-potting. Enough leaves will regrow on these plants in 3 weeks.

Plant pest control

The leaf mining moth, *Dialectica scalariella*, is often a glasshouse pest when culturing *Echium* spp. Dichlorvos (1 ml/L : usually 20 L of solution is sufficient to fumigate a large glasshouse) fumigation has been used as a means of controlling it. A with-holding period of 2 weeks is necessary to air plants before exposing them to insects.

Aphids are also a problem in plant cultures, both before and after weevil oviposition. An application of Pirimor at 1 g/L will control aphids, while not harming the beetle larvae feeding within the plant.

Mogulones cultures (autumn)

Observe weevil oviposition by placing mated pairs into individual Petri dishes with freshly cut leaves, before placing the insects onto plants. Eggs will be obvious in and around the "galls" in the petiole, as well as hidden under the dermal layer of the leaf. When these hatch into first instar larvae (see above), up to 20 may be transferred onto a rosette.

Once oviposition has been observed, the culture is most easily maintained by either rearing in pots or preferably in the field cages.

Rearing in pots

When small numbers of adults are available for a rearing culture it is preferable keep track of them through intensive pot rearing. Pot rearing will then give you a better estimate of your likely adult return in spring. Place adults onto sleeved rosettes. Individual muslin sleeves, sewn to fit the 20 cm pots, should be placed over the pot before adding the insects onto the rosette. Four adults should be used per pot (2 female and 2 male if possible). Adults should then be transferred together with sleeve to a fresh pot twice/ three a week (this gives an average of about 14 eggs laid on each rosette). This system provides the key to rearing larvae: prevent too many larvae per rosette as this quickly kills the plant. Where larvae are killing plants, it is possible to dissect the larvae from the root and place them onto the crown of a fresh rosette. Avoid this where possible, however, as it has not met with great success (on average only 2 or 3 adults have emerged from pots into which 20 larvae had been transplanted).

Rosettes that have received eggs should be kept in natural temperature and daylength in a shade house or unheated glasshouse through autumn and winter. In a heated glass house, under natural daylength a cold treatment is necessary for *M. larvatus* during the larval stage to ensure pupation and to synchronise adult emergence in spring. To apply the cold treatment, allow larvae to reach last instar in the heated glasshouse, then move the plants either outside into winter conditions, or into a cold room, for between five and seven weeks at a maximum of 8°C before placing the plants back into the heated glasshouse. This length of cold treatment is the optimal requirement to ensure synchronous emergence. Emergence should begin within 5 weeks of being placed back into glasshouse conditions (15°C/25°C night/day) and finish by about week 11. Two-thirds of the emergence occurs in the first 2 weeks. Rearing *M. geographicus* in a heated glasshouse will result in the adults emerging in 8 - 10 weeks after oviposition. To prevent early emergence (well before normal field emergence) it is not recommended they are reared in a heated glasshouse. Larval mortality of *M. geographicus* reared in pots is high because soil in the confines of a pot can become saturated over winter, drowning larvae. To prevent this, all attempts should be made to limit the amount of water pots receive over winter and improve drainage. This can be done by having the pots under some roofing (ie plastic sheeting, laserlite sheeting or any material that allows light through) or in a unheated glasshouse through the wet and cold months of winter. It is important to maintain maximum light to the pots as this will increase plant vigour and water uptake, decreasing the chance of water-logging. This can be done by moving the pots to a northerly wall of a building or in a unheated glasshouse.

While availability of efficient controlled temperature and day-length facilities could generate more than one weevil generation per year, this is not recommended as laboratory cultures are at risk due to equipment failure and loose the seasonal synchrony necessary for autumn releases.
Don't waste your time with this technique!

Keep watering to a minimum. Ideally, water just before the soil is dry enough to induce wilting. This is necessary to reduce the likelihood of plant disease (ie. root rot), and water logging of the soil (which can kill larvae and pupae).

Rearing in field cages/insect houses.

Rearing in large field cages, insect houses or free releases are the recommended methods of producing adults. *E. plantagineum* is sown from seed or planted directly in the soil on site. Here plants can be grown and ready for the insects as they become active, removing our dependence on the autumn break. Insects are released on the plants and allowed to breed naturally without the regular interference from being reared on pots. The location for the on site release should be well drained, soil that regularly becomes boggy over winter should not be used. The rearing of *M. larvatus* should be kept separate from the root feeding insects, *M. geographicus* and *L. echii* if you are rearing in a cage. *M. larvatus* attacks *E. plantagineum* earlier then the root feeders, killing plants while the root feeders are still eggs and immature larvae. In the confines of a cage this competition will not benefit *M. geographicus* and *L. echii* cultures.

Adult emergence and maintaining insects through summer

At the beginning of spring (later in cool climate regions) it is necessary to check plants regularly for the first adult emergence (shot holes produced by adult feeding are a good indicator). At this stage, pots should be re-sleeved (if you are collecting adults per pot data) or placed into field cages or insect houses, in order to collect the new adults. In this and the field cage method, trap plants (fresh green rosettes in pots) are included in the cage to aid collection of new generation adults. Freshly emerged adults need to feed on *E. plantagineum* for 5-6 weeks after emergence before being over-summered. Successful over-summering is the other key stage for a successful culture. It can be done: 1) in a field cage or insect house, where it is less effort and less risky, but exact weevil survival is not known, or 2) in large holding cages in the shade house, where good conditions and careful monitoring can lead to very high survival. While 98% survival has been achieved, constant monitoring is necessary for successful aestivation. It is prudent to assume and plan for no more than 60% survival after aestivation. We recommend a divided effort. Any insects over and above further culturing requirements should be released in spring onto flowering *E. plantagineum* at nursery sites.

