

PHOTOSYNTHETIC AND GLYCOALKALOID RESPONSES OF
POTATO (*Solanum tuberosum* L.) TO COLORADO POTATO
BEETLE (*Leptinotarsa decemlineata* Say) DEFOLIATION

by

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ABSTRACT

Photosynthetic and glycoalkaloid responses of potatoes (*Solanum tuberosum* L.) to varying levels of Colorado potato beetle (*Leptinotarsa decemlineata* Say) and manual defoliation were measured on 'Cal Red' and 'Russet Burbank' plants. No alteration in photosynthesis was observed on the remaining tissue of an injured leaf for Cal Red and Russet Burbank leaves defoliated by larval Colorado potato beetles nor for Russet Burbank leaves defoliated by adult Colorado potato beetles. No significant differences were observed between actual Colorado potato beetle and manual defoliation for both varieties. In both of the whole-plant defoliation studies, defoliation level consistently did not result in increased or decreased gas exchange parameters of individual leaves compared to undefoliated controls. There was no evidence of delayed leaf senescence in defoliated treatments. Plants defoliated by Colorado potato beetles had a significantly greater production of glycoalkaloids than in control and in manually defoliated plants for both skin and inner tissue. There was also a 32.6% and a 36.8% glycoalkaloid increase in skin and inner tissue of tubers from plants defoliated at high levels by Colorado potato beetles in comparison to control plants. Although a significant difference in glycoalkaloid concentration was not observed among the treatments in subsequent experiments, the skin and inner tissue of tubers from plants defoliated at high levels by Colorado potato beetles increased by 18.9% and 12.7% in comparison to tubers from control plants. For the experiments where glycoalkaloid concentrations were measured, there was a significantly greater concentration of glycoalkaloids in the skin versus the inner tissue of potatoes, in addition, for the initial glycoalkaloid experiment, the concentration of tuber extract required to reduce Chinese hamster ovary cellular proliferation by 50% was 10 times less for the skin versus the inner tissue. The dietary risk assessments revealed that the glycoalkaloid concentrations within the inner tissue of tubers from control plants, manually defoliated plants, and high Colorado potato beetle defoliated plants exceeded the toxic endpoint for all human subgroups at less than the 99.9th percentile of exposure.

INTRODUCTION

Insect herbivory is a fundamental feature of most terrestrial ecosystems, and it can profoundly affect agroecosystems. Foliage feeding insects are estimated to reduce crop yields from between 5% and 30% (Mattson and Addy, 1975), and during insect outbreaks, it is estimated that more than 70% of the primary biomass may be removed (Cyr and Pace, 1993). Although an extensive amount of research has identified the physiology, behavior, ecology, and life history of pest insects and how their numbers affect yield, but more needs to be determined on how the plant host responds to insect injury (Peterson and Higley, 2001).

Stress is the reduction of optimal physiological conditions and “injury is a stimulus producing an abnormal change in physiological processes” (Peterson and Higley, 2001). Because gas exchange processes (such as photosynthesis, stomatal conductance, and transpiration) are fundamental physiological processes that ultimately determine plant growth, development, and fitness, understanding how insect herbivory affects these processes would provide valuable perspectives on how a plant responds to different stresses and allow for integration of responses across different levels of biological organization.

In addition to alterations in primary metabolism, many plants respond biochemically to insect injury by producing secondary metabolites. Many secondary metabolites function as natural pesticides against insect herbivores. Secondary metabolites are highly diverse among taxa and their presence has led to extensive research on the origin, diversity, and role that these chemicals play in plant-insect

interactions, as well as their specificity to insects (Theis and Lerdau, 2003). Because naturally occurring pesticides may be synthesized when plants are under stress, it is expected that injury to plant tissue would instigate synthesis of higher concentrations of the naturally occurring pesticides in the injured and uninjured plant tissue. Because approximately 99% of all toxins consumed by humans are naturally occurring in plant tissue (Ames et al., 1990), insect injury to crops may increase concentrations of toxins, altering dietary risk to humans.

Photosynthesis

Photosynthesis “encompasses both a complex series of reactions that involve light absorption, energy conversion, electron transfer, and a multipstep enzymatic pathway that converts [carbon dioxide] CO_2 and water into carbohydrates” (Malkin and Niyogi, 2000). Photosynthesis involves two different phases: light reactions and “dark” reactions. The light reactions produce O_2 , ATP, and NADPH. The “dark” reactions (Calvin Cycle) “reduce CO_2 to carbohydrates and consume the ATP and NADPH produced in the light reactions” (Malkin and Niyogi, 2000). Although the second phase is called the Calvin Cycle, it does not require light nor does it need to take place in the dark.

All the reactions required for photosynthesis take place in particular locations within the chloroplast. Chloroplasts have an outer and inner envelope and all thylakoid membranes are interconnected and enclosed, creating an inner space called the lumen (Malkin and Niyogi, 2000). The internal membrane is known as the thylakoid membrane and is comprised of granal thylakoids, which are organized into stacks called grana and

stromal thylakoids, which are unstacked and exposed to the stroma. The stroma is the surrounding fluid medium.

Located in the thylakoid membranes are Photosystems I and II, which contain reaction centers that convert light energy and transfer the energy into chemical bond energy. Photosystem I lies in the stromal membrane and absorbs wavelengths at 700 nm. Photosystem II lies in the granal membrane and absorbs wavelengths at 680nm (Malkin and Niyogi, 2000).

The electron transfer chain not only involves Photosystems I and II, but cytochrome b_6f , plastocyanin, and plastoquinone. Photosystem II has two quinones bound to it. Light is absorbed in Photosystem II, and because Photosystem II has two quinones bound to it: Q_A and Q_B , two consecutive electrons are released from the light absorbing pigments (P680), transferred to Q_A , which are then transferred to Q_B , eventually yielding a fully reduced quinone (Q_B^{2-}). The Q_B^{2-} then takes on two protons (Q_BH_2) from the stromal side of the membrane, dissociates from Photosystem II, diffuses into the lipid bilayer to become a mobile electron carrier, and a free quinone refills the Q_B site on the Photosystem II complex (Buchanan et. al., 2000; Malkin and Niyogi, 2000). In the cytochrome b_6f complex, Q_BH_2 (plastoquinol) transfers its electrons to plastocyanin in the lumen. The cytochrome b_6f complex allows the transfer of two protons across the lumen for every electron transferred to plastocyanin. In addition to the oxidation of water, this establishes a proton gradient and on the surface of the ATP synthase, located in the thylakoid membrane, ADP is phosphorylated to produce ATP (Buchanan et. al., 2000; Malkin and Niyogi, 2000). Plastocyanin transfers its electrons to Photosystem I, where the electrons are transferred to a few electron carriers before

reaching Ferredoxin. Through electrostatic interactions, Ferredoxin binds to Ferredoxin-NADP⁺ reductase and transfers its electrons to NADP⁺ to produce NADPH in the stroma (Buchanan et. al., 2000; Malkin and Niyogi, 2000). Using the light energy absorbed, the light reactions generate the energy required by the Calvin Cycle.

In the Calvin Cycle, 13 steps fix CO₂ into carbohydrates in three phases: carboxylation, reduction, and regeneration. Carboxylation is the enzymatic combination of CO₂ and water from the environment with ribulose 1,5-bisphosphate (RuBP) using ribulose bisphosphate carboxylase/oxygenase (Rubisco) to produce two 3-phosphoglycerate (3-PGA) molecules (Buchanan et. al., 2000; Malkin and Niyogi, 2000). The reductive phase involves two steps: the conversion of 3-PGA into triose phosphate and triose phosphate to glyceraldehyde 3-phosphate (GAP) using ATP and NADPH synthesized during the light reactions. The GAP molecules generated can then be used to build carbohydrates such as sucrose and starch or other cellular constituents necessary to maintain the growth, development, and fitness of the plant.

