Persistence of Red Clover (*Trifolium pratense* L.) Is Highly Related to Plant Population: Preliminary Studies on Isoflavones that Could Act as Insect Deterrents

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Persistence of Red Clover (*Trifolium pratense* L.) is highly related to plant population: preliminary studies on isoflavones that could act as insect deterrents

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Introduction

Red clover (*Trifolium pratense* L.) is a valuable legume in Chile whose importance is mainly as forage for animal and seed production. Although it is considered as perennial, its production declines after the second year of established due to a complex of multiple biotic and abiotic factors (Taylor and Quesenberry, 1996, Ortega *et al*., 2014); in Chile this is due mainly to the infestation by the red clover root borer *Hylastinus obscurus* (Coleoptera: Curculionidae). Currently there is no an efficient control method against this insect. Therefore, breeding cultivars that are less attractive to the root borer and the understanding of the possible role of secondary metabolites as isoflavones in this response arises as a new alternative to reduce the damage caused by this curculionid. The purpose of this paper is to review the relationship between forage yield, plant populations and root damage in an experiment run for four years and to show preliminary information about isoflavone content of Chilean cultivars and experimental lines.

Materials and Methods

Two experiments were established at Carillanca Research Center (38°41'SL and 72º25'WL) under irrigation, one experiment to evaluate agronomic performance during four years and the other to evaluate isoflavone content of cultivars and experimental lines. The first experiment was established in the spring of 2010 and it was evaluated during four growing seasons. The trial considered the Chilean cultivars Quiñequeli INIA, Redqueli INIA and Superqueli INIA, six Chilean experimental synthetics and the introduced cultivars Starfire (USA), Tuscan (New Zealand) and Sensation (New Zealand). Sowing rate was 12 kg/ha in rows separated by 20 cm; the design was complete randomized block with four replicates and plots of 5.76 m^2 each. We evaluated plant population (plants/m^2) by non-destructive counting twice per season and forage yield (DM Kg/ha) three to four times per growing season. At the end of the experiment, a destructive sampling of 10 plants per plot was done to evaluate crown-root morphology, and root damage by the curculionid. Differences in forage yield, plant polulation, crown-root morphology and root damage were analyzed by ANOVA and means separated by LSD (p=5%). The relationship between variables was studied by linear Pearson correlations. The second experiment was established in the spring of 2013; sowing rate was 12 kg/ha in rows separated by 20 cm in a complete randomized block with three replicates and plots of 11.2 m^2 each. This trial considered the same Chilean cultivars and experimental synthetics as the former trial and standard cultivars known for its formononetin content (Grassland Pawera and G27 between them). Plots were sampled four times during the season 2014/15 (October 2014, December 2014, January 2015 and March 2015), sampling 15 plants per plot at a depth of 10 cm. After carefully washing, plants were separated in shoot (leaf + stem) and root; tap root was cut to a length of 5 cm; shoot and tap root were stabilized immediately by immersion in liquid N_2 for later liofilization. Extraction was done with a methanol solution and relative quantifications of isoflavones based on HPLC–MS peak areas normalized on the area of the recovered IS peak. HPLC analysis was performed using a Diode Array Detector and a C18 reversed-phase column according to the methodology reported by Ramos *et al*. (2008).

Results and Discussion

Plant population decreased considerably from establishment season onwards; average was 143 plants/m^2 at the establishment year and decreased to 52 plants/m^2 at the fourth season. There were significant differences in forage yield in the four seasons, being Sel Syn PREIII the one with the highest forage yield over the four seasons (Fig. 1). This experimental synthetic was similar in forage yield to Syn Int IV, Sel Syn Int IV, Syn PRE III, Superqueli INIA and Sel Syn PRE I. Correlations between plant population and forage yield were significant from the second season onwards; in the third season, Sel Syn PRE IIII has the highest forage yield and the second highest plant population, while Syn PRE III the highest population (Fig. 2).