The best holding cage for adults (for culturing) was a large plastic or fibreglass vessel, 1.5 m x 1.5 m x 0.4 m deep, filled with potting mix containing flowering *E. plantagineum* plants (8 per square metre), over which was fitted a made-to-measure fine mesh cage 1 m tall. The flowering plants provide litter for the adults to hide in and when the plants die the root allows access to aestivation sites below ground. After the flowering plants die *E. plantagineum* seedlings should be planted, taking care to avoid disturbing the dead rootstocks. The seedlings will be a guide to the amount of watering necessary for the adults, enough to keep the seedling alive without them growing rapidly. This will keep the tub with adequate moisture without drowning the adults, moderating the effects of high summer temperatures. The seedlings will also act as food when the adults come out of aestivation. If aestivation does not occur (as indicated by high rates of feeding and activity) long day lengths may need to be imposed. Holding cages should be kept out of direct sunlight (preferably in a shade house or eastern side of a building). Smaller vessels have been tried, e.g. ventilated boxes containing sand, or tote boxes, but success has been variable, as more care is required to prevent overheating and over-watering. The soil in black plastic pots can quickly heat well above normal pasture soil temperature killing adults. Soil hygiene, i.e. replacing soil in containers between years, is important to prevent the build up of predators and soil-born pathogens.

Phytoecia cultures (spring)

P. coerulescens is considered the least effective of the control agents on *E. plantagineum* because of the limited recorded impact on seed production (Smyth and Sheppard *unpublished*). No effort should be put into laboratory rearing and all adults should be free released. Caging increases their mortality as adults will continually fly into the cage. The adults establish easily without a cage and disperse quickly producing a site that might be harvested after a couple of years.

Meligethes cultures

Rearing using potted plants is not recommended as insect house rearing is less effort, however the rearing on potted plants method (as used in quarantine) given below has proved useful with small numbers of insects.

Rearing using potted plants

Place eight to ten freshly flowering plants of *E. plantagineum* into a large cage (1×1×1.5m) made of fine gauze (mesh size of ~1 mm) with 50-100 *Meligethes* adults at ambient spring daylength. Plants can be changed once or twice a week depending on the temperature and number of cymes on the flowering plants (where plenty of flowering plants are available, changes twice a week are recommended). Egg production can range from 2-8 eggs per day per female, depending on temperature so ensure there are sufficient cymes for the ovipositing adults. A maximum of 10-15 larvae per cyme will allow enough plant resource for development to pupae. Plants are removed from the oviposition cage and adults are collected by sharply tapping the flowering stems. This causes most adults to drop off. The plant can then be taken from the cage and remaining adults removed by pooting and returned to the oviposition cage. Most of which are found in the terminal buds and calices of flowering cymes. Care should be taken

as adults can be very active fliers above 25 °C. Changing plants can be continued throughout the oviposition period. Plants with eggs are then placed in a separate gauze cage on trays with drainage holes filled with sand. Such trays provide pupation sites and should be large enough to catch all mature larvae dropping from the plant. Some larvae may pupate in the soil of the pot. It is important to minimise watering while *Meligethes* is pupating to prevent waterlogging of the sand/soil. Complete egg to mature larval development can take as little as a month, so towards the end of this period, watering should be kept to a minimum. If all the rearing plants are dead in a cage, a flowering trap plant should be put in the cage to attract newly emerged adults. This rearing method is particularly effective when there are small numbers of adults by maximising the distribution of eggs over culture plants and thereby limiting larval competition for plant resources.

The partial second generation complicates the rearing process. This can be prevented by keeping pupae and newly emerged adults in 12:12hr or decreasing daylength. These adults require a winter (current data suggest this is a minimum of 10-14 weeks at 5-10°C) to stimulate oviposition. During this period adults have been stored in Decor™ cylinders (20 cm high x 10 cm diameter), one quarter filled with a moistened perlite/vermiculite mixture. Cut cymes of *E. plantagineum* were added and changed as necessary. Following winter the adults can then be moved to an oviposition cage with bolting *E. plantagineum* plants where it may take two to three weeks for oviposition to start.

Suggested insect house rearing protocol

Although rearing of *Meligethes* has not yet been completed in the field this should not be difficult. We have already started this in a insect house with *E. plantagineum* in the ground. *Meligethes* adults were released onto the plot in spring allowing oviposition to occur naturally. Flowering plants should be maintained through February to ensure coverage of the entire oviposition period and a food source for newly emerged adults. Flowering trap-plants can be maintained on the plot through to March to allow late summer collection of most adults. Seed can then be sown (or rosettes potted out) in this plot to ensure flowering *E. plantagineum* for the next season. The rosettes act a refuge and food source for *M. planiusculus* over winter. In spring flowering plants of *E. plantagineum* will attract ovipositing adults which can be collected for field release/ redistribution.