Sources are leaf or leaf-like structures that carry out the majority of photosynthesis and transfer photoassimilates, sucrose and/or starch, to sinks. Sinks are young leaves, stems, roots, fruits, or seeds and the photoassimilates within sinks may be utilized for respiration and for the synthesis of other molecules required for their growth. Sucrose is synthesized in the cytosol of mesophyll cells in source leaves. Most of the sucrose synthesized during photosynthesis is directly transferred into the phloem and exported into sink leaves where it is then used for respiration. The last phase of the Calvin Cycle is the regeneration phase and involves the regeneration of RuBP, which is required to continue the process of carbon fixation and carbohydrate synthesis.

Stomatal Conductance

For photosynthesis to occur, atmospheric CO₂ must diffuse into the leaf through the stomata and reach the carboxylation site of Rubisco. The appropriate gradients are required for the diffusion of CO₂ to maintain carbon fixation and variations in these gradients highly influence the diffusion rates (Taiz and Zeiger, 1998). Carbon dioxide is first absorbed through the stomatal pore into the substomatal cavity and intercellular air spaces between the mesophyll cells, eventually diffusing into the mesophyll cells, through the cytoplasm, and across the chloroplast membrane before reaching the carboxylation site of Rubisco (Taiz and Zeiger, 1998).

Higher stomatal conductance increases the diffusion of CO₂ into the leaf via the stomata. The increased diffusion of CO₂ into the leaf favors higher photosynthetic rates due to the continuous influx of carbon available for fixation in the Calvin Cycle as described above. In C₃ plants, which are most crops including potatoes (*Solanum tuberosum* L.), increasing the CO₂ levels beyond the level fixed by photosynthesis and produced by respiration causes an increase in photosynthesis and is limited by the ability of the enzymes in the Calvin Cycle to regenerate RuBP. If CO₂ concentrations are low, however, photosynthesis is limited by the carboxylation capacity of Rubisco (Taiz and Zeiger, 1998).

Transpiration

Water transpired by a leaf evaporates from the wet mesophyll cells into the intercellular air spaces to exit out of the stomatal pore. Because water, a liquid, and CO₂,

a gas, share the same pathway of release and absorption, and because CO₂ diffuses through the air 1.6 times more slowly than water, plants have to overcome substantial water loss to maintain adequate influx of CO₂. At high relative humidity, “the diffusion gradient that drives water loss is about 50 times greater than the gradient that drives CO₂ uptake” (Taiz and Zeiger, 1998). Therefore, a plant reduces its stomatal resistance allowing for a greater concentration of CO₂ to diffuse into the leaf. Substantial water loss may still occur even when stomatal resistance is lowered. In sunny conditions where there is sufficient water available to the plant, it is advantageous for the plant to decrease stomatal resistance to allow for the increased diffusion of CO₂ to support photosynthetic demands (Taiz and Zeiger, 1998). In sunny conditions where there is lack of water, it is advantageous for the plant to open the stomatal pore only slightly or keep it closed entirely (Taiz and Zeiger, 1998); slight and complete closure of the stomata would inhibit photosynthesis due to the reduction in carbon available for fixation. In addition to a plant reducing its stomatal resistance, at night when photosynthesis is limited and CO₂ demand is limited, the stomatal pores are kept as small as possible (Taiz and Zeiger, 1998).

Insect Injury Guilds

There are different types of injury to a plant that insects may impose and they may be grouped into injury guilds based upon the appearance of the injury alone. Examples of these injury guilds include leaf skeletonizing, seed feeding, leaf mining, stem boring, and fruit scarring. Because different species may impose similar injury types and because these injury types elicit a similar response in plants, those species may be placed in injury guilds based upon physiological response. The injury guilds

aforementioned were later augmented to incorporate plant physiological responses from each injury type. These injury guilds include fruit feeders, stand reducers, assimilate sappers, turgor reducers, plant architectural modifiers, and leaf-mass consumers (Boote, 1981; Pedigo et. al., 1986). Incorporating the injury guilds described above, Higley et al. (1993) reclassified the insect injury guilds to include leaf senescence alterators, leaf-mass reducers, leaf photosynthetic-rate reducers, light reducers, assimilate removers, water-balance disruptors, population or stand reducers, seed or fruit destructors, architecture modifiers, and phenological disruptors.

Insect Injury Guilds and Photosynthetic Rates

Photosynthetic rate is the “net amount of carbon fixed per unit leaf area per unit time” (Taiz and Zeiger, 1998) and therefore determines the amount of carbon available to the source leaf. Physiological factors that could affect photosynthetic rate include “increased assimilate demand after defoliation, reduced competition between leaves for mineral nutrients necessary for cytokinin production, and delayed leaf senescence” (Peterson, 2001).

Variable photosynthetic responses to insect herbivory have been observed among different combinations of plant taxa and types of injury insects impose (Welter 1989). A large amount of research has been conducted on the photosynthetic and other gas exchange responses of soybean (*Glycine max* L.) injured by different species of insects. Most of the research has involved the leaf-mass consumption injury guild (Peterson et al., 2004). The Japanese beetle (*Popillia japonica* Newman), the corn earworm (*Helicoverpa zea* Bodie) (Aldea et al., 2005), the cabbage looper (*Trichoplusia ni* Hübner), the green

cloverworm (*Plathypena scabra* F.), and the Mexican bean beetle (*Epilachna varivestis* Mulsant) would all be classified as leaf-mass consumers and each can remove soybean (*Glycine max* L.) leaf tissue. Although each species is a leaf-mass consumer, all except the Mexican bean beetle elicit a similar photosynthetic response (Peterson et al., 1998).

The Japanese beetle, the corn earworm (Aldea et al., 2005), the cabbage looper, and the green cloverworm caused no change in photosynthetic rates of remaining uninjured tissue on an injured leaf (Hammond and Pedigo, 1981; Ostlie and Pedigo, 1984). The only change observed was a transient increase in water loss, suggesting that the photosynthetic pathway is not affected by defoliation of insects. Reductions in photosynthetic rates that have been observed after leaf-mass consumption injury (Delaney and Higley, 2006; Peterson et al., 1998) may also be due to the disruption of water and nutrient transport, changes in the performance of photosynthetic enzymes, and diversion of resources to defenses. At the canopy level, reductions in photosynthetic rate have also been observed, but many of these reductions were temporary and were caused by a reduction in leaf-area indices, smaller leaf size, and decreased light interception (Peterson, 2001).

Many studies have indicated that removal of either partial or entire leaves by insects increases photosynthetic rates (Welter, 1989). Other studies have observed no changes in photosynthetic rates on the remaining leaf tissue of leaves injured by insects (Poston et al., 1976; Welter, 1989, 1991; Higley, 1992; Peterson et al. 1992; Peterson and Higley, 1996; Burkness et al., 1999) suggesting that photosynthesis is unaffected by leaf-mass consumers. Changes in photosynthetic rates observed are caused from the reduction in leaf photosynthesizing tissue and not from an increase or decrease in the

photosynthetic capacity of the remaining leaf tissue. In alfalfa and soybean defoliator systems, defoliation of whole plants or plant canopies resulted in a delay of normal, progressive leaf senescence (Higley, 1992; Peterson et al., 1992).

Sources, Sinks, and Photosynthetic Rate

To further explain plant response to insect injury, it is best to divide the plant into two tissue types based on physiological function: sources and sinks. Sources are leaf or leaf-like structures that carry out the majority of photosynthesis and transfer photoassimilates, sucrose and/or starch, to sinks. Sinks are young leaves, stems, roots, fruits, or seeds and the photoassimilates within sinks may be utilized for respiration and for the synthesis of other molecules required for their growth.

Sink size and activity affects the mobility of photoassimilates. Sink activity is “the rate of uptake of assimilates per unit weight of sink tissue” and sink size is “the total weight of the sink tissue” (Taiz and Zeiger, 1998). By altering the size and activity of a sink, the rate of transport of photoassimilates may be altered. The larger the sink, the greater the photoassimilates that may be stored and the greater the activity of a sink, the greater the number of photoassimilates processed. The quicker sinks metabolize photoassimilates, the greater the amount of photoassimilates can be transported (Taiz and Zeiger, 1998). If the source to sink ratio is altered, so that the number of sources per sink is reduced, an increase in the photosynthetic rate of that single source leaf would be observed. The photosynthetic rate increases when the sink demand increases, but when the sink demand decreases, the photosynthetic rate often decreases (Taiz and Zeiger, 1998).

