

The influence of pollen competition on gene flow in small patches of *Lolium rigidum* Gaud. (annual ryegrass)

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Summary *Lolium rigidum* Gaud., annual ryegrass, is a serious weed in southern Australian cropping systems. This species is wind pollinated obligate and an out-croser. Many farmers are concerned that they can do little to prevent herbicide resistant genes being carried by wind-borne pollen introducing herbicide resistance onto their properties, despite costly *in situ* management practices. Essential data on pollen movement and gene flow is required in order to understand the level of risk posed by pollen carrying herbicide resistance genes.

Gene flow has been previously shown to be affected by the distance between the donor and acceptor plants and the density of acceptor plants. However, information about the influence of localised pollen competition is scanty. This study examines the effect of the density of acceptor plants on gene flow between biotypes susceptible and resistant to fluzifop-p-butyl herbicide. The results show that small populations of susceptible plants can effectively flood the area with enough pollen to reduce gene flow from a single resistant plant to extremely low levels.

Keywords Pollen competition, *Lolium rigidum*, herbicide resistance, fluzifop-p-butyl, gene flow.

INTRODUCTION

Herbicide resistant *Lolium rigidum* Gaud. now infests many grain-growing fields in Western Australia (Owen *et al.* 2007). While the intensive use of herbicides by grain growers drives herbicide resistance evolution, the rate of resistance evolution may be modified by gene flow. The introduction of herbicide resistance alleles into a population from outside has the potential to increase the rate of resistance evolution or to ensure evolution occurs where it might not otherwise have done so. There is a belief among some grain growers in Australia (Llewellyn *et al.* 2002, Llewellyn and Allen 2006) that herbicide resistance will arrive from their neighbours. Such beliefs, if acted on, will negate the adoption of prophylactic herbicide resistance management strategies.

Gene flow between weed populations could occur by seed or pollen movement. Movement of *L. rigidum*

seed could occur through contaminated sowing seed, hay or machinery; however, pollen is also thought to be an important vector for the movement of herbicide resistance genes in *L. rigidum* (Llewellyn and Allen 2006). Research has established that successful cross-pollination can occur between herbicide-susceptible and herbicide-resistant *L. rigidum* from pollen that has drifted up to three kilometres (Rognli *et al.* 2000, Watrud *et al.* 2004, Bussi *et al.* 2007). Pollen dispersal shows a leptokurtic distribution, with neighbouring plants receiving most of the pollen released from both wind- and insect- pollinated species (Gleaves 1973, Handel 1983).

Pollen competition is an important factor in pollen-mediated gene flow. It is important to consider the relative sizes of donor and acceptor populations when considering the risks posed by gene flow. By combining information on both distance and pollen competition, the risk of gene flow can be elucidated and management strategies better designed. This study examined the effect of pollen competition on isolated small *L. rigidum* populations with the plants in close proximity to each other to determine the dynamics of pollen competition in *L. rigidum*.

MATERIALS AND METHODS

Two biotypes of *L. rigidum* were used for pollen competition experiments in 2005 and 2006. The biotypes were VLR1, susceptible to fluzifop-p-butyl, and WLR96, resistant to fluzifop-p-butyl. Both these biotypes had been maintained by line breeding at the Waite Campus, University of Adelaide for many years.

In 2005 an experiment varying the ratio of susceptible to resistant *L. rigidum* biotypes was undertaken at the Waite Campus. VLR1 and WLR96 biotypes were grown as single plants in 255 mm pots, filled with cocoa peat and hand watered as required. Five treatments, run in triplicate, were set up as artificial patches. Each patch was isolated from neighbouring patches by 5 m and surrounded by plastic. Some pollen movement between the patches was possible because the patches were exposed to natural wind

movement. The immediate area was surveyed for *L. rigidum* plants, which were removed by hand pulling when located to minimise any cross contamination from volunteer plants.

Treatments consisted of a control (two VLR1 plants, side by side to measure the background level of resistant pollen movement between patches), and four treatments of a single WLR96 plant surrounded by one, five, 10 or 20 VLR1 plants. Seed was harvested from each VLR1 plant and bagged separately. The bags were left in a warm dry environment over summer to dry completely and break seed dormancy.

The following winter all seed from each plant was sown separately onto one or more labelled seedling trays and grown to the two leaf stage. The number of seedlings per plant was recorded before spraying with fluzifop-p-butyl at 53 g a.i. ha⁻¹, plus 2% oil (DC Trate). Seedlings that continued to grow two weeks after spraying were recorded as resistant.

