Will the red apion (*Apion miniatum*), a potential biocontrol agent for doublegee (*Emex australis*), establish in Australia?

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Summary Red apion, *Apion miniatum*, attacks *Emex spinosa* in the eastern Mediterranean region. Under laboratory situations it also readily attacks *Emex australis*, a major pest within southern Australia. Since 1998, CSIRO has been rearing and releasing the red apion on *Emex australis* infestations predominantly within the Western Australian grain growing area. To date 42 releases in autumn and 10 releases in spring have been made, varying in size from 60 to 2000 insects. At most sites, insects released in autumn breed up well during the plant's growing season. In the most favourable region, there was a 52 fold increase in numbers during the year of release, and up to 113 000 red apion went into aestivation per site. In field cages the insects have survived Perth's harsh summer conditions to breed the following season. At the field release sites however, we have been unable to detect more that a few insects the following season. It is not known if they have survived, and dispersed so that we cannot find them, or if they have died. This paper summarises the release program to date and discusses future strategies.

Keywords *Emex* species, *Apion miniatum*, release numbers, release strategy, biological control, Australia.

INTRODUCTION

The ultimate aim for all biological programs is for agents to establish throughout the pest's geographical range and to reduce the impact of the target pest species on agriculture or the environment. Many programs fail because the agent cannot establish. In weed biocontrol 59% of releases fail (Shea and Possingham 2000). Habitat instability is also a factor, with establishment rates within annual field crops being half that from more stable habitats (Beirne 1975). The probability of establishment increases with the number of individuals released whereas releasing agents at multiple sites safeguards against extinction due to unfavourable localised conditions (Hopper and Roush 1993). Typically there are limited numbers of individuals available for release, which restricts both the number of sites and the size of each release.

Doublegee (*Emex australis* Steinh.) is an annual southern African weed that is found throughout the grain-growing regions of Western Australia (WA). These areas have a Mediterranean climate, with the plant persisting as seeds in summer. Pre-season rains result in mass germination of annual plant species. Doublegee's large tap root enables individuals to survive until the true winter rains arrive and allows it to dominate pastures in these years (Parsons and Cuthbertson 1992). Seed banks can be 10,000 m^{-2} and persist for more than eight years (Gilbey 1996). Normally doublegee is not a strong competitor and is readily out-competed by pasture legumes and annual grasses (Panetta and Randall 1993). This characteristic should favour a biological control agent having an impact (Burdon *et al.* 1981).

A weevil, *Apion miniatum* Germar (red apion), attacks *Emex spinosa* (L.) Campd. in the Negev desert region of Israel. The climatic conditions there closely match the drier, more northern grain growing regions of WA. Red apion was found to attack *E. australis* as well as a few weedy, exotic species of *Rumex*, which are declared targets for biological control (Scott and Yeoh unpublished data). It has been approved for release in Australia.

Establishing widespread populations of a univoltine insect, with inherent relatively low fecundity, on a target that is an annual within an annual crop is a challenge that is only likely to succeed within a structured approach. The aim of this release program was to determine the optimum size for releases of red apion. Subsequent use of this information, within a re-distribution program, will maximise the rate of spread of red apion nationally (Shea and Possingham 2000).

METHODS AND RESULTS

The red apion life cycle Females lay eggs at a rate of an egg a day from late autumn through to early spring. All immature stages of development, including pupation, occur within the host plant. There is a long larval developmental period (567 degree-days above a lower developmental threshold of 8°C, unpublished data) with eggs laid late in the season failing to complete development in shorter growing seasons. Upon emergence in spring, the light tan adults feed on *Emex* foliage, changing to red in the process, before they aestivate. Fully fed adults show no negative geotaxic tendencies and do not burrow into soil. They initially fly upwards and in the field cages, after hitting the walls, they are gregarious and negatively phototaxic. If placed upon rough-barked trees, they burrow out of sight into crevices. Nothing is known about the natural aestivation behaviour of red apion. Since its habitat is devoid of green, annual vegetation in summer, it is assumed that they disperse to shrubs/trees to seek shelter and then in autumn seek new *Emex* infestations.

