Integration of herbicide treatments with the plume moth for horehound control

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Summary

The plume moth *Wheeleria spilodactylus* (Curtis) is becoming widespread and demonstrating a useful impact on horehound *Marrubium vulgare* L. There are circumstances in which herbicide may be used to control horehound when *W. spilodactylus* is present and there is a need for more information on how the biological control agent may respond to herbicide if the outcome of these treatments is to be predicted. Herbicide applied to *M. vulgare* will prevent flowering for some time even if the plant is not killed. Previous work found that lack of *M. vulgare* flowers to feed on significantly shortened the lifespan of adult *W. spilodactylus*. The present study confirms this finding and shows that flowers of other species are not of any benefit in increasing the lifespan of *W. spilodactylus* that are deprived of *M. vulgare* flowers. Another important question is whether adult female *W. spilodactylus* will avoid herbicide sprayed *M. vulgare,* in favour of untreated plants several metres away, when selecting oviposition sites. Four separate trials at different times after herbicide application found that by three or four weeks after herbicide was applied female *W. spilodactylus* released on herbicide treated plants did move within a few days to adjacent untreated plants. The findings are discussed in relation to potential effects of herbicide treatment on population size and impact of *W. spilodactylus* in the field after herbicide is applied in different ways.

Introduction

The horehound plume moth *Wheeleria spilodactylus* (Curtis) is now established at a large number of locations across South Australia, Victoria, New South Wales and Tasmania (Wills 2000). At a number of sites the density of *W. spilodactylus* larvae appears to be sufficient to cause significant damage to horehound (*Marrubium vulgare* L.), particularly reduction in flowering following larval feeding in shoot tips. Complete defoliation and death of established plants has been observed at one site in Victoria. Biological control is generally thought of as the appropriate technique for weeds in situations where issues of accessibility and/or cost make other measures unattractive, and this is certainly the case for *M. vulgare* on large areas of public land and on low-value pastures and rangeland. Despite the apparently increasing success of *W. spilodactylus*, integration of this agent with herbicide application is of interest in the following situations: small areas of high-value natural ecosystems, or degraded systems that still contain rare species may require removal of *M. vulgare* in the short to medium term and managers may be unwilling to delay in the hope that biological control alone will be sufficient. Where insufficient funds or poor access prevent treatment of a large *M. vulgare* infestation but treatment around the edges is required to halt spread on to adjacent land, maintain clear areas along walking tracks or similar purposes. Where *M. vulgare* has encroached into higher value pastures for which herbicide application may be economically viable, including places where *M. vulgare* is not the major weed of concern but may be treated as a consequence of treating other weeds. Roadsides where routine herbicide treatments are made for a variety of weeds and applications will not be halted because of the success of *M. vulgare* biological control.

A further possibility is that herbicide treatment may be seen as a more attractive option to achieve rapid control if *W. spilodactylus* can be shown to provide post-treatment suppression of *M. vulgare*. Thus situations where herbicides would not previously have been used because of the difficulty of follow-up work and the expected rapid recovery of *M. vulgare,* would then be considered as worthwhile to treat.

A wide range of herbicides containing different active ingredients are registered for *M. vulgare* control and it was not feasible to conduct experiments on all of them. The work described here was done using 2,4-D amine as a representative of the widely used phenoxy herbicides; it is not registered for control of *M. vulgare* in all States of Australia and it is likely that the outcomes would be similar with many of the other registered products. Use of 2,4-D amine in this study in no way implies endorsement or recommendation of products containing this active ingredient in preference to other products that are registered for control of *M. vulgare.*

Glasshouse experiments have been carried out previously to investigate the effect of herbicide treatment on eggs and larvae of *W. spilodactylus*, and of lack of *M. vulgare* flowers on the adult moths (Ainsworth 1999). The conclusions from these experiments can be summarized as: adult moths will have shortened lifespan and reduced reproduction if deprived of *M. vulgare* flowers; female moths prefer healthy to adjacent herbicide-treated *M. vulgare*; development of eggs and larvae can continue on *M. vulgare* stressed by herbicide. The present paper describes further studies that

i. determine whether adult moths can switch to flowers of other species, and
ii. define in more detail the tendency of adult moths to select healthy *M. vulgare* rather than herbicide-treated plants.

An attempt is then made to predict in broad terms when herbicide should be applied in the field for optimum effect.

Methods

Moths used in the experiments were wild progeny of the 'Old French strain' as discussed by Clarke *et al.* (2000); they emerged from pupae collected in the field at Jack Smith Lake in South Gippsland in December 1998 and January 1999.