Longitarus echii

The jumping behaviour of adult *L. echii* makes them difficult to work with. Adults also spend a large part of the year (>6 months) underground which has led to very poor recovery rates when collecting emergence from inoculated pots as success of the culture conditions can only be known *post hoc*. These factors result in a very low return for effort put into a culture. We have reared only 8 adults per female while rearing on pots. To maximise adult numbers, ground rearing should be used in a insect house, large field cage or even a free release on site. Procedures for rearing *L. echii* this way are the same for *Mogulones spp.* If resources permit, cultures of each of the root feeding insects should be kept separate. If not *M. larvatus* should be excluded from the culture as it will dramatically reduce plant quality for *L. echii*.

Phase 2: Field Release of Insects into Nursery Sites.

Mogulones spp.

The effort involved in successfully over-summering these insects will mean that most releases from cultures into nursery sites will take place using freshly emerged adults in spring. Such sites should not be harvested for the redistribution of agents for a further 18 months (ie. in autumn two calendar years hence). While harvesting a site for redistribution first becomes possible at this stage from a practical stand point, it is probably better to wait until the following spring (ie 2 years after release) when fresh adults have emerged. At this stage a larger number of adults may be more easily collected with a beating tray and pooter, rather than rummaging around on your hands and knees during the previous autumn for fewer adults.

Spring releases can be made at sites with large infestations of medium to large *E. plantagineum* plants. The number of insects released at a nursery site depends on the success of the culture. For spring releases 100+ insects are preferable to ensure establishment, assuming plenty of rosettes are present in autumn. Recent surveys suggest that while small releases appear to "establish" in the first 2 years, many of these fail to perpetuate so larger releases are recommended where possible. In January the soil surface can be disturbed (without trampling the adults!) to encourage a good 'crop' of *E. plantagineum* to germinate following the late summer/autumn rains. The autumn density of *E. plantagineum* must be sufficient to support the ovipositing adults that have passed the summer. If this is not achieved the insects may have problems locating suitable *E. plantagineum* plants.

Mark the site ready for redistribution in autumn or spring. Free releases of insects have been shown to be very successful in autumn or spring, providing the buffer zone is maintained free of stock.

Phytoecia coerulescens (spring)

Field releases of *P. coerulescens* should be made following adult emergence in early spring on a patch of large bolting *Echium* spp. Do not put in a cage.

Meligethes (spring)

The standard gauze cages will not restrict *Meligethes* because of its small size. We recommend releases into a finer mesh (~ 1 mm) inner sleeve as free released adults tend to quickly leave the release site. The best time for release will be in early spring with recently over-wintered adults. It appears several hundred adults are necessary as smaller releases do not seem to have established. The release site should be free of grazing to maximise larval survival. An alternative would be to release freshly emerged adults in late spring provided *E. plantagineum* is still flowering at the release site. Release at this time may be necessary if facilities are not available to over-summer and over-winter the adults. Again disturbance at the site should be limited to the amount necessary to ensure a flowering population of *E. plantagineum* the following year.

Longitarus echii

Field releases of *L. echii* can only be made in autumn/winter when adults emerge from the soil. Releases of 100 adults may be sufficient as this has been successful in the insect house at CSIRO Canberra, but larger releases are recommended at this stage. Newly emerged adults were seen until late April after the plot was watered, but may not appear until June/July. Caging is not necessary as caged and uncaged releases last year have both resulted in good attack rates (establishment won't be confirmed until the autumn break at these sites). It is more important to make the release in a patch of healthy *E. plantagineum* rosettes that has been fenced off to exclude grazing. The release site may need to be cleared over-summer to allow germination of *E. plantagineum* for the next season.

The Paterson's curse insect release form

Why?

The first purpose of the form is record keeping. Other than pure record keeping, the information will be used for a number of purposes including mapping to produce distribution maps of agent release. Information collected on factors such as Soil type, Aspect, Patch size and grazing regime will allow for analysis of how these factors effect establishment and rate of spread. This information will then be developed into release strategies that optimise an agents potential.

Who needs to use this form?

The release form, including the attached legend is to be used by both State and local coordinators such as shire weeds officers and Landcare coordinators.

How it works

The release form is to be filled out and kept by the coordinator directly associated with the release for use in monitoring establishment. A copy of the form will then be forwarded to the state coordinator who will in turn forward the information (preferably electronically in either Microsoft Access or Excel) to Anthony Swirepik at CSIRO Entomology in Canberra where a central data base will be maintained. Ideally each state coordinator will also maintain a data base for his/her own use. At the end of each release cycle a copy of the updated data base will be made available to each state. The monitoring process will simply involve the local coordinator visiting the release site annually for the first two years in order to assess establishment/failure and hence the need for a re-release.

Paterson's curse insect release form

The details required for sections **State Coordinator** and **Local Coordinator, Cooperator / Landholder** are largely self explanatory.

State and Local Release site number: The data base requires each release by a particular coordinator to have a unique number. Practically this means associating a number to each release that has already been made and sequentially numbering each release thereafter.