Founding colony A total of 533 individuals was collected between 1993 and 1995 from sites between the Gaza strip and the West Bank, Israel (31–32°N, 200–600 mm annual rainfall). These were imported to quarantine in Perth for host-specificity trials. Subsequently 520 of their offspring were removed from quarantine and used for mass rearing.

Mass rearing Initial mass rearing, using potted and caged plants, was both laborious and inefficient (population increase 3 fold), because red apion killed the plants even at low densities. Hence two field cages (6 × 10 m) were used. In the first year this was very successful, producing a 50 fold increase. Problems with inter-specific competition (from clover and medics) affected production for a couple of seasons despite selective applications of glyphosate and hand weeding. Fumigation with methyl bromide and sowing of doublegee in rows, in conjunction with hand weeding, is now proving successful. The red apion available for release (year) were as follows: 7430 (1998), 9660 (1999), 4000 (2000), 3000 (2001) and 18,000 (2002).

Release strategy Release sites were concentrated within WA because the red apion biotype was collected from Israel in a region climatically similar to the Mullewa – Geraldton area (using the computer-based system, CLIMEX). Releases consisted of approximately 62, 125, 250, 500, 1000 or 2000 individuals per site. They were distributed within 3 regions (Figure 1) and individual releases were at least 3 km apart. Northern release sites (28–31°S, 255–510 mm rainfall) were within the Mullewa – Geraldton area. Eastern releases (31–34°S, 255–510 mm rainfall), consisted of WA's eastern and southern grain growing regions. Southern release sites (34–35°S, >510 mm rainfall) are influenced by coastal weather patterns and are predominantly used for grazing beef and sheep. A release site in Perth is within this group.

In 1998, 10 spring releases were made onto tree trunks near doublegee infestations. There were three eastern, two northern and five southern sites (average 730 ± 110 red apion per site). No subsequent spring releases have been made. Autumn release sites were distributed evenly over regions in 1998–1999. In order to increase the probability of establishment, later releases were all 1000 or more insects and avoided the southern release region. In 2000, two releases of 2000 insects were made in the eastern region. In 2001, releases of 1000 insects were made at one eastern and two northern sites. In WA, 52 releases (average of 460 red apion per release) have been made at 47 sites. Three autumn releases (total 840 insects) were sprayed/overgrazed with no offspring resulting. The