When all but one source leaf of a soybean plant was shaded for approximately eight days, a decrease in starch concentration of source leaves and increases in photosynthetic rate, activity of Rubisco, sucrose concentration and transport to sink, and orthophosphate concentration all were observed in the source leaf (Thorne and Koller, 1974). For plants that mainly store starch, there is a drastic reduction in the photosynthetic rate when sink demand decreases, and this may be caused by the accumulation of assimilates such as starch, sucrose, or hexoses in the source leaf (Taiz and Zeiger, 1998).

There are three mechanisms which may explain decreases in photosynthetic rates when sink demands decrease. When sink demands are low, starch accumulation in the source leaves could disrupt the chloroplasts by either interfering with CO₂ diffusion or by blocking the absorption of light (Taiz and Zeiger, 1998). Synthesis of sucrose is often reduced with low sink demands, causing less phosphate to be available for exchange in the chloroplasts. If starch synthesis, which releases orthophosphates in the chloroplast, could not recycle phosphate fast enough, ATP and CO₂ fixation would decline, and the lack of free orthophosphates within the chloroplasts would cause a reduction in the rate of photosynthesis (Taiz and Zeiger, 1998). High sugar levels in source leaves as a result of inhibition of export to sink leaves may also decrease the rate of transcription of mRNA encoding photosynthetic enzymes through a feedback loop (Taiz and Zeiger, 1998).

Signals between source and sink leaves may be physical, chemical, hormonal, or through carbohydrate concentrations. Physical signals between source and sink leaves may involve turgor pressure. The signal using turgor pressure may pass through the sieve elements from sink to source leaves. Photoassimilates are transferred from source leaves

into the phloem. From the phloem, sinks unload the photoassimilates and if the unloading was rapid, there would then be a reduction in turgor pressure in the sieve elements of sinks. This reduction in turgor pressure would then be transmitted to source leaves. If the phloem unloading was not rapid, there would be an increase in turgor pressure, and this would also be transmitted to the source leaves. Thus source leaves would reduce photosynthetic rates. Turgor pressure across plasma membranes has been shown to affect the activity of the proton pumping ATP-ase in the membranes. Using the taproot of a sugar beet incubated in a mannitol solution, the turgor pressure was reduced, and there was an increase in proton release (Taiz and Zeiger, 1998).

Hormonally, shoots can produce growth regulators such as auxin and roots can produce growth regulators such as cytokinin. These regulators are easily transferred either via the phloem into the roots or via the xylem up into the shoots (Taiz and Zeiger, 1998). Gibberellic acid and abscisic acid also are transported via the plants vascular system. Although plant hormones may not have a direct effect on photosynthetic rate, they do control growth and related developmental processes such as sink growth and leaf senescence (Taiz and Zeiger, 1998). Carbohydrates such as sucrose have been shown to have inhibitory and stimulatory effects on gene expression (Taiz and Zeiger, 1998), potentially affecting the transcription of molecules needed to either stimulate or inhibit photosynthesis.

Potato

The potato (*Solanum tuberosum* L.) is in the Solanaceae family and was first cultivated by people in the high Andes of western South America at least 7,000 years ago

(Petroff, 2002). From Peru, Chile, and Colombia, Spanish and English explorers introduced potatoes to Europe in the mid 1500's (Petroff, 2002). Today, the world produces approximately 350 million tons of potatoes per year with a U.S. annual consumption per capita of 61 kg fresh weight (Lachman et al., 2001). Potatoes are the fourth largest crop grown in the world and are exceeded in total hectares only by rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and corn (*Zea mays* L.) (Petroff, 2002). They are grown primarily for human consumption, providing a major source of energy and protein (Lachman et al., 2001)

Potato plants are perennial in nature and survive year-to-year as a tuber. However, in commercial production potatoes are grown as an annual crop by planting certified, disease free, seed potatoes each year (IPMPWUS, 1986). There are four stages of growth and development for potato plants: vegetative growth, tuber initiation, tuber growth, and maturation (IPMPWUS, 1986). The vegetative growth stage begins when approximately three sprouts emerge with eight to 12 leaves to form the main stems (IPMPWUS, 1986). When the plant begins to flower, tuber growth is initiated. As tubers begin to develop at the tips of the stolons, more vegetative growth is observed (IPMPWUS, 1986). More carbohydrates are produced during photosynthesis than are required to sustain growth of the plant which allows for tuber enlargement. Maturation is the final growth stage and is characterized by the thickening of the tuber skin and senescing of potato plant tissue (IPMPWUS, 1986).

Late season cultivars are indeterminate and have a vine type of growth with three stages of flowering (IPMPWUS, 1986). The primary flowering typically coincides with the tuber growth phase and after the plant has grown eight to 12 leaves, a branch

develops from the axillary bud producing eight to 12 more leaves to initiate secondary flowering. The process continues until after tertiary flowering when growth of the plant stops. Indeterminate varieties continue to produce tubers until disease, weather conditions, or lack of nutrients and water terminates their growth (IPMPWUS, 1986).

Early season cultivars are determinate and have a bush type growth (IPMPWUS, 1986). Branches may develop after primary flowering, but always they develop from the nodes on the main stem. Determinate varieties tend to initiate tuber growth earlier and stop the growth of new foliage (IPMPWUS, 1986). Unlike indeterminate varieties, which typically complete their growth cycle in 100 days, determinate varieties complete their growth cycle in 60 to 80 days.

Colorado Potato Beetle

The Colorado potato beetle (*Leptinotarsa decemlineata* Say) was discovered in the Rocky Mountain region of North America in 1824, defoliating weeds such as buffalo bur (*Solanum rostratum* Ramur) and nightshades (*Solanum dulcamara* L.) within the Solanaceae family (Muka and Semel, 1983). Within 30 years, as settlers moved westward and started planting the cultivated potato, Colorado potato beetles shifted their preference of Solanaceae weeds to potato, a Solanaceae crop. In 1859, larvae and adult Colorado potato beetles were first recognized as a pest by settlers in the Eastern Rocky Mountain range (Ragsdale and Radcliffe, 2002). By 1874, Colorado potato beetles had migrated eastward an average of 85 miles per year, following potatoes grown on farms and in gardens in the Great Plains and Ohio River Valley (Ragsdale and Radcliffe, 2002).

Since reaching the East Coast, Colorado potato beetles have been introduced to Europe and now can be found as far east as the Ural Mountains.

Adult Colorado potato beetles have black spotted orange heads and their stout, oval bodies have 10 black-and-yellow alternating longitudinal lines on their elytra. The insect overwinters in the adult stage approximately 5-20 cm below the soil surface (Dwyer et al., 2001). In the spring, adult Colorado potato beetles emerge from the soil and search for suitable hosts. Once the beetles find a suitable host, they feed, mate, and lay eggs. Females, over a four week period, can lay 400-600 yellowish-orange eggs in rows of 10-30 clusters on the underside of leaves (Dwyer et al., 2001), and over a lifetime, the female may lay more than 3500 eggs (Stetter Neel, 1992). Eggs hatch within 4-10 days, and the neonates immediately start feeding. The dark orange larvae are humpbacked with two lateral rows of black spots. Within a 2-3 week period, the dark orange larvae undergo four molts, getting larger with each molt (Dwyer et al., 2001). On the final molt, the larvae construct a small chamber in the ground to pupate and emerge as adults in 5-10 days. Depending on the season, as many as three different generations of Colorado potato beetles may occur.

Colorado Potato Beetle Injury and Potato Response

Colorado potato beetle adults and larvae defoliate potato leaves. Defoliation often affects the production, transport, or allocation of resources within the plant, reducing the final yield of the crop due to decreased growth. Dripps and Smilowitz (1989) found that injury from Colorado potato beetles during the tuber growth or full-bloom phase caused a greater reduction of leaf, stem, and tuber growth than injury during the vegetative phase

or before bloom. Decreases in vegetative and tuber growth at the flowering stage may be caused by a decrease in leaf area and thus photosynthesis and carbohydrate production in plants with high levels of defoliation. A decrease in the production of carbohydrates at a growth stage where carbohydrate production is critical to maintain growth, decreases total yield.