Moth feeding on flowers of different species

Adult moths of both sexes were caged individually from shortly after emergence and maintained at 25°C with a 14 hour photoperiod. Each cage contained a non-flowering *M. vulgare* shoot and, separately, a shoot of either non-flowering *M. vulgare*, flowering *M. vulgare* or a flowering shoot of *Cassinea aculeata, Hypochoeris glabra, Lavendula angustifolia, Echium plantagineum* or *Rubus fruticosus*. Four of the test species chosen are common plants that are often found near *M. vulgare* infestations and the fifth (*Lavendula angustifolia*) is more likely to be an acceptable alternative to *M. vulgare* because both are in the family Lamiaceae. Moths were checked daily except over weekends, and their survival and location in the cage recorded. A wick to provide water was inserted with the non-flowering *M. vulgare* shoot in every cage. Plant material was changed as soon as it became wilted.

Adult avoidance of herbicide-treated M. vulgare for oviposition

Plants used in this experiment were pot grown in a glasshouse and had been pruned of old flowering stems and fertilized with a slow release NPK product three weeks before the experiment. Plants were randomly allocated to spraying with either tapwater at 400 L ha^{-1} or 0.5 kg ha^{-1} 2,4-D amine (active ingredient) in the same volume, using a tracked boom sprayer. The rate was chosen on the basis of having previously been found to cause severe but slowly-developing damage to *M. vulgare* grown in pots. Nine plants from each treatment were randomly allocated to be used immediately post-spraying or one, two or three weeks later. The experiment was performed in three identical rooms each 2.9 m (north-south) by 3.1 m (east-west) and 2.4 m high. Use of plants in the open was not possible because of the occurrence of wild *W. spilodactylus* at the site. A transparent roof allowed for natural daylight and the north-facing wall was of mesh so that the temperature remained close to that outdoors. Three plants of each spray treatment were placed close to the east or west wall and three plants of the other treatment opposite, giving a minimum of 2.3 m between the two groups. During the first trial two rooms had the unsprayed plants on the west side and one room had sprayed plants on the east side; this was changed at the start of each new trial. Recently emerged moths were released on the unsprayed plants in each room and the location of the moths observed over the next week. Between eight and eleven females were released into each room with at least the same number of males. After each week the plants and any surviving moths were removed and the eggs laid on all plants recorded. New plants and moths were then placed in each room, with the side used for each treatment reversed each time.

Results

Moth feeding on flowers of different species

Figure 1 shows a clear response of male moths to availability of *M. vulgare* flowers with mean lifespan increased from 5.5 days without flowers to 16.1 days with *M. vulgare* flowers. The Kruskall-Wallis non-pararametric test was used due to heterogeneous variances and showed a significant effect of flower type (including the no flower treatment) on male lifespan (P=0.005). Flowers of species other than *M. vulgare* did not increase longevity compared to no flowers. Female moths showed no significant effect of flower availability on longevity when all treatments were considered together (P=0.32 Kruskall-Wallis), although the longevity with *M. vulgare* flowers was clearly higher than longevity without any flowers. Mean female lifespan showed some sign of being higher in the presence of flowers from all the other species compared to no flowers. Records of moth location within cages showed that female moths spent seven times longer on flowering compared to non-flowering *M. vulgare* shoots when offered this choice. When offered non-flowering *M. vulgare* or flowers of another species the females spent at least twice as long on the non-flowering *M. vulgare* as on the other species, suggesting that the alternative flowers were not attractive to female moths. Male moths showed variable and weak preferences between non-flowering *M. vulgare* and other flowers, with a much greater time (at least 76% of observations) spent on the top or sides of the cage.

Adult avoidance of herbicide-treated M. vulgare for oviposition

Distribution of eggs between herbicide sprayed and untreated plants is shown in Figure 2. The number of eggs laid per room in each week varied from 493 to 1317 with no particular pattern. Significant departures from a null hypothesis of number of eggs being independent of herbicide treatment, i.e. expected equal number on each, were tested for each room within each trial independently using the G-test, without adjustment to allow for the multiple comparisons. The null hypothesis of equal eggs per treatment is conservative in terms of detecting avoidance of herbicide sprayed plants, because the moths were originally released on the herbicide sprayed plants and would therefore have been expected to lay more eggs on them if no preference existed. Plants placed on the west side of the rooms appeared to be more attractive to moths than plants on the east side, perhaps due to an attraction to the warmer sunlit side of the room early in the day. Due to the unequal number of rooms with each treatment placed at the favoured side, the results are presented on a per room basis rather than averaging the outcome across all three rooms for each week. Oviposition decisions by the female

