Factors affecting the establishment of a classical biological control agent, the horehound plume moth (*Wheeleria spilodactylus*) in South Australia.

Submitted by

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General Summary

Natural enemies have long been used for biological control (Simmonds *et al.* 1976) but predicting optimal conditions for successful establishment is still an uncertain process (Memmott *et al.* 1998; Grevstad 1999; Shea and Possingham 2000). Criticisms of planned introductions include perceptions of unnecessary risks associated with the release of 'another exotic' into the environment (Simberloff and Stiling 1996; M°Evoy and Coombs 1999) and the apparently low success rate achieved by such programs when compared to their cost (Julien 1989; Williamson 1996). On average, one in four agents achieve major impacts on the target species (Julien 1989; Williamson 1996). However, especially in Australia where alternative forms of control can ultimately be more expensive in financial cost and/or in damage to the indigenous environment, or just impractical, biological control may be the only viable option. Consequently, understanding the factors that affect the initial establishment of a biological control agent forms the first step in the process that will lead to improving the success rate for selected agents.

Although every biocontrol program is unique, classical biological control is the ideal ground to test some of the concepts and theories surrounding population establishment. In this project, the horehound plume moth (*Wheeleria spilodactylus* Curits), an agent introduced to control the invasive weed horehound (*Marrubium vulgare* L.), was used as a model system to investigate the following factors believed to influence the successful establishment of an introduced natural enemy:

1. Initial population size, host plant quality and time of release;
2. Interaction of population growth rates with decreased genetic diversity;
3. Expression of outcrossing depression for allopatric lines of horehound plume moth;
4. Expression and/or purging of inbreeding depression.

The final area of investigation was to retrospectively test the use of generic population viability analysis (PVA) and decision making tools for determining optimal release strategies for the

horehound plume moth in South Australia and to compare the outcomes with the empirical data collected during the course of this project.

The effects of initial population size and host plant quality on population establishment and growth were examined using replicates of 4 different founder sizes of Spanish collections of the horehound plume moth released at two different locations within South Australia in the spring of 1999. These locations were characterised by either high or low plant quality areas. Time of release was studied at high plant quality locations only, with releases of 4 different founder sizes made in the spring, summer and autumn of 1999/2000. Census data provided population growth rates for each release and were used to determine the relationship between release size, host plant quality, season of release and successful establishment. The existing hypothesis that there was a positive relationship between release size and likelihood of establishment was supported by the observed results. This was evident for both high and low host plant quality. The quality of the host plant was positively associated with successful establishment of colonies. Because season of release influenced host plant quality this indirectly affected the successful establishment of colonies.

Outcrossing allopatric lines of horehound plume moth was undertaken between cultures originating from France and Spain. The F_1 generation was bred in the laboratory from reciprocal crosses of equal founder size. Pure lines, for use as controls, were also bred using the same numbers of adults as foundation stock. The F_1 offspring were then released in the field and census data after one generation used to determine if outcrossing depression or vigour could be detected. The results suggested that the outcrossing of the allopatric lines produced an effect of vigour.

Individual horehound plume moth larvae were harvested from the field release sites for DNA analysis. Initially, it was hoped these would be analysed using microsatellites isolated and developed from a partial total genomic DNA library as well as with the multilocus technique of amplified fragment length polymorphisms (AFLP). However, during the course of the project analysis of microsatellite DNA was abandoned due to difficulties involving isolation of suitable markers. AFLP analysis was then developed to investigate the genetic diversity for each release

site. The AFLP data suggested that random founder effects and genetic drift were the primary factors in genetic differentiation for all releases. Contrary to what might be expected from the biocontrol literature, there was neither evidence of selection processes, nor any evidence of assortive mating in the outcrossed field population. The data showed that there was a positive association between the number of polymorphic sites and average genetic diversity across the loci and population replacement rates, indicating that slower population growth could be expected to result in lower genetic diversity.

The attempted purging of lethal alleles to eliminate potential inbreeding depression was undertaken with full- and half-sib mating programs for Spanish horehound plume moth. By generation two, there was a decrease of 24% in the number of viable offspring per female when compared to random outcrossed pairs. The number of lethal equivalents per gamete for the horehound plume moth was calculated to be 3.6. This figure is of a similar magnitude to that observed for many mammalian species, as well as for the lepidopteran species, *Dryas iulia* and *Heliothis virescens* (Roush 1987; Haag and Dearaujo 1994; Ralls *et al.* 1988).

PVA was undertaken using a generic software package that contained a component allowing the incorporation of inbreeding effects. Life cycle data and environmental variation were estimated from previously published studies (Weiss and Lippai 1996; Clarke 2001) and from observations made during the inbreeding experiment. The field experiments undertaken over the 2000/2001 spring-to-spring time period provided data for comparison with simulated populations. PVA for simulated populations was run for high and low host plant quality and varying founder sizes, with and without inbreeding incorporated. The predicted outcomes suggested that the inclusion of inbreeding effects for reasonable release numbers had no effect on the likelihood of establishment. Simulations predicted the detrimental effect that large variation in seasonal environmental conditions and host plant quality had on successful establishment. The predicted survival rates of horehound plume moth populations concurred with the observed field data. Using the predicted and observed probability of establishment and the optimal release strategy model developed by Shea

and Possingham (2000) also produced similar decisions on which release strategies would be preferred for high and low plant quality locations.

The main conclusions of this study on the horehound plume moth were:

1. There was a positive relationship between release size and probability of establishment;
2. High host plant quality and spring releases (after winter but while host plant quality was still good) improved establishment;
3. Random founder effects and genetic drift had more influence on the genetic profile of established colonies than selection;
4. The outcrossing of allopatric lines resulted in outcrossing vigor;
5. Inbreeding as a method of purging lethal alleles was unlikely to be successful;
6. Generic PVA reflected the observed outcomes;
7. Decision-making tools to determine optimal release strategies were useful when combined with PVA.

When releasing biological control agents, it was apparent that the economic savings come through a reduction of rearing costs. The establishment of harvestable nursery sites early in the program would be of more benefit than varying the number or size of initial releases. The study indicated that procedures for the successful establishment of the horehound plume moth could be improved. The variation in establishment rate in the different regions with contrasting plant quality showed that predictable local geographical, physical and climatic factors affected plant growth habits and so influenced the establishment of horehound plume moth colonies. This has a direct bearing on the efficacy of the control agent across the target regions. The use of generic software to predict the outcomes of various scenarios, as well as release strategies, for the horehound plume moth indicated that the use of decision-making tools could have enhanced the distribution and spread of this biological control agent. These results provide the impetus for other species to be investigated to determine if there are general rules regarding the regional effects on host quality and timing of release on successful establishment. It is also hoped that the use of predictive decision-making

software will continue to develop, thereby providing improved management tools for future biological control programs.

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