Potato Biochemical Response to Colorado Potato Beetle Injury

Plants have developed both physical and chemical defense mechanisms to reduce injury by insects and/or the impact of the injury by insects. Plant compensation is for every unit of injury, there is no plant damage. In some plant species, insect injury will affect the level of plant compensation, so that if the growth rate is high, the demand for photoassimilates will be high, increasing the overall photosynthetic rate (Delaney and Macedo, 2001). Plants can also induce resistance through the production of allelochemicals that may act as attractants and/or deterrents to beneficial and non-beneficial insects and/or use degrees of tolerance where they can endure or compensate for varying levels of injury (Delaney and Macedo, 2001).

Primary metabolites are compounds involved in photosynthesis, respiration, solute transport, translocation, nutrient assimilation, or differentiation (Taiz and Zeiger, 1998). Unlike primary metabolites, secondary metabolites have no known direct function in growth and development, but function as biochemical defenses against injury and stress (Taiz and Zeiger, 1998). Secondary metabolites are highly diverse among taxa and their presence has lead to extensive research on the origin, diversity, and role that these chemicals play in plant-insect interactions, as well as their specificity to insects (Theis

and Ler dau, 2003). Through natural selection, secondary metabolites are thought to have evolved with little metabolic costs and toxicity to the plants themselves (Theis and Ler dau, 2003).

Many of these secondary compounds function as natural pesticides within plants, and there is increasing interest to enhance these natural pesticides for commercial use (Fenwick et al., 1990). Plants are being bred to contain not only a greater diversity of natural compounds, but also greater quantities (Hlywka et al., 1994). At certain concentrations, these compounds are toxic to humans and livestock (Theis and Ler dau, 2003).

There are three types of secondary compounds: terpenes, phenolics, and nitrogen-containing alkaloids. Although terpenes are the largest class of secondary metabolites (Thies and Ler dau, 2003), glycoalkaloids, in North America are thought to be the most highly consumed natural toxin (Hall, 1992). However, little is known about the human dietary risks associated with consumption of these chemicals and how those risks are altered by insect injury.

In potatoes, glycoalkaloids serve as natural defense mechanisms against pathogens and insects (Lachman et al., 2001). Because naturally occurring pesticides may be synthesized when plants are under stress, it is expected that injury to plant tissue would instigate synthesis of higher concentrations of the naturally occurring pesticides in both the injured and uninjured plant tissue. Hlywka et al. (1994) found tubers from plants subjected to Colorado potato beetle defoliation contained higher glycolalkaloid concentrations than tubers from undefoliated plants. In field experiments, Hlywka et al. (1994) observed a 37.53% increase in glycoalkaloid concentrations of tubers from

‘Superior’ plants defoliated at high levels by Colorado potato beetles in comparison to control plants. In tubers from ‘Superior’ plants manually defoliated, there was a 13.33% increase in glycoalkaloid concentration in comparison to tubers from control plants. Hlywka et al. (1994) argued that potato plants protected by pesticides may result in a higher degree of safety by reducing the natural production of glycoalkaloids in tubers when plants are stressed.

Glycoalkaloids

In the potato plant, glycoalkaloids are found in high concentrations in the leaves, stems, and sprouts. Lower concentrations of glycoalkaloids can be found in the skin of tubers and areas where sprouts emerge (Lachman et al., 2001). Friedman and Dao (1992) found that leaves of red, white, and Russet Burbank potatoes grown in a greenhouse had a concentration of glycoalkaloids 10 times greater than the tubers and a sprout glycoalkaloid concentration nearly 68 times greater than the tubers. Phillips et al. (1996) observed a greater concentration of glycoalkaloids in the leaves compared to tubers from the same plants; however, there was a great deal of variability among leaf glycoalkaloid concentrations within the same variety of plants. In tubers, the greatest concentration of glycoalkaloids was found in the skin (Bejarano et al., 2000), and the greater the concentration of glycoalkaloids present in tubers, the more bitter the taste (Lachman et al., 2001).

Although there are many glycoalkaloids, α -chaconine and α -solanine account for up to 95% of the total glycoalkaloids present (Friedman and McDonald, 1997); α -

solanine is found in greater concentrations than α -chaconine, and α -solanine has only half as much specific activity as α -chaconine (Lachman et. al., 2001). Other glycoalkaloids that are present, but in much lower concentration, are β - and γ - solanines and chaconines, α - and β -solamarines, and aglycones demissidine and 5- β -solanidan-3-*a*-ol (Friedman and McDonald, 1997). In wild potato, leptines, commersonine, demissine, and tomatine are also present (Friedman and McDonald, 1997).

Roddick and Rijnenberg (1986) observed a degree of synergism between α -solanine and α -chaconine on the lysis of phospholipids/sterol liposomes, which was dependent upon the concentrations of α -solanine and α -chaconine with the greatest effect observed when α -chaconine comprised over 40% of the glycoalkaloids present. In addition, Roddick and Rijnenberg (1986) tested the synergism between α -solanine and α -chaconine, α -tomatine, digitonin, and β_2 -chaconine, and observed synergistic effects only between α -solanine and α -chaconine.

Factors that increase glycoalkaloid levels in tubers include tuber exposure to light, bruising, cutting, rotting by fungi or bacteria, and other forms of mechanical damage (Lachman et al., 2001). Lachman et al. (2001) found that between damaged tubers and undamaged tubers, damaged tubers had 89% higher glycoalkaloid content and in 1994, when weather conditions were unfavorable and dry, the glycoalkaloid content was 71% higher than the content in 1995. In a greenhouse study, tubers harvested from a “hot” chamber versus a “cool” chamber contained a greater concentration of glycoalkaloids (Lachman et al., 2001). In addition to growing conditions, handling, and storage, tubers

exposed to light often turn green and can have especially high glycoalkaloid concentrations (Friedman and McDonald, 1997).

Health Risks

Tablestock tubers typically have about 75 mg/kg fresh weight (FW) or 500 mg/kg dry weight (DW) total of α -chaconine and α -solanine (Zeiger, 1998). Neither α -solanine nor α -chaconine are regulated in the US. The U.S. Department of Agriculture recommended food-safety level for glycoalkaloids is 200 mg/kg FW or 1000 mg/kg DW (Bejarano et al., 2000; Zeiger, 1998). Most commercial tablestock tubers contain between 20 and 130 mg/kg FW (Zeiger, 1998) or 133 and 867 mg/kg DW. Levels of α -solanine greater than 140 mg/kg FW or 933 mg/kg DW taste bitter, and levels greater than 200 mg/kg FW or 1333 mg/kg DW cause a burning sensation in the throat and mouth (Lachman et al., 2001). Dry weights of potatoes typically are calculated by assuming a fresh weight reduction of 85% (Eborn, 2000).

In humans, the toxic dose for glycoalkaloids is 2 to 5 mg/kg of body weight (BW) and the fatal dose is 3 to 6 mg/kg BW (Morris and Lee, 1984). According to Friedman and McDonald (1997), the toxic effect level seems to be closer to 1.0 mg/kg BW, but there are few human toxicity studies. Hellanäs et al. (1992) used seven volunteers who abstained from eating potatoes for two days and were then given potatoes containing glycoalkaloids at a dose of 1 mg/kg BW per individual. Six out of the seven subjects experienced burning sensation of the mouth and light to severe nausea and one of the six experienced diarrhea; the first initial symptoms were observed 30 minutes after

consumption and lasted for approximately four hours. Animal studies have shown that in mouse, rat, and hamster tissue, α -chaconine and α -solanine reached their highest concentrations within 6 to 14 hours of ingestion and in less than 35 hours, peak concentrations in the blood were reached (Zeiger, 1998).

The symptoms of “solanine” poisoning include nausea, vomiting, diarrhea, stomach and abdominal cramps, headache, fever, rapid and weak pulse, rapid breathing, hallucinations, delirium, and coma (Friedman and McDonald, 1997). Effects on the nervous system include increased heart, pulse, and respiratory rates, sedation, and coma (Zeiger, 1998). Effects from cell membrane disruption include internal hemorrhaging, edema, diarrhea, constriction of the abdominal muscles, and lesions in the stomach and duodenum of the large intestines. Teratogenic effects have been observed mainly in the central nervous system and include exencephaly, cranial bleb, encephalocele, and anophthalmia (Zeiger, 1998). Alpha-chaconine seems to exert teratogenic effects at lower concentrations than α -solanine (Zeiger, 1998).