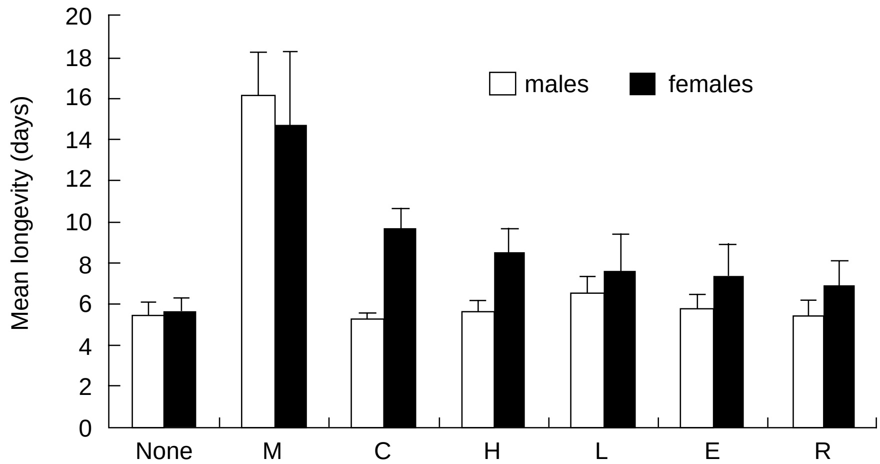

Figure 1. Longevity of moths provided with flowers of different species. Bars are means of 10 moths of each sex per plant species, vertical lines represent standard errors. Kruskall-Wallis test showed a significant effect of plant species on longevity of males (P=0.005) but not females (P=0.32). M = *M. vulgare,* C= *C. aculeata,* H = *H. glabra,* L = *L. angustifolia,* E = *E. plantagineum,* R = *R. fruticosus.*

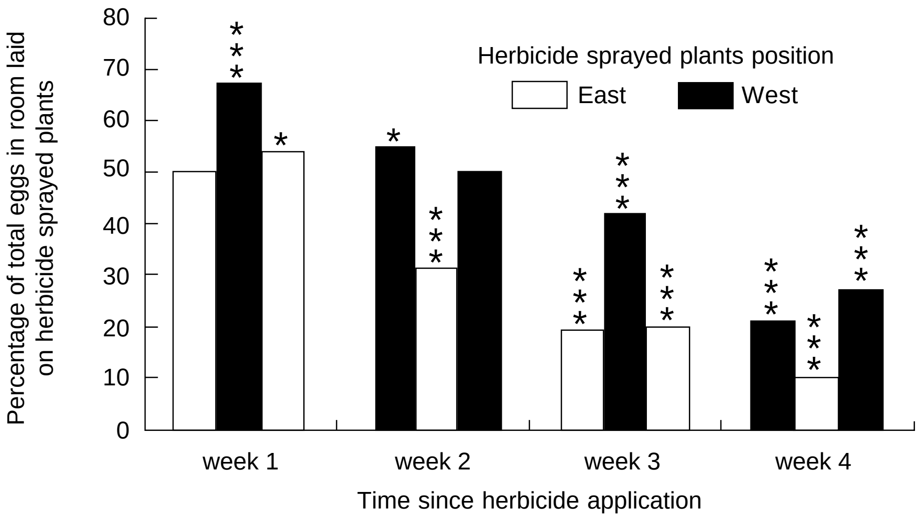

Figure 2. Oviposition preference in four separate trials at different times after herbicide treatment. Asterisks indicate significantly more or less than 50% of eggs in the room laid on herbicide sprayed plants (* P<0.05, *** P<0.001). Placement of herbicide sprayed plants in each room alternated as indicated.

moths resulted in clear preference for unsprayed *M. vulgare* in the trials conducted three and four weeks after the plants had been sprayed, despite the attraction to the west side of the rooms. No decreased oviposition on herbicide sprayed plants was evident in the first two weeks, except in the second room in the second week, where the herbicide sprayed plants were on the less favoured east side of the room. However, records of the locations of moths showed that the proportion of moths observed on the herbicide sprayed plants decreased within both the first and second weeks, to less than 20% by the last day, so that if these trials had been prolonged to the end of the oviposition period of the females the outcome would have been different.

Live flowers on the herbicide treated plants decreased with time since spraying so that by the fourth week there were no flowers on the herbicide treated plants in any room.

