

## Viral Effects on Canna Leaf Antioxidants

The types and levels of flavonoids in foods is an important topic of scientific study because of their known benefits to human health. In addition plant flavonoids are known for their antioxidant properties providing beneficial effects for control of oxidative stress related diseases including atherosclerosis and cancer. Clinical tests have shown that polyphenols such as catechin or quercetin scavenge reactive oxygen species (ROS). Canna lilies are grown in the US primarily for use in landscapes and gardens, but in certain South American countries, canna leaves are used for wrapping and steaming vegetables and rice, the same way that banana leaves or grape leaves or corn husks are used. During cooking flavonoids can leach out of the leaves into the foods imparting unique flavors, and potential dietary benefits. The goal of this research is to evaluate the antioxidant activity of canna leaf extracts in tissue cultured cells. This project has two goals: 1) evaluate the antioxidant activity of canna leaf extracts using the hybrid variety 'Red Futurity', and 2) determine if plant virus infected canna leaves show altered anti-oxidant levels using the same antioxidant activity assays tested in objective 1.

Preliminary tests by an undergraduate REU student determined that leaf extracts of the canna variety 'Red Futurity' are rich in antioxidants and can attenuate pro-inflammatory responses in RAW264.7 cells (a murine cell type that is commonly used to study inflammatory responses). However, when Canna Yellow Streak Virus (CaYSV) infects Red Futurity cannas, the plant's leaves are no longer their signature red, but are green. The agricultural rule-of-thumb is that if it's infected, you throw it out. However, there is *no significant scientific evidence* to prove that infected plants have altered health benefits. The question of whether plant viruses threatening agricultural production also alter the nutritional value of the foods that we eat is an important and fundamental scientific question. This project is also a great opportunity for undergraduate student training in both CASNR and HES. Viruses could be part of the natural selectors that is actually shifting the Canna genome in a positive direction. The purpose of this experiment is to test whether virus-infected Canna leaves are more or less effective in regulating cellular anti-inflammatory responses than their healthy counterparts. The outcomes of this work will lead to a larger project that could significantly decrease agricultural waste across the world.

In order to test this, canna leaves will be dried and ground into a powder, then re-suspended in a water-methanol mixture. RAW 264.7 cells will be co-treated with lipopolysaccharide (LPS) and extracts of healthy or virus-infected canna leaves. LPS is a well-known endotoxin that initiates the pro-inflammatory response and increases expression of four pro-inflammatory cytokines: IL1b, IL6, TNFa, and INOS. The Canna extract should attenuate the pro-inflammatory response. By subjecting RAW 264.7 to both the healthy Canna extract, and the infected canna extract and comparing the results, we should be able to determine the effect of viral infection on a plant's anti-inflammatory response.

RAW 264.7 cells are macrophage-like cells that are similar to microglial cells (the macrophages of the brain). When the microglial cells become inflamed or damaged, they become star shaped, and can no longer function. This degeneration is thought to be a contributor to neurological disorders, such as Parkinson's disease. Adding antioxidants and anti-inflammatory chemicals could help the microglial cells return to their proper shape, and theoretically slow the deterioration of the brain. Judging by the reaction of RAW 264.7 to Canna extract, which is full of antioxidants and anti-inflammatory chemicals, this reversal of microglial cellular degeneration could be reversed.