Tissues that were observed to accumulate α -chaconine and α -solanine were abdominal fat, adrenals, blood, brain, heart, kidney, liver, lungs, muscle, pancreas, spleen, testis, thymus, and thyroid. Alpha-chaconine and α -solanine remained unchanged or as solanidine when excreted in urine and feces (Zeiger, 1998).

Peeling reduces the quantity of glycoalkaloids in tubers 30 to 80% (Zeiger, 1998). Alpha-chaconine and α -solanine are not broken down from cooking or frying because they are heat stable and only begin to break down between 230 to 280°C (Bejarano et al., 2000).

Although the potential hazard associated with human consumption of plants injured by insects has been recognized (Hlwyka et al., 1994), there has not been an analytical consideration of the human dietary risks associated with increased production of glycoalkaloids as a result of Colorado potato beetle injury.

Risk Assessment

Risk assessment is a science-based framework for the objective evaluation of risk (NRC 1983). It involves three major steps: problem formulation, data analysis, and risk characterization (USOSTP, 1999). Problem formulation establishes the objective and scope of the risk assessment, whereas the data analysis phase incorporates two steps: hazard identification and exposure assessment. In terms of human dietary risk assessment, hazard identification identifies the inherent ability for a substance to cause harm to a human subgroup and may either be an estimate or based on a direct measurement. Hazard identification often utilizes dose-response relationships to determine the dose required to elicit a harmful response. Exposure assessment identifies the human subgroups at greatest risk for adverse health effects, the route of exposure (dermal, oral, or inhalation), the type of exposure (acute, subchronic, or chronic), and the duration of exposure; it also is the phase where exposure levels are determined based on consumption data and potential changes in the exposure level due to food preparation and storage practices. Risk characterization is the phase where the estimate of risk to the human subgroups is determined by integrating the information gathered in the data analysis phase; this involves the identification of areas where assumptions were made,

areas of uncertainties, and areas where more information is required to refine the estimates of risk.

GAS EXCHANGE RESPONSES OF POTATO TO COLORADO POTATO BEETLE DEFOLIATION

Abstract

Gas exchange was measured on potato leaves (*Solanum tuberosum* L.) on two varieties in response to varying levels of simulated and actual Colorado potato beetle (*Leptinotarsa decemlineata* Say) defoliation. For larval and adult Colorado potato beetle single-leaf defoliation experiments, no alteration in photosynthesis was observed on the remaining tissue of an injured leaf for Cal Red and Russet Burbank leaves defoliated by larval Colorado potato beetles nor for Russet Burbank leaves defoliated by adult Colorado potato beetles. No significant differences were observed between actual Colorado potato beetle and manual defoliation for both varieties. This indicates that artificial defoliation techniques may be appropriate to measure certain responses of potato to Colorado potato beetle injury and adult and larvae Colorado potato beetles may be assigned to the same injury guild. There was a significant alteration in photosynthetic rate for Cal Red leaves defoliated by adult Colorado potato beetles, but there were fewer replications of defoliation levels with that variety. In both of the whole-plant defoliation studies, defoliation type or level consistently did not result in increased or decreased gas exchange parameters compared to undefoliated controls. Although there were significant differences between gas exchange variables in upper and lower leaves in this study, there was no evidence of delayed senescence in defoliated treatments. Although, there was a significant difference among defoliation levels in both whole plant studies, defoliation type or level consistently did not result in increased or decreased gas exchange

parameters compared to undefoliated controls. Therefore, Colorado potato beetle injury on Cal Red and Russet Burbank potato plants most likely can be classified as leaf-mass consumption.

Introduction

Understanding the physiological responses of plants to insect injury is important for understanding plant population dynamics, life histories of both plants and insects, and for determining yield loss relationships and economic injury levels in agroecosystems. Plant gas exchange incorporates photosynthesis, stomatal conductance, respiration, and transpiration; each is a critical process for plant growth, development, and fitness.

Variable photosynthetic responses to insect herbivory have been observed among different combinations of plant taxa and types of insect injury. Photosynthetic rates are often dependent upon the type of insect injury imposed (Welter, 1989; Higley et al., 1993; Haile, 2001; Peterson, 2001).

Injury Guilds

Most studies have focused on the effects of plant gas exchange from insects classified as leaf-mass consumers (defoliators), which consume large amounts of leaf tissue in a relatively short time (Welter, 1989; Peterson and Higley, 1993; Peterson and Higley, 1996). Leaf-mass consumption is just one type of insect injury guild. Currently, several insect injury guilds have been identified based on differences in physiological responses of the host plant (Boote, 1981; Pedigo et al., 1986; Higley et al., 1993). These injury guilds include: leaf senescence alteration, leaf-mass consumption, leaf

photosynthetic-rate reduction, light reduction, assimilate removal, water-balance disruption, population or stand reduction, seed or fruit destruction, architecture modification, and phenological disruption.

Effects of Insect Injury on Photosynthetic Rates

A large amount of research has been conducted on the photosynthetic and other gas exchange responses of soybean (*Glycine max* L.) injured by different species of insects. Most of the research has involved the leaf-mass consumption injury guild (Peterson et al., 2004). In many of these studies, the only change observed after defoliation was a transient increase in water loss, suggesting that the photosynthetic pathway is not affected by defoliation. In two greenhouse experiments, soybean leaves at the V5 and R2 growth stages were defoliated by the green cloverworm (*Plathypena scabra* (F.)), painted lady butterfly (*Vanessa cardui* (L.)) larvae, and hole punches from a paper hole puncher and cork-borer punch. Each experienced reductions in photosynthetic rate 12 hours post injury, but no differences 24, 48, 72, or 96 hours post injury (Poston et al., 1976). In a study by Hammond and Pedigo (1981), soybean leaves defoliated by green cloverworm larvae, hole punches, and excision of whole leaves resulted in a significantly greater water loss in leaves defoliated by green cloverworm larvae and by a hole punch than excision of an entire leaf.

Although leaf-mass consumption typically does not alter photosynthetic rates of the remaining leaf tissue, reductions in photosynthetic rates after leaf-mass consumption that have been observed may be due to interactions between intrinsic and extrinsic factors. Intrinsic factors that may alter photosynthetic rates include increased assimilate

demand after defoliation (Neales and Incoll, 1968), reduced competition between leaves for mineral nutrients necessary for cytokinin production (Wareing et al., 1968), and a diversion of resources to defenses. Extrinsic factors that may alter photosynthetic rates include alterations in sunlight penetration, water and nutrient availability (Welter, 1989; Peterson et al., 1992), alteration of source-to-sink ratios, temperature, and atmospheric carbon dioxide concentrations. At the canopy level, reductions in photosynthetic rate have also been observed, but many of these reductions were temporary and were caused by a reduction in leaf-area indices, smaller leaf size, and decreased light interception (Peterson, 2001).

Secondary Compounds and Photosynthetic Rates

Plants have evolutionarily developed both physical and chemical defense mechanisms to reduce injury by insects. Plants can induce resistance through the production of chemicals that may act as attractants and/or deterrents to beneficial and non-beneficial insects and/or use degrees of tolerance where they can endure or compensate for varying levels of injury (Delaney and Macedo, 2001).

Primary metabolites are compounds involved in photosynthesis, respiration, solute transport, translocation, nutrient assimilation, or differentiation (Taiz and Zeiger, 1998). Unlike primary metabolites, secondary metabolites have no known direct function in growth and development, but have been observed to function as biochemical defenses against injury and stress (Taiz and Zeiger, 1998). Secondary metabolites are highly diverse among taxa and their presence has led to extensive research on the origin, diversity, and role that these chemicals play in plant-insect interactions, as well as their

specificity to insects. Through natural selection, secondary metabolites are thought to have evolved with little metabolic costs and toxicity to the plants themselves (Theis and Lerdau, 2003).