Release Details:

***Echium* Species**: There are the two main species of *Echium* to choose from *Echium plantagineum* (Paterson's curse), and *Echium vulgare* (Viper's Bugloss). Please specify! Initials will do.

Release date: obvious!

Number Free released:

Number Caged:

At the moment two strategies have been and continue to be adopted for releasing insects, caged and free release. Please record the number you have released in either fashion.

Site Status: A release site should fall into one of six categories. They are:

Site Status	Definition
Potential	Used for recording the details of a potential site
Released	Used once insects have been released
Recovered	Records that insects have been found at the release site after one generation (usually 12 months after release)
Established	Insects observed at a release site after two generations (usually two years)
Failed	Used to record the absence of insects at a release site at any point in the monitoring cycle
Re-released	Insects have been re-released after a "failed" release

Visit date: This box is used as a means of recording the date of a visit that has failed to confirm establishment of an agent but which has left you unconvinced that the insect has "failed".

Date established: The date of the visit when establishment of an agent has first been recorded.

Insect species: There should be six agents available for redistribution during the life of this program. The list of agents are:

Latin Name	Common name
Mogulones larvatus	Crown Weevil
Mogulones geographicus	Root Weevil
Longitarsus echii	Root Flea Beetle
Longitarsus aeneus	Root Hair Flea Beetle
Meligethes planiusculus	Flower Beetle
Phytoecia coreulescens	Stem Boring Beetle

Please check the appropriate box!

The Paterson's curse insect release form *continued*

Site Details

Grazing

Grazing should generally fall into one of the categories listed below in table 3.

Grazing Animal
Sheep
Cattle
Horse
Sheep+Cattle
All of the above
Ungrazed
Other, Please specify

Grazing system

The bulk of graziers use a continuous or set stocking rate, the aim of this section of the form is to identify any effects associated with a different method of grazing management.

Choose from

Continuous/Set stocking rate
Rotation
Strip
Time Controlled grazing
Other - please specify

Fencing

Please state if a release site is fenced or unfenced.

Soil Type

A farmer (cooperator) will generally know the soil type(s) of their property. Ask them, and record the soil type of the area that is infested by Paterson's curse.

Drainage

Is the site on the flat or on a hill? Ask the farmer if the area on which the release is to be made becomes water logged during winter.

Aspect

Aspect is obviously not an issue on flat ground. On a hill however it could be an important variable in determining insect establishment, ie a release site on the south west side of a hill will receive much less sun during winter than a site facing north east. Please record the direction the site faces.

Shade

Shade or full sun like aspect may be a variable which determines insect establishment. Please record shade details, eg release site is an old sheep camp under a tree.

Patch size

Paterson's curse typically grows in patches, estimate the size of the patch the release is being made on and record it.

Density

Patch density should be recorded in one of three states, light, medium, or heavy. A light infestation should be scored if there are gaps of greater than 1m between plants. A medium density infestation has plants less than one metre apart and may form small clumps. A heavy infestation should be scored if there is a uniform cover with the majority of plants touching.

Irrigation

Record the use or absence of irrigation

Pasture Composition

The information collected in this section relies heavily on the farmer/manager having made a rough assessment (or having a rough idea) of his/her pasture though out the year. A good manager should be able to give a reliable indication of pasture composition changes from season to season, a less skilled manager may not be able to. Managers will be encountered that have a limited knowledge of the components of their pasture and the ecological processes involved in the recruitment of their pasture. In this situation managers should be encouraged to develop some pasture assessment skills, as the skill is essential in order to effectively manage a pasture / grazing system. As biocontrol workers it is largely out of our scope to provide this sought of training to farmers, however there are a number of grazing programs such as Prograze and Pasture Plus that provide such training (see attached contact details).

The information is to be scored as a percentage, so that for each season there is a total of 100%. The information is useful from the purely biocontrol point of view as variation in pasture composition yields different competitive forces within the pasture, which in turn may have an effect on insect establishment.

The information may also yield a broad scale picture of the variables driving pasture systems dominated by Paterson's curse, which in turn will allow us to plan and implement integrated management strategies.

The Paterson's curse insect release form *continued*

Global Position

Where possible please provide these grid references so that a map of the distribution of each species of biocontrol agent may be produced.

Prevailing wind direction

In broad terms it is probably fair to say that in most places in temperate Australia the prevailing winds are associated with the seasons. Please ask the farmer the direction of the prevailing wind during each season and record it. For example if I were recording the direction of the prevailing wind for Canberra I would record: Summer NW-NE; Autumn NW-SW; Winter SW; Spring SW.

Insect dispersal

Revisiting sites one year and two years after release will be required to monitor insect recovery and establishment. During this visit information on the rate of **insect dispersal** may be easily and quickly recorded. To do this simply go to the release point and look for evidence of insect attack on Paterson's curse. Once you have established attack and therefore the **recovery** or **establishment** status, walk in four directions (North, South, East and West) away from the release point until you find no more attacked plants. Record direction and distance (m) from release point to the last observed attacked plant on each axis.

