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INTRODUCTION

- Perennial forage legumes are an important source of nutrition for dairy cattle but may also be a source of **phytoestrogens** [1,2]
- Phytoestrogens** are naturally-occurring compounds in plants that protect them against environmental stress
- The molecular structure and shape of phytoestrogens (below) mimic the sex hormone estrogen (17- β -estradiol) and may therefore have important implications for both cows and humans that consume dairy products [3]
- When dairy cows eat large quantities of phytoestrogenic legumes their reproductive health may be impacted (e.g., reduced fertility and conception rates) [4]
- Phytoestrogen content varies between legume species and may be impacted by growing conditions, such as whether or not insects are feeding on the plant [4] (Fig 1).

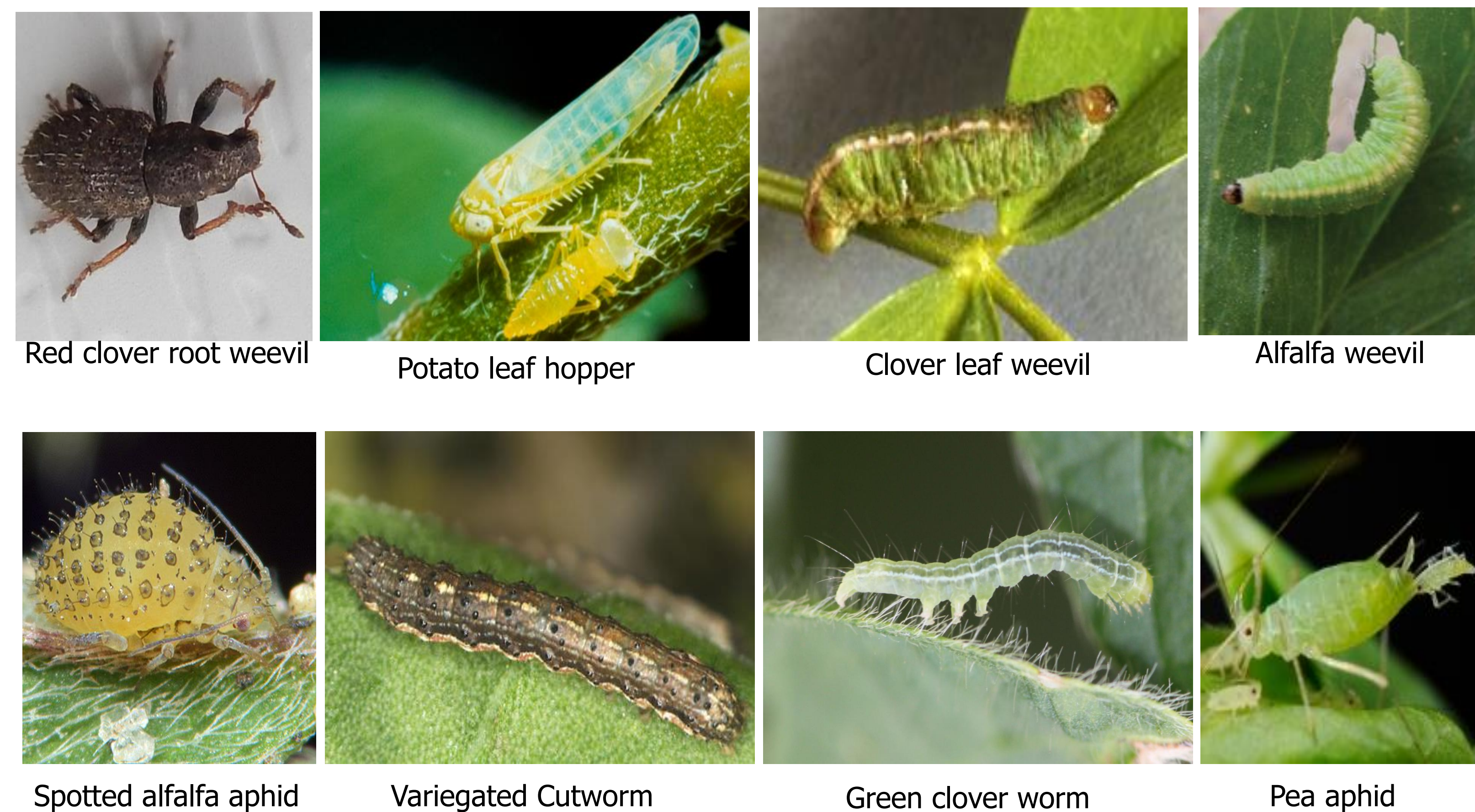
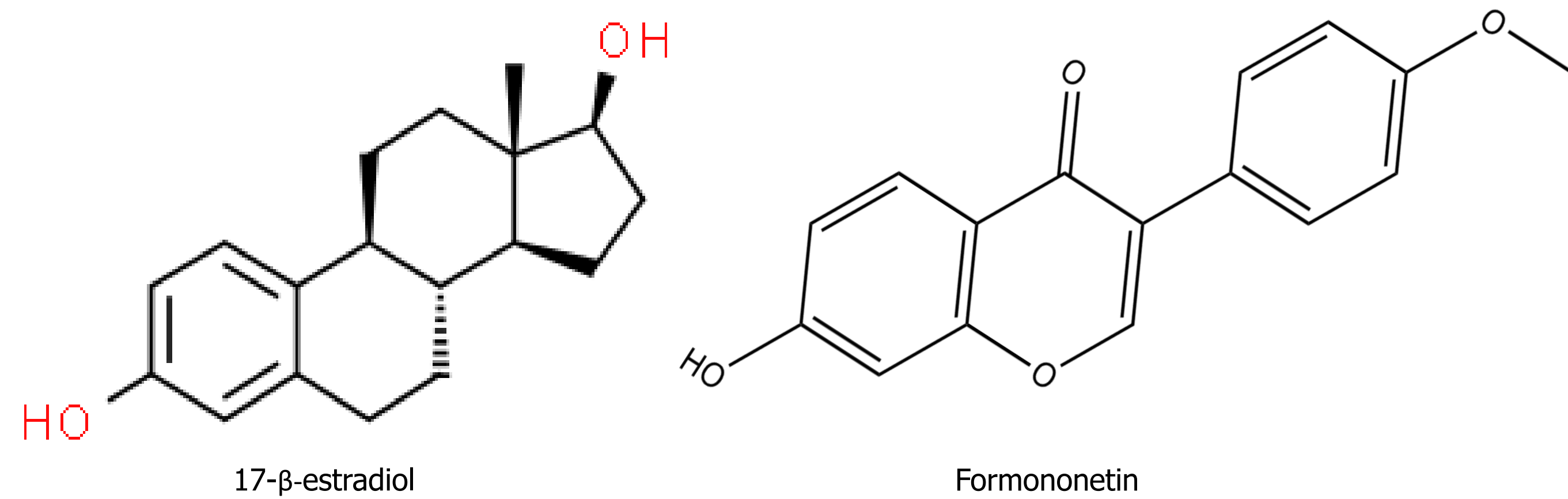