Discussion

A previous study (Ainsworth 1999) of the longevity of *W. spilodactylus* with or without *M. vulgare* flowers found similar results for males as were recorded here: lifespan 16.8 days with *M. vulgare* flowers, 6.2 days with no flowers. Females in the earlier study lived for substantially longer than males: 33.9 days with *M. vulgare* flowers, 8.6 days with no flowers, whereas the results presented here (Figure 1) show lower figures. With only 10 moths of each sex per treatment there is the possibility that a few weak females randomly assigned to the *M. vulgare* flowers treatment had a large influence on the results. Despite the suggestion of slightly longer survival by females with access to alternative nectar sources it does not appear probable that any large benefit could be obtained from such sources, especially in view of the apparent low attractiveness of these flowers compared to non-flowering *M. vulgare*. Herbicide treatment that prevents *M. vulgare* flowering is therefore likely to reduce the fitness of adult moths and thus decrease the number in the next generation unless dispersal to flowering *M. vulgare* occurs.

The previous work on oviposition preference of *W. spilodactylus* when offered sprayed or unsprayed plants also found avoidance of sprayed plants that increased as the herbicide effects developed (Ainsworth 1999). Cages used in the earlier tests separated the plants by a maximum of about 0.3 m and the present experiment was intended to investigate the response with more widely separated alternative oviposition sites. *W. spilodactylus* is not usually observed to be very mobile and one possibility was that it would not commonly cross several metres of open space to reach unsprayed *M. vulgare*. Results shown in Figure 2 demonstrate that movement over this distance does occur once herbicide effects have developed, although it appears that herbicide-damaged plants would probably still have some eggs laid on them by moths that emerged after spraying, because movement to the unsprayed plants takes place over a period of several days. Female *W. spilodactylus* could be moving in response to greater availability of flowers on the untreated plants or because the leaves of the herbicide treated plants are perceived as unsuitable oviposition sites and this triggers a searching response, or both factors could be involved.

McMillan (1990) found that herbicide treatment of *M. vulgare* in autumn was preferable, with effects developing more slowly than in spring treatments but the eventual control being better. Treatments in winter or summer are not usually attempted because cold or water stress respectively reduce herbicide effectiveness. An autumn herbicide application that killed most of the *M. vulgare* would have severe effects on the *W. spilodactylus* population if oviposition by the last adults before winter had already been completed. Even if a small number of plants survived, death of older shoots would be expected to lead to loss of many eggs and first instar larvae. Earlier autumn spraying would be preferable as it would allow adult *W. spilodactylus* to avoid sprayed plants to some extent, and increase oviposition on nearby unsprayed plants. Obviously this option relies on substantial amounts of *M. vulgare* remaining untreated, either because resources or inaccessibility do not permit total treatment or as a deliberate measure to conserve *W. spilodactylus*.

Spring herbicide application would ideally be timed so that the majority of the overwintering population were at the pupal stage. Emerging adults would then presumably behave as in the trial described here, moving within a few days to unsprayed *M. vulgare*. Once again, adjacent unsprayed *M. vulgare* would be required. Treatment later in spring once the first spring adults had laid most of their eggs seems likely to be less successful; rapid development of herbicide effects would cause large losses from the new *W. spilodactylus* generation. An important unknown in considering the above options is whether the dispersal to healthy plants observed here would be repeated over a larger distance and what costs in terms of decreased oviposition time or increased predation might be incurred by the moths. The trial in an enclosed room also did not take into account the possibility that many moths would fail to find nearby unsprayed *M. vulgare* and would be lost by dispersing away from available host plants. Where relatively easily treated *M. vulgare* is adjacent to more inaccessible plants the herbicide control of the former may lead to later suppression of the remainder of the infestation by the displaced *W. spilodactylus*.

An alternative to reserving unsprayed areas of *M. vulgare* would be to devise a herbicide treatment which many of the plants survive, although badly damaged, and which also allows survival and development of *W. spilodactylus* larvae so that the population of the agent remains high enough to continue suppression of the weed. Some glasshouse experiments (Ainsworth 1999) show that this outcome can, in principle, be achieved. Before this approach could be recommended the reliability of the desired sublethal effect in field conditions and the effectiveness of subsequent biological control would need to be investigated.

Integrating biological control agents with herbicides will not always be possible and will depend upon the biology of the agent and the weed and the characteristics of the appropriate herbicides. It seems, however, that there is scope for integrated control of *M. vulgare* in areas where herbicide use is affordable.

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