Contact Details

Prograze: Cameron Allen
Prograze Coordinator
Phone (063) 913 951
Fax (063) 913 899

Pasture Plus: Kondinin Group -
(09) 478 3343 Western Australia
(069) 21 4047 Wagga Wagga

Sustainable Grazing Systems Key Program
Ian Simpson
SGS Extension Facilitator
Phone (063) 614 491
Fax (063) 612 862

Paterson's curse agent release form

Coordinator

State | Surname | Given

State Coordinator :

State release number:

Local Coordinator :

Local release number:

Cooperator/Landholder

Surname | Given name | Property | Town

Mailing Address | Postcode | Phone

Release Details

Echium Species

Release date

Number Free released

Number Caged

Site Status

Visit date

Date Established

Insect species

- Mogulones larvatus
- Mogulones geographicus
- Longitarsus echii
- Longitarsus aeneus
- Meligethes planiusculus
- Phytoecia coreulescens

Please mark a box

Site Details

Grazing

Soil Type

Aspect

Patch size

Irrigation:

Grazing system:

Fencing

Drainage:

Shade:

Density:

Global position

Longitude

Latitude

Prevailing wind direction

Summer

Autumn

Winter

Spring

Pasture Composition

This section requires you to rank the relative proportions (as a percentage) of the five pasture component groups throughout the year. To do this it will be necessary to speak with the farmer, asking him/her to recall the broadscale, seasonal changes in pasture composition throughout the year.

	Autumn	Winter	Spring	Summer
Bareground				
Annual grass				
Perrenial grass				
Broad leaf weeds				
Legumes				

Insect dispersal

North

East

South

West

Phase Three: The Distribution Network

Checking for establishment of the Agents

Mogulones larvatus

See Paterson's curse monitoring form and associated documentation.

It is of interest to participants and the project as a whole, to collect data that indicate the successful establishment and impact of the agents. The data must record both the rate (% of plants attacked) and level (number of weevil larvae per rosette) of attack. The amount of time available to collect such data will vary. The following sample methodology would be the minimum sufficient to provide useful data.

Mogulones geographicus

As time allows 25-50 rosettes chosen at random from within a 5m wide area surrounding the release area should be carefully dug up with surrounding soil and placed in individual bags. Random sampling can be achieved by tossing a quadrat over your shoulder and removing *E. plantagineum* from the quadrat area. These samples should be dissected as soon as possible after collection; counting the number of larvae, pupae or fresh adults present and measuring the maximum root crown diameter just below the lowest leaf bud. This will provide an estimate of attack rate and attack level, by the weevils, as well as the plant parameters that will allow us to estimate the reduction in seed production due to the insect. Relating plants sampled and *Mogulones* per plant to the quadrat area will allow the calculation of *Mogulones* per unit area. It is important to toss the quadrat at least 10 times to maximise the accuracy of the sample.

Measuring the long term impact of the agent at a site from one year to the next, would be the next level of assessment this is done by measuring the density of flowering plants once a year in Nov.-Dec. (depending on region/season). We also use additional more accurate methods, such as comparison of reproductive effort of attacked and undamaged plants, or by measuring the seed bank. We advise groups interested in undertaking any such studies to contact us directly, because there are many pitfalls in collecting such data.

Phytoecia coerulescens

Attack to plant stems by *P. coerulescens* is easily observed. Cutting dead stems back to 15 cm above the ground in summer will reveal the mine of the larvae as a hollow stem filled with feeding damage (darker brown than the cream pith of an unattacked stem). At this time insects are in the stem-base or roots and will not be disturbed by these activities. If this assessment is left too late, stems may be hard to find and insect damage may become indistinguishable from natural decay. It can be safely assumed that at least one adult will emerge from each attacked plant found. Up to three mature larvae have been observed in a single large rootstock. A sample of 50 dead plants at the end of the flowering period will provide a reasonable estimate of abundance levels, if these plants are selected at random both for position in the release area and for size. Again, a quadrat will provide a random sample of plants and allow the calculation of *Phytoecia* per unit area. A sample of attacked root stocks (ca. 20) can be dug up and dissected to assess larval survival and to check on the presence of parasite attack on this agent (parasites cause 40-80% of larval mortality in the native range). Record the number of attacked and unattacked plants and stems sampled in these assessments as well as the number of larvae per plant following dissection.

Meligethes planiusculus

Recording *Meligethes* density in the field is most easily done by counting eggs and larvae on flowering cymes. To do this accurately a sample of 20-40 cymes are collected at random fortnightly and dissected (within 48 hrs to prevent desiccation). If too time consuming, a single sample at peak flowering (1.5 months after the start) will suffice. If plant density and cymes per plant are measured, *Meligethes* number per unit area can be assessed.

Secondary distribution of insects from release sites

It is requested that a "Paterson's curse insect release form" containing the details of each release (for both nursery and secondary distribution sites) be forwarded to the Division of Entomology (see contact page 8). It is preferable that each State collaborator maintain their own version of the database and forward an updated version to the Division, rather than sending completed release forms. A copy of the sheet is included as part of this package.

Mogulones spp.