In 2006, the experiment described above was repeated with the following changes; a single WLR96 plant surrounded by one, two, three, four, five, eight or 10 VLR1 plants. These changes provided a focus on the area where the asymptote was reached in 2005. After separating seed from debris the total seed weight was recorded for each VLR1 plant. The number of seed in one gram of seed was recorded for each plant and sown onto separate seedling trays. The seedlings were counted and treated with fluzifop-p-butyl and survivors assessed as described above. SAS Jmp V4.0 was used to fit the regression curve describing % survival and Microsoft Excel was used to display the data and regressions.

RESULTS

In both 2005 and 2006, the percentage of resistant seed-set on the susceptible plants declined rapidly with an increasing number of susceptible plants present. When there were five susceptible plants in the patch, less than 10% of the seed was resistant (Figures 1 and 2). With 20 susceptible plants in the patch, less than 1% of the seed was resistant.

For both years combined, the regression describing the relationship between percent survival and the number of susceptible plants in a population was:

$$\% \text{ survival} = 148.4 * e^{(-0.77x)}$$

where x is the number of susceptible plants in a patch. Extending this model suggests that if the incoming pollen density is 100 times less than the resident pollen density, the frequency of pollination events will be considerably lower than 10⁻¹⁰.

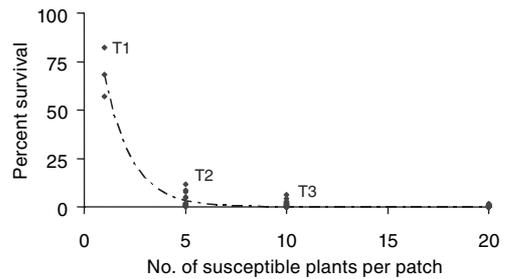


Figure 1. Percent of ARG seedlings surviving field rates of fluzifop-p-butyl in the 2005 experiment with increasing number of susceptible plants per resistant plant in each isolated patch Treatment 1, 1R:1S; Treatment 2, 1R, 5S; Treatment 3 1R, 10S; Treatment 4 1R; 20S, where R = resistant biotype; S = susceptible biotype.

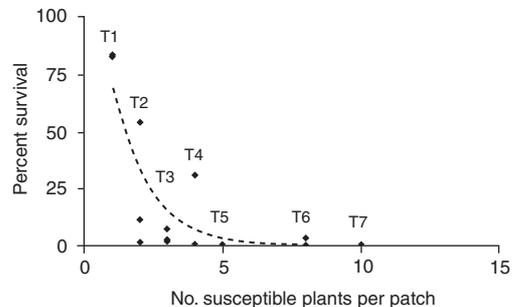


Figure 2. Percent of ARG seedlings surviving field rates of fluzifop-p-butyl in the 2006 experiment with increasing number of susceptible plants per resistant plant in each isolated patch Treatment 1, 1R:1S; Treatment 2, 1R, 2S; Treatment 3 1R, 3S; Treatment 4 1R; 5S, Treatment 4 1R; 8S; Treatment 4 1R; 10S, where R = resistant biotype; S = susceptible biotype.

DISCUSSION

This research suggests that pollen competition from within the field can have a dramatic effect on the impact of pollen arriving from over the fence. Effective pollination (i.e. gene flow) is also strongly affected by resident plant density. Once the number of susceptible plants in a patch was greater than five, the level of effective pollination with the herbicide resistance gene was very small. Therefore, a small amount of pollen entering a field with a large resident population of *L. rigidum* is likely to have little effect on increasing the frequency of herbicide resistant individuals in that field. However, if the resident population is very

small, i.e. one or two *L. rigidum* plants present in the field, pollen from outside will have greater effect on resistance frequencies.

This confirms earlier studies of pollen contamination during seed multiplication of different grass cultivars that have shown the level of contamination can be reduced by reducing the size of the pollen donor field or by increasing the number of receptive plants (Griffiths 1950).

The answer for managers is not simple. Herbicide resistance can come over the fence in pollen, but because of the number of resident *L. rigidum* plants present in most fields, pollen movement is likely to have minimal impact on the selection for resistance. Management of pollen movement of herbicide resistance is difficult as effective means of managing pollen ingress on farms are not readily available. The movement of herbicide-resistant seed in hay, machinery or sowing seed may be more important to the spread of resistance over long distances. It is also a problem that can be more easily managed by grain growers.

ACKNOWLEDGMENTS

Tony Cathcart and Will Pryor for their technical assistance and Peter Boutsalis for his excellent advice.

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