Fig 1. Insects that commonly feed on perennial forage legumes. Sources for images: https://pubs.nmsu.edu/_a/A338/; <https://cropwatch.unl.edu/2017/scouting-advised-alfalfa-and-clover-leaf-weevils/>; www.ispotnature.org/communities/uk-and-ireland/view/observation/819385/clover-root-weevil-sitona-hispidulus; <https://extension.sdstate.edu/green-cloverworms-causing-defoliation-soybean/>; <http://bugguide.net/node/view/1627390>; <https://bugguide.net/node/view/1627390>

OBJECTIVE

- Our goal is to quantify phytoestrogens in two species of perennial legumes and determine if insect herbivory influences their concentrations.

ACKNOWLEDGEMENTS

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METHODS

We grew these forage legumes:

- Red Clover 'Freedom'
- Kura Clover 'Rhizo'

Experimental Design: Completely randomized design (CRD)

After 8 weeks, we introduced corn earworm onto half of the plants, and the other half experienced no herbivory

Seven days later, we harvested the plants and measured their phytoestrogen levels



Fig 2. Corn earworm, *Helicoverpa zea*, is a herbivore that feeds on a wide range of plant species. Insects were introduced at their 3rd instar stage (bottom right) <https://www.benzonresearch.com/post/corn-earworm-helicoverpa-zea>



Fig3. Different steps of the experiment: (a) Plants were grown in insect habitat cases; (b) Plants just before insects were released; (c) Insects feeding on leaves and stems; (d) An insect-damaged leaf; (e) Whole plant damaged by insects