A clearer indication of weevil establishment is eggs or damage to plants in the autumn/winter, 18 months after spring releases, when their presence is most obvious. This is the time to assess the nursery site for possible redistribution of the adult weevils. If damage is extensive around the release site and egg laying adult weevils are easy to find around the base of rosettes, sufficient insects can probably be collected to make two further releases. This should be done immediately while the insects are still laying eggs on the fresh young autumn rosettes. Fewer insects (eg. 50-100) are necessary for autumn releases as the eggs are laid immediately. A few releases with larger numbers of adults (200-500) is encouraged to check if population increase is enhanced. If damage at any release site is not extensive supplimentary releases may be required.

Redistribution to secondary release sites should be made in a large patches of *E. plantagineum* rosettes. The release should be free of grazing in the first 2 years to promote establishment. Over summer the plot may need to be cleared to increase *E. plantagineum* germination in the following autumn.

Insect collections and subsequent releases should be made in this fashion each autumn/spring, when sufficient insects are available, until the local demand for the agent is met.

Meligethes planiusculus

Meligethes adults are more inclined to fly between plants than *Mogulones* spp. Therefore once populations start to build-up spread will be far more rapid than for the weevils. It is important, therefore, to ensure release sites are not monocultures and are separated by 10's of kilometres to ensure a fast coverage of the distribution of the weed. A *Meligethes* release should be excluded from grazing so as to ensure establishment. Site selection for redistribution must take this into account, while at the same time ensuring local weed presence will continue in the future.

Secondary releases of *Meligethes* will be most successful in early spring. Adults can be observed in the field feeding on the first bolting stems of *E. plantagineum.* At this time adults can be easily and quickly collected by tapping/beating stems of *E. plantagineum* over a tray and is most efficiently achieved in early morning before they heat up and fly off. The adults can then be pooted from the tray and kept cool. Several hundred adults will be required to make a secondary release. If these can not be easily collected then the site should be left, or given supplimentary insects, and left until next year.

Longitarus echii

The best indication of beetle establishment is larval damage to plants in the spring, 18 months after autumn/winter releases, when their feeding damage is most obvious. The time to assess the nursery site for possible redistribution of the adult beetles though is some time after autumn rains. If adult activity is extensive around the release site and adults are easy to find, sufficient insects can probably be collected for one or two further releases. This should be done immediately while the insects are still laying eggs on rosettes.

Redistribution to secondary release sites should be made in a large patches of *E. plantagineum* rosettes. The release should be free of grazing in the first 2 years to promote establishment. Over summer the plot may need to be cleared (or grazed for a short period) to increase *E. plantagineum* germination in the following autumn.

Insect collections and subsequent releases should be made in this fashion each autumn/winter, if sufficient insects are available, until the local demand for the agent is met.

Monitoring forms for Paterson's curse crown weevil

The monitoring form is to be used at 10 - 15 release sites per state to provide more detailed information on both Paterson's curse and insect populations. The information to be collected is a direct extension of the information being collected for **Date establishment** and **insect dispersal** on the release form.
Data collection involves counting plants/attacked plants in 9 quadrats over the same transect used on the release form for **Insect dispersal.**

When to monitor

Monitoring for the crown weevil should be done during autumn or early winter. Owing to the variation in seasons between states the timing of monitoring will be left to the individuals involved. Ideally though monitoring may commence 6 - 8 weeks after the autumn break by which time blackened crowns caused by the crown weevils should be evident.

What to do

Monitoring form 1 - (The nine quadrat method)

Once you have established attack and therefore the **recovery** or **establishment** status, walk in four directions, the axis of a cross (North, South, East and West) away from the release point until you find no more attacked plants. In the simplest field situation sampling could be done on two axis of across. Obviously a variety of field conditions will be meet where you won't be able to impose the transects in the shape of a cross, use you common sense to make the sampling method to work as effectively as possible. Record the length (m) of each of the 4 transects. When you have determined **the length of a transect**, divide the length by 3 to calculate the **quadrat interval** (the number of quadrat intervals along a transect), record in metres.

Begin sampling at one end of the transect by placing a 50 cm^2 quadrat (or the appropriate sized quadrat depending on plant density) on the ground. **Do not actively select attacked** plants, the samples must be taken randomly. Simply count the number of plants in each quadrat. Once the number of plants has been recorded go back over the quadrat and count the number of plants that display the tell tale black goo that signifies crown weevil attack, record the figure. Repeat this procedure three times along the first transect at the calculated quadrat interval and then two times for each of the remaining three transects (so as to avoid re sampling the release point)

Monitoring Form 2 - (The 21 quadrat method)

The procedure is the same in this method is the same as above except that on the first of the four transect you will sample six quadrats and on the remaining three transects you will sample five quadrats. The number of quadrats sampled increases with distance from the release point. For example if a transect is 25 metres long walk 12.5 metres to the mid-point of the transect. You then sample two quadrats a couple of metres either side of the transect line. You then move 12.5m to the outer edge of the insect attack, where three quadrats need to be sampled. One on the transect line, and two 3 or 4 metres either side of the transect line.

Note that the transects may not be of the same length as insect spread may vary with direction away from the release point. Remember that in this instance **quadrat interval** must vary with **transect length.**

Pasture Composition

Gather pasture composition information on a seasonal basis as you did when the release was initially made. Information is to be collected during each year that you return to a release site.