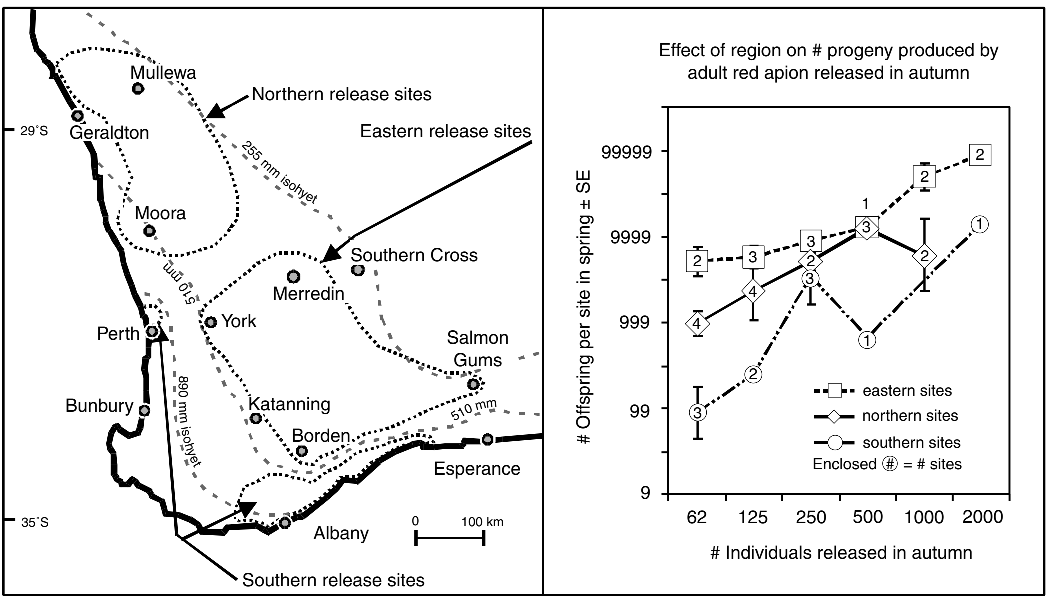

Figure 1. Release areas within WA and progeny production within these regions.

size and general location of the remaining 38 autumn releases (total 15,940 insects) are shown in Figure 1. Additionally five 1998 autumn releases (total 595 insects) occurred in NSW/SA. There was no spring assessment, hence these have been excluded from this report.

Criteria for selection of red apion release sites Sites chosen had high densities of doublegee with remnant vegetation close by (<100 m) and were protected from both grazing and applications of herbicides or insecticides. The immediate release area had to be destined for pasture during the following season so that the progeny would not be resource limited. The insects were released in autumn as sexually active adults over >100 m² within a patch of doublegee that extended for at least 1500 m². The resources immediately available to red apion (autumn release sites only) at the time of release are summarised in Table 1.

Assessment of progeny reared during the season Autumn release sites were revisited in spring to estimate the number of offspring produced. Quadrats (0.176 m²) were thrown within the original release area (n=10) to estimate the number of doublegee plants m^{-2}. Random plants were selected (n=20) from the same area (closest crown to a thrown object) and dissected to estimate the number of offspring per plant. Similar estimates of plant and insect densities were conducted at 5–10 m intervals, moving progressively outwards from the release area, in four different directions until no further insects were detected. Ten quadrats were thrown and 10 random plants were dissected at each distance in each sector.

Individuals released in autumn at the eastern sites produced twice as many offspring as those released in the northern region, which in turn were twice as productive as those in the cooler coastal southern region (Table 1, $F_{2,35}$=16.7, P<0.0001). At one eastern site, 2000 insects produced 113 000 offspring. The average eastern site had a spring population of 28,211 adults, with the lowest productivity being 3246 insects. To the north the range in spring-population sizes was 346 to 16,199 with an average of 6018 individuals. One site in the southern region produced 15,384 offspring (from 240 parents) in contrast to another site where 1920 parents produced 14,327 offspring. Most southern sites had very poor offspring:parent ratios (<7:1) and spring populations of <1000 red apion.

For the 35 sites surveyed both in autumn and spring, offspring production was found to be correlated (P<0.05) to the initial density of doublegee plants (r=0.34), initial percentage ground cover of doublegee plants (r=0.34), the percentage ground cover of other plants (r=-0.41) and the density of doublegee plants in spring (r=0.43). Inclusion of these variables as covariants, when predicting the effect of regions on red apion reproductive outputs, did not however account for any significant variation with most variability still explained by the site's region ($F_{2,28}$=6.27, P=0.005).

Assessment of establishment Surveys were conducted during spring/early summer. Doublegee near remnant vegetation was inspected for signs of adult feeding damage and random plants were dissected to find larvae or tunnels More than one person hour per site was spent looking for signs of establishment.

It is too soon to assess last year's three sites (average spring populations of 32,260 offspring). From all the other sites, red apion have only been detected at one site (spring population = 14,327 in 1999), where one person day's searching found two larvae. None were detected at this site in the following year.

Table 1. Comparisons of resource availability at the time of release and the production of progeny (means ± SE). Raw data was normalised by either log $(x+1)^l$, $\sqrt{(x+0.5)}^s$ or arcsine $\sqrt{x}^r$ transformations. Means followed by the same letter are not significantly different (Tukey HSD test: 5%). N.B. Three destroyed sites not included. Plant densities not assessed at four sites in spring. Offspring densities not assessed at one site.