RESULTS

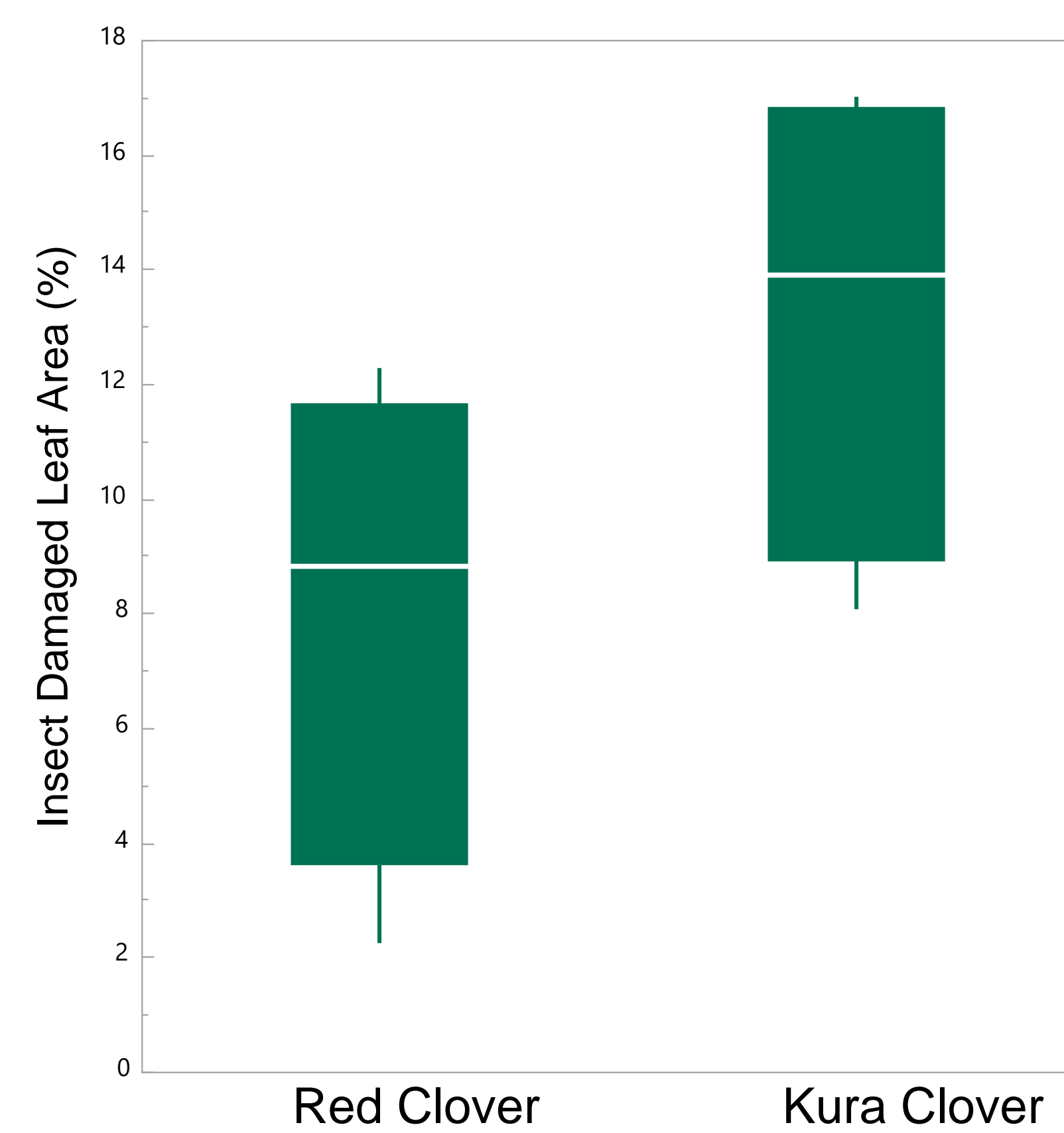


Fig 4. Relative amount of leaf damage caused by herbivory (% of leaf area consumed) for each legume species. Corn earworm damage on kura clover plants was ~5% greater than red clover