Many of these secondary compounds function as natural pesticides within plants. Milkweed (Asclepiadaceae) species have two well-known defense mechanisms: (1) leaf tissue containing one to several mild to severely toxic cardenolides, and (2) leaf tissue containing latex that dries on insect mouths to disrupt feeding. Defoliation using a cork-borer resulted in an eight-fold increase in cardenolides (Malcolm and Zalucki, 1996), and although latex and cardenolides do not always occur together, in species in which they do occur together, the cardenolides may be found in latex fluid. Delaney and Higley (2006) observed reductions in photosynthetic rates of common milkweed (*Asclepias syriaca* L.) on remaining leaf tissue of individual leaves injured by leaf-mass consumers. Cutting all of the lateral veins on milkweed leaves did not result in as significant of a decrease in photosynthetic rate compared to midrib vein disruption (Delaney and Higley, 2006). The midrib vein contains the xylem and phloem, which are involved in the import and export of water, and disruption of the midrib vein in milkweed resulted in localized decreases in photosynthetic rate in the tissue distal from the wound site as a result of disrupting the xylem and/or phloem (Delaney and Higley, 2006). Although water loss played a role in the decreases in photosynthetic rate on the remaining leaf tissue, because the disruption of the lateral veins did not result in as severe of a decrease in photosynthetic rate as did disruption to the midrib vein, there may be a tradeoff between a plant's natural defense (i.e., high cardenolide concentrations) and the ability for the leaf to compensate for insect injury.

Colorado Potato Beetle Injury and Potato Response

In potatoes (*Solanum tuberosum* L.), glycoalkaloids are well known and highly toxic natural defense compounds against pathogens and insects (Lachman et al., 2001). Because naturally occurring pesticides are synthesized when plants are under stress, it is expected that injury to plant tissue would instigate synthesis of higher concentrations of the naturally occurring pesticides in the injured versus uninjured plant tissue. Hlywka et al. (1994) found tubers from plants subjected to Colorado potato beetle (*Leptinotarsa decemlineata* Say) defoliation contained higher glycolalkaloid concentrations than tubers from undefoliated plants.

Colorado potato beetle adults and larvae consume leaf-mass of potato. Dripps and Smilowitz (1989) found that injury from Colorado potato beetles during the tuber growth or full-bloom phase, caused a greater reduction of leaf, stem, and tuber growth than injury during the vegetative phase or before bloom. Decreases in vegetative and tuber growth at the flowering stage may be caused by a decrease in leaf area and thus photosynthesis and carbohydrate production in plants with high levels of defoliation. A decrease in the production of carbohydrates at a growth stage where carbohydrate production is critical to maintain growth, decreases total yield.

Despite the potato being the fourth most important food crop in the world and Colorado potato beetles being a worldwide potato pest, there is very little information on how potato responds to injury from this pest. This is particularly true for physiological responses such as photosynthesis. Both Colorado potato beetle adults and larvae consume whole-leaf tissue and therefore might be considered leaf-mass consumers. However, to date, the primary physiological responses have not been characterized to test this

hypothesis. Alternatively, because potato contains high levels of toxic defensive compounds (glycoalkaloids) it may respond to defoliation similar to milkweed.

Objective

The current understanding of plant responses to biotic stressors such as insects is limited, and understanding a plants physiological response to injury provides a common language and insight into managing such pests in order to optimize development, fitness, and growth. This research was conducted to characterize the primary physiological responses of potato by measuring photosynthetic rate, stomatal conductance, and transpiration at varying levels of simulated and actual Colorado potato beetle defoliation.

Materials and Methods

Plant Material

All plants were grown in a greenhouse (Montana State University, Bozeman, MT) and planted in 50:50 MSU:Sunshine #1 soil mix, and fertilized weekly with Scott's Peter Professional Peat-Lite Special 20-20-20. The Sunshine Mix #1 consisted of Canadian Sphagnum Peat Moss, perlite, vermiculite, starter nutrient charge, wetting agent, and Dolomitic lime (Sun Gro Horticulture, Inc., Bellevue, WA). The MSU soil mix consisted of equal parts of Bozeman Silt Loam Soil, washed concrete sand, and Canadian Sphagnum Peat Moss in addition to AquaGro 2000 G wetting agent blended in at 593 g/m³ of soil mix. The MSU soil mix was aerated-steam pasteurized to 80°C for 45 min.

Plants were grown at $21 \pm 2^{\circ}\text{C}$ with a photoperiod of 14:10 (Light:Dark). Two cultivars were used, Cal Red and Russet Burbank, and were obtained from VenHuizen Seed Potatoes, Inc., Belgrade, MT. Each seed tuber was cut, ensuring at least one eye per cut, and placed cut-side down in a 20-L pot filled approximately with 12 to 13 cm of pre-moistened 50:50 MSU:Sunshine soil mix and covered with 5 to 9 cm of pre-moistened 50:50 MSU:Sunshine soil mix.

Plants were watered 4 to 5 times/week and fertilized with Scott's Peter Professional Peat-Lite Special 20-20-20 bi-weekly. Once plants reached approximately 9 cm tall, in a process called "hilling," soil was added to the pots weekly until pots were full. Upon senescence, stems were cut and potatoes were harvested two weeks later. The tubers were washed, placed in brown paper bags, and stored in a dark cold room held at 4°C .

Colorado Potato Beetles

At the early vegetative stage, Colorado potato beetle eggs were obtained from the Phillip Alampi Beneficial Insect Lab, New Jersey Department of Agriculture, Trenton, New Jersey. Approximately 120 egg masses were placed on approximately 10 to 15 extra Cal Red and Russet Burbank potato plants and allowed to hatch and feed until approximately the third instar. At approximately the third instar, the Colorado potato beetles were placed on the treatment plants and allowed to defoliate.

Larval Leaf Defoliation Study – 2005

Responses of individual potato leaves to defoliation injury by Colorado potato beetle larvae were. The greenhouse bay (32 m²) was set at 21 ± 2°C and a photoperiod of 14:10 (Light:Dark). A randomized complete block design (RCBD), repeated measures two-by-five factorial with five replications was used, with five metal halide lamps (1000 W) serving as the blocking factors. The treatment factors were the two cultivars, Cal Red and Russet Burbank, and the five defoliation factors were control (no defoliation), low and high simulated Colorado potato beetle defoliation, and low and high actual larval Colorado potato beetle defoliation.

At the early vegetative stage, a leaf in the top one-third of the plant was randomly chosen and covered with a net approximately 12 cm x 12 cm (w x h) made of green or white tulle. At the mid-vegetative stage, three and six third instars were applied to the low and high defoliation treatment leaves, respectively, and allowed to defoliate overnight to allow for approximately 30% (low) and 90% (high) of the leaf area to be removed. Simulated Colorado potato beetle injury treatments were imposed with scissors so that approximately 30% (low) and approximately 90% (high) of the leaf tissue was removed in a pattern consistent with actual larval injury immediately after the larvae were removed from the other treatment plants.

Adult Leaf Defoliation Study – 2005

Responses of individual potato leaves to defoliation injury by Colorado potato beetle adults were determined. The greenhouse bay (32 m²) was set at 21 ± 2°C and a photoperiod of 14:10 (Light:Dark). A RCBD, repeated measures two-by-five factorial

with three replications was used, with five metal halide lamps (1000 W) serving as the blocking factors. The treatment factors were the two cultivars, Cal Red and Russet Burbank, and the five defoliation factors were control (no defoliation), low and high simulated defoliation, and low and high adult Colorado potato beetle defoliation.

At the early vegetative stage, a leaf in the top one-third of the plant was randomly chosen and covered with a net approximately 12 cm x 12 cm (w x h) made of green or white tulle. At the mid-vegetative stage, one and three adult Colorado potato beetles were applied to the low and high defoliation treatment plants, respectively, and allowed to defoliate until approximately 30% (low) and 90% (high) of the plant leaf area was removed. Target defoliation levels were reached after approximately 9.5 hours. Simulated Colorado potato beetle injury treatments were imposed the same as described above.