Grazing History

This section requires that we attempt to capture the grazing regime over the past year. To achieve this it will be necessary to talk with the farmer asking him/her to recall the major changes in their grazing management.

Grazing system

The bulk of graziers use a continuous or set stocking rate, the aim of this section of the form is to identify any effects associated with a different method of grazing management. Choose from

Continuous/Set stocking rate
Rotation
Strip
Time Controlled grazing
Other - please specify

Paddock/Cell size

Paddock/Cell size is useful to us when we are not given stocking rate in Dry Stock Equivalents(DSE). If we know a number of a certain type of stock plus the paddock size we can calculate DSE.

For each season we need to know the **Grazing animal, Stocking rate(DSE),** the **Duration of grazing** and the **Duration of** (any) **spell** that may be given to a pasture.

Local Coordinator Form

A copy of the Local Coordinator form should be filled out for each local coordinator so that everyone involved in the network is able to contact each other.

Paterson's curse monitoring form 1

Date:

Landholder:

Property:

Release site number:

Transect 1	Plants/Quadrat	Attacked P/Q	Plants/Quadrat	Attacked P/Q	Plants/Quadrat	Attacked P/Q
Length :						
Interval :						
Direction :						

Comments

Transect 2					
Length :					
Interval :					
Direction :					

Transect 3					
Length :					
Interval :					
Direction :					

Transect 4					
Length :					
Interval :					
Direction :					

Pasture Composition

This section requires you to rank the relative proportions (as a percentage) of the five pasture component groups throughout the year. To do this it will be necessary to speak with the farmer, asking him/her to recall the broadscale, seasonal change in pasture composition throughout the year.

	Autumn	Winter	Spring	Summer
Bareground				
Annual grass				
Perennial grass				
Broad leaf weeds				
Legumes				

Grazing History

This section requires that we attempt to capture the grazing regime over the past year. To achieve this it will be necessary to talk with the farmer asking him/her to recall the major changes in their grazing management.

Grazing system

Paddock/Cell size Hectare

	Autumn	Winter	Spring	Summer
Grazing animal(s)				
Stocking rate(DSE)				
Duration of grazing				
Duration of spell				

Paterson's curse monitoring form 2

Date:

Surname Given name

Landholder

Property

Release site number

Transect 1 Plants/Quadrat Attacked P/Q Plants/Quadrat Attacked P/Q Plants/Quadrat Attacked P/Q

Length:

Interval

Direction

Transect 2

Length

Interval

Direction

Transect 3

Length

Interval

Direction

Transect 4

Length :

Interval

Direction

Comments

Pasture Composition

This section requires you to rank the relative proportions (as a percentage) of the five pasture component groups throughout the year. To do this it will be necessary to speak with the farmer, asking him/her to recall the broadscale, seasonal change in pasture composition throughout the year.

	Autumn	Winter	Spring	Summer
Bareground				
Annual grass				
Perennial grass				
Broad leaf weeds				
Legumes				

Grazing History

This section requires that we attempt to capture the grazing regime over the past year. To achieve this it will be necessary to talk with the farmer asking him/her to recall the major changes in their grazing management.

Grazing system

Paddock/Cell size Hectare

	Autumn	Winter	Spring	Summer
Grazing animal(s)				
Stocking rate(DSE)				
Duration of grazing				
Duration of spell				

Local Coordinators

Surname:

Given name:

Address:

Town:

State:

Phone:

Fax:

Email:

Soil Core Protocol

When

Soil cores should be taken when sampling for insect establishment/dispersal in late autumn/early winter.

Why

At the moment we have agreed to take one years soil cores in order to estimate a base line seed bank for our 10-15 detailed monitoring sites. If feasible we may continue to core on an annual basis. This will be discussed and decided at the next annual distribution project meeting.

How

Soil cores should be taken at random from a fifty metre square plot, that surrounds the release site being monitored. There are three main options for selecting the random position of a soil core, you may choose to follow a set of random coordinates, use a regular grid pattern that covers the entire plot or you can simply throw the dolly (the sleeve that fits over the soil corer that you hit) over your shoulder after having taken each core. The later is the method CSIRO use. An important thing to remember is that when throwing the dolly, ensure that you are moving around the plot rather than around a smaller sub-set of the plot.

Once you know where you want to put the core you hit the dolly repeatedly with a 1 kg hammer until the corer has travelled 10 cm into the soil. At this point remove the corer, throw the dolly over your shoulder and take the corer to the stand. The stand is most effective when used as a pair. Worker one places the corer into the stand, while the second worker places a plastic bag underneath the corer. Worker one then removes the soil from the corer with the plunger that sits in the back of the stand. Worker two then seals the plastic bag with either a rubber band or by tying a knot. Place the completed core into a labelled bag or container large enough for 50 cores. Repeat 50 times. This process shouldn't take more than 30-50 minutes with 2 people.

Once back at the lab place your cores in a cool room where they will keep for up to 12 months awaiting processing.

Processing

At CSIRO we like to think of soil core processing as a good job for the dead of winter or the height of summer, when it is preferable to be in the comfort of an air-conditioned lab. In summer we use cold water in winter, warm. If you are processing cores from a clay soil it is a good idea to soak an appropriate number of cores, in their bags with warm water. This helps to break up the clay before sieving.

