USE OF GENETIC MARKERS TO QUANTIFY POLLEN COMPETITION AND IMPLICATIONS FOR SEED YIELD IN TRIFOLIUM REPENS

A.H. Marshall[1], A.A. Cowan[1], T.P.T. Michaelson-Yeates[1] and M.G.Rodet[2]

[1]Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Dyfed, Sy23 3EB, UK.

[2]Station de Recherche de Zoologie et D'Apidologie, INRA-Avignon, Domaine Saint-Paul, 84143 Montfavet CEDEX

ABSTRACT

Genetic markers within white clover (*Trifolium repens* L.) were used to study competition between pollen sources. An experiment using homozygous red leaf markers as pollen donors and normal white 'V' leaf mark as pollen donors and recipients, showed that incompatible pollen did not prevent compatible pollen from being effective in fertilisation. Stigmas were found to be receptive to pollen some 40 hours after the initial application of compatible pollen. More than one application of pollen and a mix of pollen from different sources was found to maximise the number of seeds/floret, confirming observations from field experiments. The implications for seed yield are discussed.

KEYWORDS

White clover, *T.repens*, genetic markers, pollen competition, seeds/ pod

INTRODUCTION

Pollination by bees is essential for seed production by most insect-pollinated plant species. In self-incompatible species, pollen must be transferred from the anthers of one genotype to the stigma of another. White clover (*Trifolium repens* L.) is an insect-pollinated self-incompatible species pollinated in the UK by the honey bee (*Apis mellifera* L) and species of bumble bee (*Bombus* spp.) (Free, 1993). Seed yields are variable and often low (Marshall, 1994). Insect pollinators deposit pollen from a number of different sources on the stigma during a single visit (Marshall et al., 1995) and more than one visit may occur. This may result in both incompatible and compatible pollen deposited on the stigma during a single pollinator visit or from different visits. As competition between pollen grains can influence the proportion of ovules forming seeds (Marshall and Ellstrand, 1988; Stanton, 1994), this has implications for pollen germination, fertilisation and the number of seeds/pod. This paper describes experiments in which novel genetic markers within white clover (*T.repens*) were used to study competition between pollen and consequences for seed yield.

METHODS

Experiment 1: Plants of normal white 'V' leaf mark white clover and red leaf plants were grown in a bee proof glasshouse at 20½C. At time 0, white 'V' leaf mark plants were pollinated with compatible or incompatible pollen and at susequent time intervals (0, 8, 16, 24, 32 and 40 hours), red leaf pollen (compatible) was applied. For each treatment 6 inflorescences of 20 florets were pollinated. Pods were dissected when ripe and the number and weight of seeds/pod determined. All seeds were removed and germinated in a glasshouse and scored for the red colouration of the first trifoliate leaf.

Experiment 2: Five fixed isoenzyme banding selections of the white clover cv.S100 (Michaelson-Yeates, 1986) were used. In a bee proof glasshouse maintained at 20½C, plants of one selection were the pollen recipient (female) and the others pollen donors. On each recipient plant, 14 inflorescences reduced to 20 florets received different pollination treatments. These applied a) equal loads of pollen of the same and different banding pattern and b) different pollen loads. Inflorescences were harvested when ripe, pods dissected and number of seeds and ovules/ pod determined. All seeds were sown in a glasshouse and at the first trifoliate leaf stage, the isoenzyme banding pattern determined by electrophoresis (Michaelson-Yeates, 1986).

RESULTS AND DISCUSSION

Genetic markers are a valuable tool in the study of pollen competition. The genetic background of the red leaf plants means that progeny derived from crosses between these and 'normal' plants have red leaves. In experient 1 incompatible pollen on the stigma did not prevent subsequent compatible pollen being effective in fertilisation. Following the application of compatible pollen, the number of red-leafed progeny decreased from 50% at time 0 (both pollen types applied at the same time) (Figure 1), until none were observed after 40hours. This indicates that the application of compatible pollen did not prevent other pollen grains from germinating and successfully fertilising ovules until 40hours after the initial pollen was applied. However, the number of seeds/pod decreased as the time interval between the pollination treatments increased (data not shown). Similarly, plants which were selfed before the application of compatible pollen had fewer seeds/pod than those plants which initially received compatible pollen. Clearly both pollen type and interval between pollen applications can influence fertilisation efficiency.

In experiment 2 the number of seeds/pod significantly increased as the number of pollen applications increased (Table 1). However, two applications of pollen were sufficient to saturate the stigma and further applications did not result in any further significant increase in seed set. This confirms field studies showing that frequent bee visits to inflorescences significantly increased seed set (Rodet, pers. comm). This was not always related to the quantity of pollen present but to a presumed increased diversity of pollen on the stigma. Results from experiment 2 have also shown that pollen from a diversity of sources tended to increase the number of seeds/pod compared to pollen from a single source (seed set; one phenotype 1.8, mix of phenotypes 2.1, sed 0.11**). Evidence from white clover (Marshall et al.,1995) and other species (Marshall and Ellstrand, 1985) has indicated that natural pollinators deposit a mix of pollen grains on the stigma which from this present work is clearly beneficial to the number of seeds/pod.

Stigmas of white clover are receptive to pollen from several bee visits and pollen from a number of sources is beneficial to the number of seeds/pod. The proportion of ovules forming seeds was seldom more than 50%, indicating that other factors contribute to the failure to maximise the number of seed/pod in white clover.

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Table 1
Effect of pollen load on the number of seeds/pod in white clover (*Trifolium repens* L.)

	Number of pollen applications				
	1	2	3	4	s.e.d[z]
Seeds/pod	1.5	1.9	2.1	2.1	0.16***

[z] Standard error of the differerence of the mean (n=240)

Figure 1
Effect of time interval between application of ‘normal’ white leaf mark pollen and red leaf pollen application on the proportion of red leaf and white leaf mark seedlings.