For both larval and adult Colorado potato beetle experiments, a portable photosynthesis system (Model LI-6400, LI-COR, Inc., Lincoln, NE) with CO₂ injector and light source (to allow for stable CO₂ and light concentrations) was used to measure plant-gas exchange (photosynthesis, stomatal conductance, and transpiration). These variables were measured 24 and 48 hours after injury. All measurements were taken on a 6-cm² leaf area with the following settings: light source at 1200 $\mu\text{mol}/\text{m}^2/\text{s}$ photosynthetic photon flux density, 400 μmol of CO₂/m²/s, and chamber humidity between 40 and 60%, from mid-morning to mid-afternoon.

After the final measurements were recorded, the leaves were removed, labeled, and scanned using an HP Scanjet 7400c scanner (Hewlett-Packard Company, Palo Alto, CA) with the HP Precision Scan Pro 3.02 software (Hewlett-Packard Company, Palo

Alto, CA). Percentage of actual leaf area removed was measured using the image analysis software Sigma Scan Pro 5.0 (SPSS Inc., Chicago, IL) and Adobe Photoshop CS2 9.0 (Adobe Systems Inc., San Jose, CA).

Manual Defoliation Study – 2004

Responses of individual potato leaves over time to whole-plant defoliation injury were determined in two experiments conducted in 2004 and 2005. In a greenhouse (32 m²) set at $21 \pm 2^{\circ}\text{C}$ and a photoperiod of 14:10 (Light:Dark), five replicates of Russet Burbank cultivar of the following treatments—control (no defoliation), low, medium, and high manual defoliation—were arranged in a two-by-four repeated measures factorial within an RCBD; low defoliation corresponded to approximately 30% leaf-mass removal, medium defoliation corresponded to approximately 60% leaf-mass removal, and high defoliation corresponded to approximately 90% leaf-mass removal. Metal halide lamps (1000 W) served as the blocking factors.

Plants were defoliated over a 10-day period at the mid-vegetative stage, removing the following percentages of leaf-mass corresponding to their treatment levels (day 1 to 10): 1%, 1%, 5%, 5%, 10%, 10%, 30%, 30%, 4%, and 4%. Leaf-mass removal was followed according to these percentages to mimic larval Colorado potato beetle defoliation from the first to fourth instars just prior to pupation.

The LI-6400 portable photosynthesis system was used to measure photosynthetic rate, as described above, on a leaf in the bottom third and a leaf on the top third of the plant. These gas exchange variables were measured one day after the cessation of

defoliation and thereafter weekly until the initiation of senescence (i.e., 10 weeks of measurements).

Manual and Colorado Potato Beetle Defoliation Study – 2005

A greenhouse bay (32 m²) was set at $21 \pm 2^{\circ}\text{C}$ with a photoperiod of 14:10 (Light:Dark). A RCBD, repeated measures two-by-two-by-five factorial with five replications was used, with five metal halide lamps (1000 W) serving as the blocking factors. The treatment factors were the two cultivars, Cal Red and Russet Burbank, the two leaf factors were upper and lower leaves, and the five defoliation factors were control (no defoliation), low, medium, and high Colorado potato beetle and manual defoliation.

At the early vegetative stage, all plants were individually covered with nets approximately 91 cm x 40 cm x 40 cm (h x w x d) made of white tulle. Approximately 120 egg masses were placed on 10 host Cal Red and Russet Burbank potato plants and allowed to hatch and feed until the larvae reached the third instar. At the mid-vegetative stage, just before flowering, approximately 15, 20, and 25 third instars were applied to the low, medium and high treatment plants, respectively, and allowed to defoliate. Once the low, medium, and high treatment plant leaf area was reduced by approximately 30% (low), 60% (medium), and 90% (high) the Colorado potato beetles were removed. The manually defoliated plants were defoliated with scissors throughout the same period to simulate the percentage and patterns of leaf-mass removed by the Colorado potato beetles.

The LI-6400 portable photosynthesis system was used to measure gas exchange rate on a 2-cm² leaf area using the procedure described above. A leaf in the bottom third

and a leaf in the top third of the plant was measured each time. Gas exchange parameters were measured one week following the application of Colorado potato beetles and weekly thereafter until the initiation of senescence (i.e., six weeks of measurements).

Statistical Analyses

Control and treated groups were compared ($\alpha = 0.05$) using an analysis of variance (ANOVA) and interaction plots fitted with standard error. Data were analyzed using R 2.0.1 (R: A Language and Environment for Statistical Computing; The R Foundation for Statistical Computing, 2004, Vienna, Austria). To meet normality, the necessary data were log transformed. Linear and non-linear regression analyses were conducted to determine the relationship between percent defoliation and gas exchange parameters using SAS system for Windows V8 (SAS Institute, Inc. ©1999-2001, Cary, NC).

Results

Larval Colorado Potato Beetle Leaf Defoliation Study – 2005

The mean ($\pm$ SE) for high Colorado potato beetle defoliation, low Colorado potato beetle defoliation, high manual defoliation, and low manual defoliation were $51.4 \pm 6.1\%$, $26.8 \pm 1.4\%$, $47.8 \pm 2.4\%$, $14.3 \pm 1.8\%$. There was no significant block, variety, defoliation, or time effect on photosynthetic rate (Table 1, Figures 1, 2). There was a significant interaction between variety, defoliation, and time ($F = 2.592$, $df = 4$, $P = 0.044$). Because there were no other interaction effects and because there were no variety or defoliation main effects, it is unlikely the three-way interaction has biological

Table 1. ANOVA for Larval Colorado Potato Beetle Leaf Defoliation Experiment (2005).

		Photosynthesis	Stomatal Conductance	Transpiration
	df	p-value	p-value	p-value
Block	4	0.5063	0.344	0.146
Variety	1	0.6467	0.0006	0.0013
Defoliation	4	0.5511	0.715	0.7804
Time	1	0.0566	0.0001	< 0.0001
Variety x Defoliation	4	0.6291	0.2661	0.319
Variety x Time	1	0.4971	0.0483	0.0734
Defoliation x Time	4	0.4405	0.2473	0.2841
Variety x Defoliation x Time	4	0.0439	0.2977	0.2407