You will need: Two sieves one of 3-4 mm diameter and one of 1 mm diameter, a sink, and a tap that you can connect a piece of hose or tubing to. The washing process begins by stacking the two sieves one a top the other (large diameter on top) and then placing the soil from one bag into the top sieve. You then set your tap running at a steady rate (not too hard as you will loose the sample over the sides of the sieve), you then, with the aid of the water break up any clumps, washing all of the material capable of going through the top sieve into the bottom sieve. Discard the contents of the top sieve. Repeat the above procedure for the bottom sieve.

Once you have a clean sample wash the contents into the lip of the sieve before washing it into a tray for sorting. We use shallow freezer containers, however any flat vessel will do. It is good to have about 1-2 cm of water over the top of the sample in the sorting tray so as to float off any remaining organic load.

If you are processing as a pair it is best to have one worker washing constantly while the other sorts. When the washer builds up a large enough backlog they stop and sought for a while. Once the backlog clears the tasks may be reversed. When processing alone it is a good idea to build up a number of cores 10-20 before stopping the washing process and beginning the sorting.

Sorting

We all know what *Echium* seed look like, so recognising them in the bottom of a tray should not present a problem. Work through the soil/sand in the tray with a pair of forceps in a structured fashion so that no seed are missed. When you find a seed place it on the rim of the tray or in a Petri-dish or the like. Once sorting is finished count the seed you have collected and record the number on the data sheet provided. The seed don't need to be kept so you may then discard them or use to grow plants for rearing. You can get through 50 cores or more per day processing soil cores this way.

Paterson's curse soil core form

Date of field collection

Lanholder

Release site number

Date processed

Property

Processed by

Core		Core		Core		Core	
1:		13:		26:		38:	
2:		14:		27:		39:	
3:		15:		28:		40:	
4:		16:		29:		41:	
5:		17:		30:		42:	
6:		18:		31:		43:	
7:		19:		32:		44:	
8:		20:		33:		45:	
9:		21:		34:		46:	
10:		22:		35:		47:	
11:		23:		36:		48:	
12:		24:		37:		49:	
		25:				50:	

Combining Biological Control With Current Farming Practices

Biological control agents are just one tool in integrated weed management. Current weed control practices must continue, although a knowledge of how they might affect biological control agents is important. Biological control will always be working to assist your efforts either on your property or indirectly somewhere else to reduce the total infested area

Herbicides

Chemical control of *E. plantagineum* has been demonstrated to be most effective after the conditions are too cold for further germination (May/June). After this time *E. plantagineum* is at a competitive disadvantage compared to clover and other pasture species (Piggin 1979). Delaying chemical application will also benefit the root weevils as they have a maximum amount of time to develop before their food supply is cut. If sufficient stock are available, we consider Piggin's (1979) spray-graze method would be most compatible with biological control. [ie. light application of herbicide, MCPA or 2,4-D, followed by 7 day break and then graze with sheep (preferably) at 5-7 times normal stocking rate until curse is eaten out or over-grazing of desirable pasture is imminent]. Experimental results show that MCPA and 2,4-D have no direct effect on the survival of the weevils (Smyth & Sheppard 1996). The heavy grazing should cause little harm to ovipositing adult weevils and minimal harm to both their mature larvae and pupae or *P. coerulescens* in the soil. Obviously there will be some loss of insufficiently developed weevil larvae within the crowns and roots of plants that are killed by spray-grazing, however this loss will be outweighed by the benefits of weed control (Your local District Agronomist, Noxious Plants Advisory Officer or Land Protection Officer etc. should be able to provide further relevant information).

Cultivation

In the initial years of insect establishment it would be clearly unwise to cultivate the release area as this may wipe out the populations of these beneficial insects.

Once there is a widely established weevil or longicorn beetle population, cultivation to re-seed pasture before autumn break should pose little threat. Even if some of the over-summering weevils and longicorn beetles were killed by cultivating at this time, many can re-invade from adjacent areas. Cultivation at this time may also be beneficial to the weevils in a year with an early autumn break, as this favours *E. plantagineum* (Piggin 1976). Many weevils should be able to complete development on the new rosettes between germination and application of chemical control (eg. spray-graze in May/June).

Cultivation after the autumn break is ill advised both for the control of *E. plantagineum* and the survival of weevils, which will remain vulnerable in the soil until mid-spring. Cultivating sites with *E. plantagineum* on a annual basis, even if done in summer, will also hinder biological control, as such regular disturbance will prevent the agent populations from reaching the maximum levels necessary to limit the weed.

Spraying for Red Legged Earth mite

Spraying "Le-mat" for the control of Red legged earth mite will have no effect on the *Echium* agents, as the spray is highly mite specific . General systemic insecticides such as "Rogor" are not compatible with biological control.

Hay-making

Hay-making will not reduce the effect of the *Echium* weevils as this takes place during their inactive phase. *P. coerulescens* populations may suffer if hay-making takes place while *E. plantagineum* is in flower.

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Contact Officers:

Anthony Swirepik or Matthew Smyth
Phone: 06 246 4252 Fax: 06 246 4000
CSIRO Division of Entomology
GPO Box 1700
Canberra ACT 2601