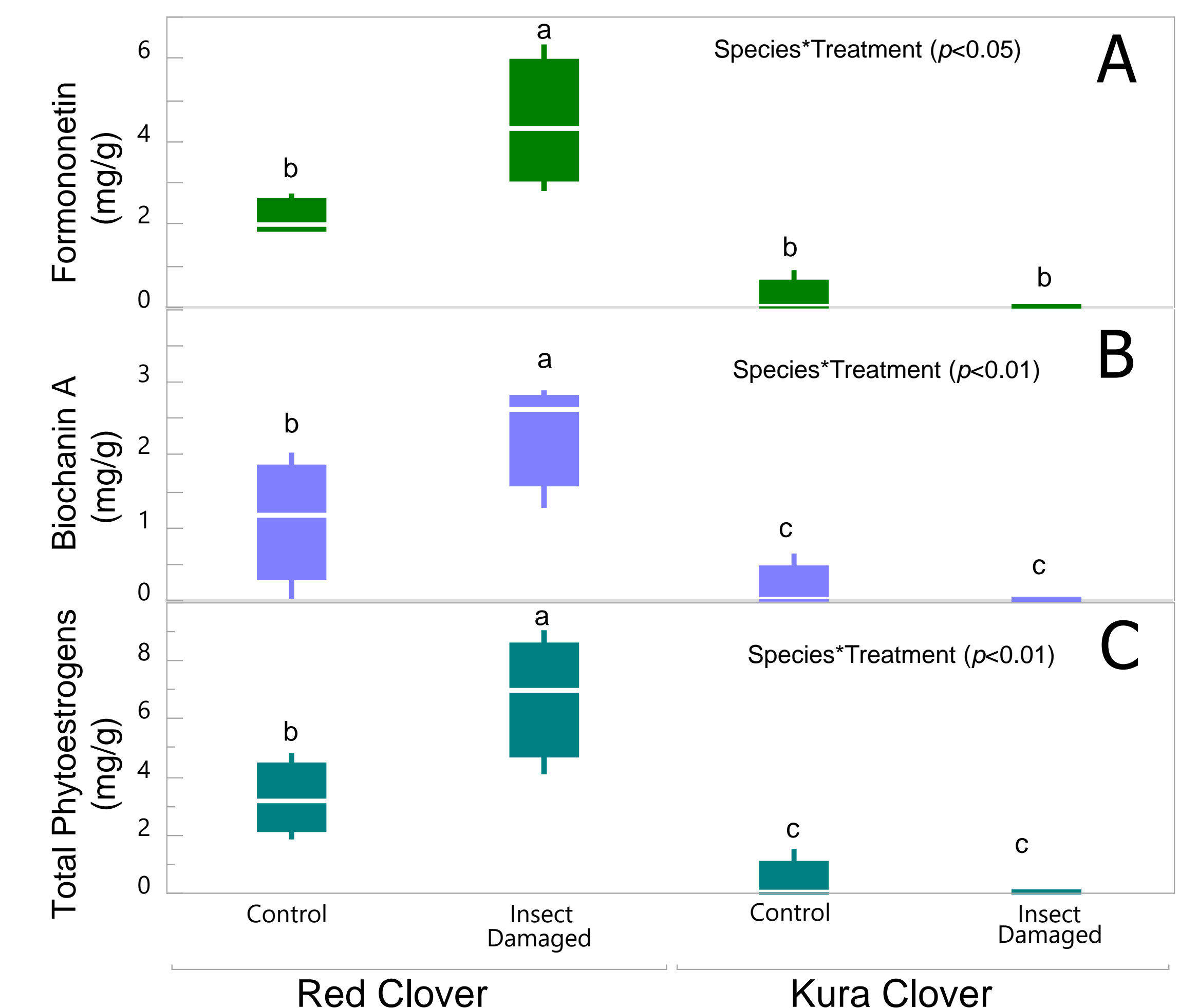


Fig 5. Red clover plants had elevated levels of two important phytoestrogens (A & B) and greater total phytoestrogen concentrations (C) when insects fed on leaves. Kura clover had very low levels of phytoestrogens regardless of insect herbivory

- Insect herbivory may increase phytoestrogens in certain forage legumes
- Insect herbivory doubled the phytoestrogen content in red clover, while kura clover did not produce these compounds
- We are currently working to quantify phytoestrogens and their environmental drivers in a range of forage legume species – two important pieces of information for dairy producers and consumers

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