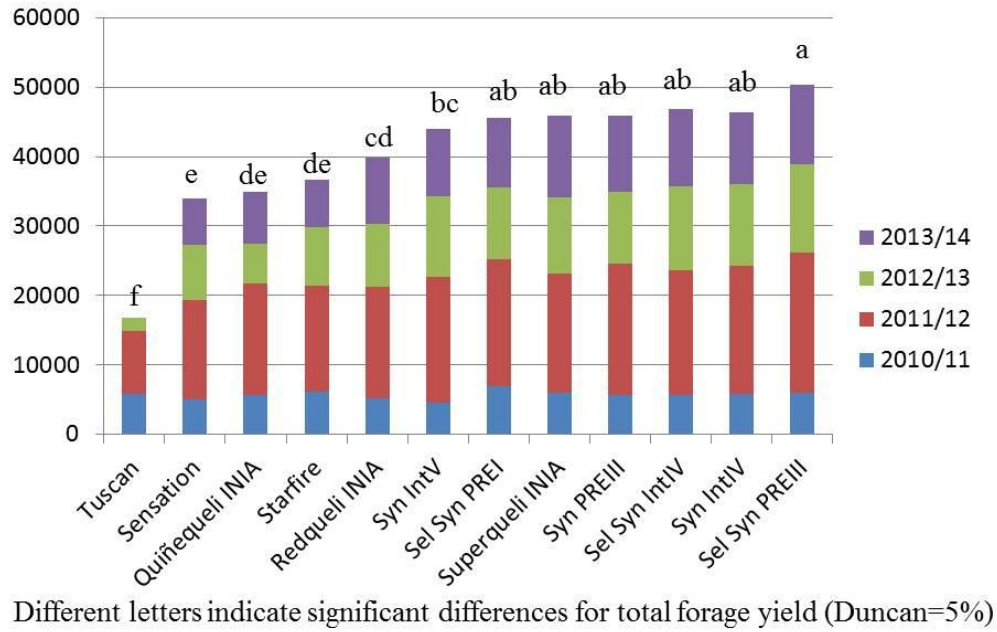

Fig. 1: Forage yield (DM kg / ha) per season of 12 Red Clover cultivars and synthetics. INIA Carillanca, irrigated trial sown in 2010.

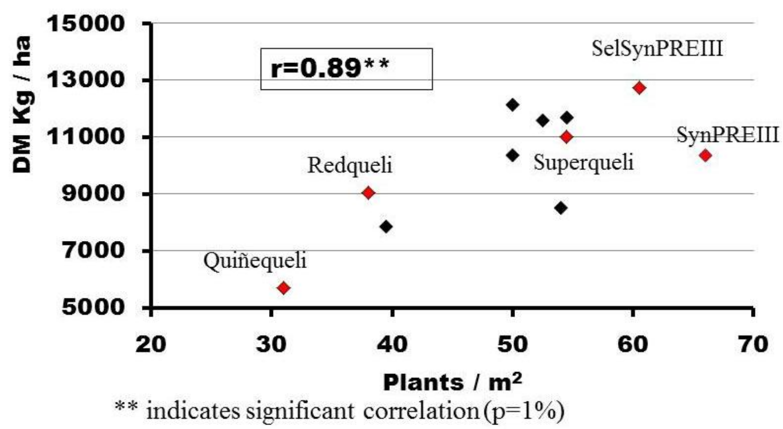

Fig. 2: Relationship between plant population (plants / m^2) and forage yield (DM Kg / ha) of Chilean Red Clover cultivars and synthetics at the third season. INIA Carillanca, irrigated trial sown in 2010.

Sampling of plants at the end of the trial showed a high proportion of the survival plants damaged by the curculionid (70% on average) and there were non significant differences between cultivars. Average number of insects per plant was 2.6, showing a high deterioration of the plants at this stage. There were no significant correlations at this late stage between root morphology (crown diameter, weight of root sections-tap root, adventitious, lateral) and survival of plants.

In the other experiment, we evaluated the level of the Isoflavones Daidzin, Genistin, Ononin, Daidzein, Sissotrin, Genistein, Formononetin and Biochanin. In the roots, sampling of early spring gave a total average concentration of isoflavones of 0.962%, while in the shoots average was more than twice that value (2.096%).

In the roots, the isoflavone with the highest concentration was Formononetin (38% of the total Isoflavones of roots), while in the shoots it was Genistein (31% of the total), while Formononetin was the second (22% of the total). We need to analyse the Isoflavone of the other three sampling dates of the season in order to get conclusions about the genetic variation of these secondary metabolites and their possible relationship with the curculionid.

Conclusion

There was an important reduction in plant population from the first season to the fourth season and most of the roots were infested by *Hylastinus obscurus* at the end of the experiment. Forage yield from the second season onwards was highly correlated to plant population. Isoflavone concentration in early spring was higher in shots than roots and there was variation of the type of isoflavone according to the part of the plant and cultivar; however, further results of the other periods of the season need to be obtained in order to reach conclusions about their relationship with the curculionid.

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