Assessed in autumn	Eastern sites (n=12)	Northern sites (n=15)	Southern sites (n=8)	All sites (n=35)
# doublegee plants m^{-2} ^{s}	57.6 ± 14.0 a	49.5 ± 9.6 a	28.4 ± 5.5 a	47.4 ± 6.5
leaves/doublegee plant ^{l}	4.7 ± 1.1 a	6.2 ± 1.0 a	3.6 ± 0.7 a	5.1 ± 0.6
% cover (doublegee) ^{r}	15.8 ± 5.0 ab	22.4 ± 4.2 a	5.8 ± 1.6 b	16.3 ± 2.7
% cover (other plants) ^{r}	20.9 ± 5.0 a	42.6 ± 5.5 b	49.2 ± 4.3 b	36.7 ± 3.6
# doublegee per apion ^{l}	78.6 ± 55.6 a	30.8 ± 9.9 a	13.0 ± 2.6 a	43.2 ± 19.5
Assessed in spring	**Eastern sites (n=13)**	**Northern sites (n=15)**	**Southern sites (n=10)**	**All sites (n=38)**
# doublegee plants m^{-2} ^{l}	35.0 ± 10.0 a	16.9 ± 2.6 a	12.1 ± 3.9 a	21.8 ± 3.9
# offspring per parent ^{s}	52.6 ± 7.5 a	24.6 ± 5.4 b	9.8 ± 6.1 c	30.3 ± 4.6
# offspring per site ^{l}	28,211 ± 958 a	6018 ± 1429 b	3460 ± 1910 c	12,937 ± 3793

DISCUSSION

Doublegee occurs within a habitat that is stark and scorched for several months of the year. Many insects burrow into the soil, have highly resistant life stages or disperse to more suitable habitats to avoid such extreme environmental conditions. Red apion appears to have adapted a transient approach, dispersing to shelter over summer and back to new infestations of doublegee in autumn. This dual dispersal phase (both prior to mating) decreases insect densities and therefore increases the chances that virgin insects will not encounter each other. With small populations, this quickly leads to localised extinction (Hopper and Roush 1993). With this perspective, the release numbers needed to get establishment are more likely to be akin to those used for arthropod parasitoids than for arthropods of weeds. For many weeds, establishment of arthropod populations has been achieved with very small release numbers (e.g. 30–100 weevils), although to get consistent establishment and to enhance the rate of control, larger releases (400 or more) are now recommended (McFadyen 1998, Swirepik and Smyth pers. comm.). Hopper and Roush (1993) recommend >1000 per release for consistent establishment of lepidopteran parasitoids.

Previous attempts at establishing biological control agents on doublegee in Australia are thought to have failed because agents were unable to survive the summer heat (Scott 1992). Because of this, releases of red apion have intentionally been made in the cooler southern coastal regions of WA as well as at a few uniform rainfall or irrigated sites in eastern Australia. During the breeding season, red apion performed poorest in the cooler conditions. Selective mortality of attacked doublegee, whilst under interspecific competition, is known to affect offspring production in our mass rearing cages. It is suspected of being partly responsible for the results seen at the southern release sites, but other unknown reasons are also contributing. Red apion's native habitat appears as harsh as any areas in Australia and the authors have observed individuals surviving over summer without host plants or irrigation in field cages set up in Perth and so believe that physiologically red apion can endure Australian conditions. It is possible that red apion has in fact established. It is common for agents to be undetectable for many years (McFadyen 1998).

On the assumption that establishment so far has failed due to a combination of releases sizes being too small and insect dispersal, we are currently conducting two massive releases. At each site, there will be four populations (1 km apart), each founded by 2000 autumn released insects. It is assumed dispersing insects will reunite with others from the neighbouring populations. Our current breeding program will only allow for two such releases a year. Establishment at a few field sites will however offer massive breeding colonies from which red apion can be harvested for redistribution.

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