Factors affecting red clover and alfalfa phytoestrogen content

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Phytoestrogens are produced by a large number of plant species, including legumes in which they are the most abundant. While in farm animals phytoestrogens cause reproductive disorders, interest for these same molecules is increasing as they have recently been reported to be beneficial to human health by preventing several diseases or mitigating symptoms associated with menopause. Red clover (*Trifolium pratense* L.) and alfalfa (*Medicago sativa* L.) are two important forage crops currently being used as a source of phytoestrogens by the nutraceutical industry to treat a variety of health concerns. A series of experiments were conducted to determine the effects of management on phytoestrogen content of these two species.

The concentrations of four phytoestrogens (daidzein, genistein, formononetin, and biochanin A) were quantified in field-grown red clover. Plots were established in 2002/3 at two locations in Montreal, QC. Variation in phytoestrogen content as a factor of cultivar, harvest, year, and plant part was determined. Differences were observed among 20 cultivars evaluated, total phytoestrogen content ranging between 7,786 and 10,744 mg kg^{-1} DM. Phytoestrogen content varied among years and harvests, being 17% greater in 2003 than in 2002, and 76% higher in the first of two cuts in the seeding year. When harvested at the early flowering stage, total phytoestrogen content was on average 445% greater in leaves than petioles, flowers, and stems. Preliminary results from greenhouse experiments suggest that foliar application of natural elicitors such as acetic acid may in some instances increase the content of some phytoestrogens in red clover.

In other studies, we determined the concentrations of four other phytoestrogens (coumestrol, apigenin, luteolin, and quercetin) in field-grown alfalfa. Differences between plants of different stage of maturity, between plant parts, and canopy segments were assessed. The concentration of individual phytoestrogens in whole herbage varied between 15 and 225 mg kg^{-1} DM and was strongly affected by stage of maturity. Coumestrol and apigenin concentrations were highest at early vegetative stages, luteolin and quercetin at early vegetative and late flowering stages. All phytoestrogens were found in lowest concentrations at the early flowering stage. Concentrations of luteolin, quercetin, and apigenin were 225, 410, and 690 % greater, respectively, in flowers than in leaves or stems; coumestrol concentration was similar between plant parts. Concentrations through the herbage canopy varied and were greatest at >60 cm from the soil surface for apigenin and coumestrol, but greatest at >60 and 0-20 cm for quercetin and at 0-20 cm for luteolin.

Results suggest that if red clover and alfalfa are to be used as sources of beneficial phytochemicals for the production of herbal supplements or nutraceuticals, current agronomic practices may need to be adapted. A better understanding of the impact that agronomic practices and the environment have on plants' phytochemicals is an essential component, along with biochemical and molecular studies, of programs aiming at developing plants with high levels of beneficial phytochemicals.