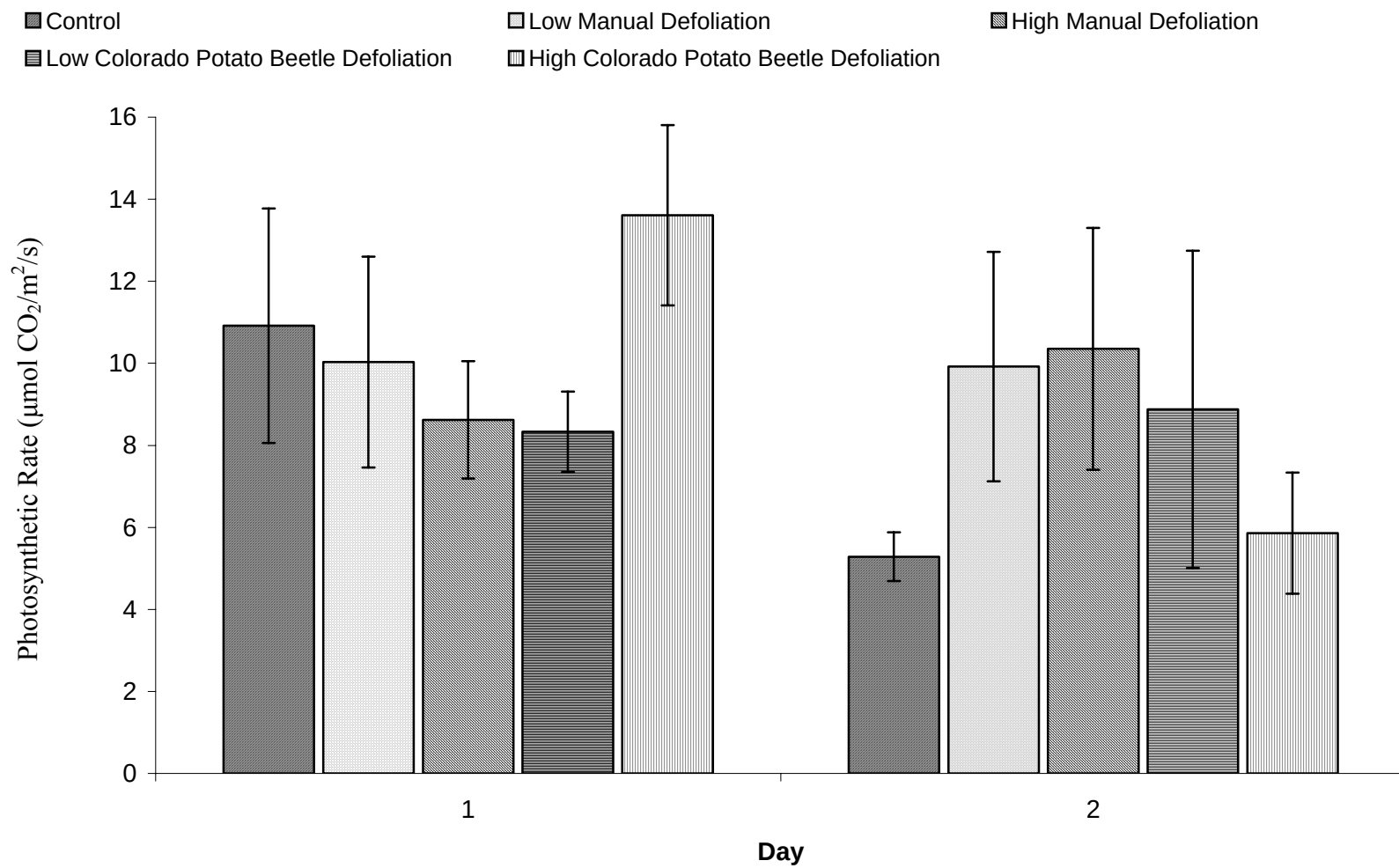


Figure 1. Photosynthetic Rates of the Larval Colorado Potato Beetle Leaf Defoliation Study (2005) on Cal Red plants.

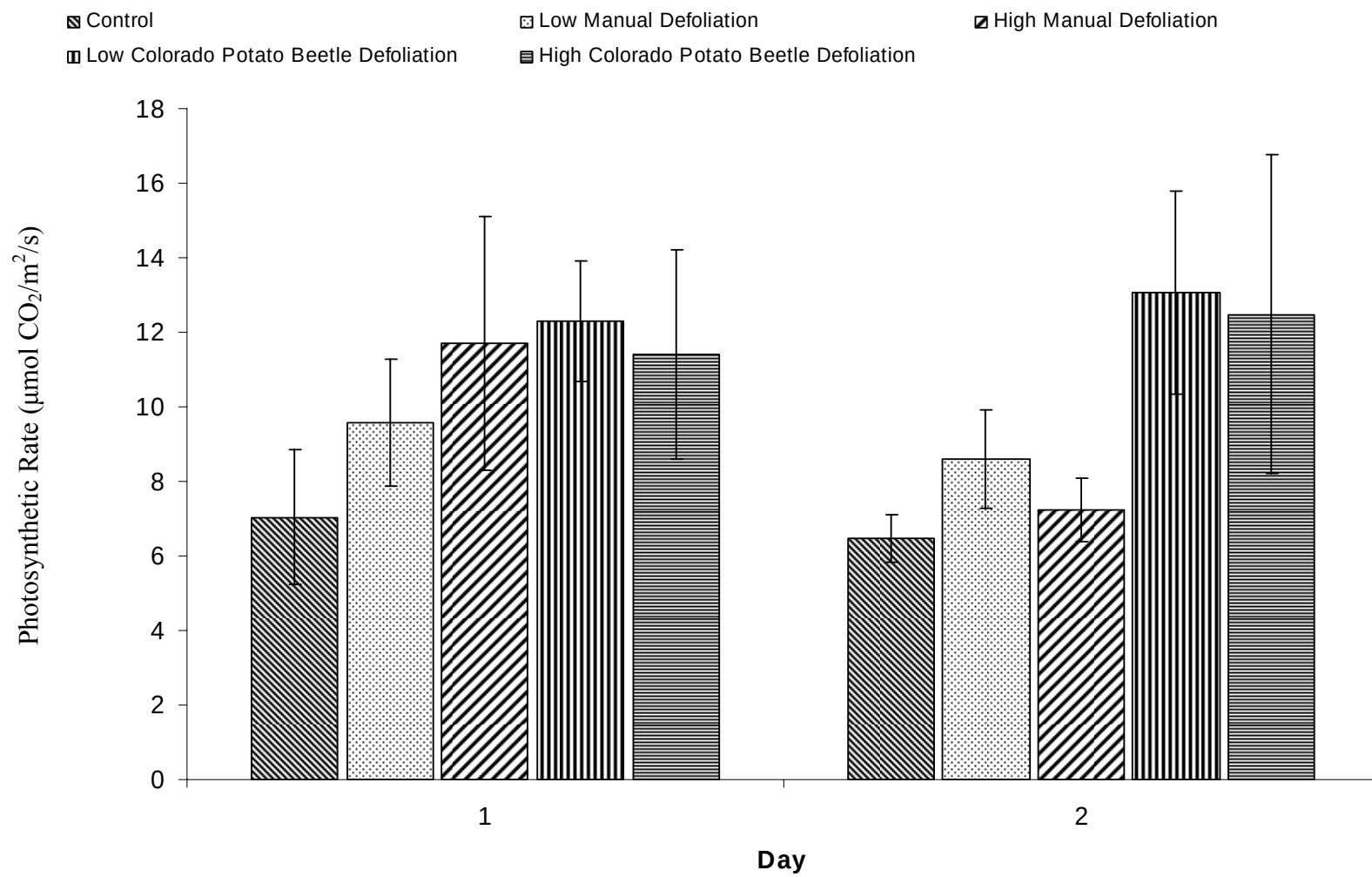


Figure 2. Photosynthetic Rates of the Larval Colorado Potato Beetle Leaf Defoliation Study (2005) on Russet Burbank Plants.

relevance. Linear and quadratic relationships between percent defoliation and photosynthesis were not significant.

Stomatal conductance was log transformed and variety ($F = 13.077$, $df = 1$, $P = 0.0006$), time ($F = 18.133$, $df = 1$, $P = 0.0001$), and the interaction between variety and time ($F = 4.04$, $df = 1$, $P = 0.048$) had a significant effect on stomatal conductance (Table 1). Linear and quadratic relationships between percent defoliation and conductance were not significant.

Transpiration was log transformed and variety ($F = 11.27$, $df = 1$, $P = 0.001$) and time ($F = 57.905$, $df = 1$, $P < 0.0001$) had a significant effect on transpiration (Table 1). Linear and quadratic relationships between percent defoliation and transpiration rate were not significant.

Adult Colorado Potato Beetle Leaf Defoliation Study – 2005

The mean ($\pm$ SE) for high Colorado potato beetle defoliation, low Colorado potato beetle defoliation, high manual defoliation, and low manual defoliation were $55.7 \pm 12.0\%$, $32.2 \pm 11.4\%$, $47.5 \pm 2.5\%$, $16.9 \pm 1.9\%$. There was no significant block, variety, defoliation, or time effect on photosynthetic rate (Table 2, Figures 3, 4) The interaction between variety and defoliation had a significant effect on photosynthetic rate ($F = 4.621$, $df = 4$, $P = 0.005$) (Table 2). As defoliation level and type increased (i.e., more severe), Cal Red plants' photosynthetic rate decreased (Figure 3); this same trend was not observed in Russet Burbank plants (Figure 4). Linear and quadratic relationships between percent defoliation and photosynthesis were not significant.

Table 2. ANOVA for Adult Colorado Potato Beetle Leaf Defoliation Experiment (2005).

		Photosynthesis	Stomatal Conductance	Transpiration
	df	p-value	p-value	p-value
Block	2	0.2326	0.385	0.3117
Variety	1	0.1417	0.0121	0.0277
Defoliation	4	0.2651	0.4824	0.5152
Time	1	0.5953	0.0395	0.0345
Variety x Defoliation	4	0.0054	0.1676	0.1095
Variety x Time	1	0.4523	0.8275	0.7971
Defoliation x Time	4	0.3659	0.1227	0.1094
Variety x Defoliation x Time	4	0.1597	0.5789	0.4954

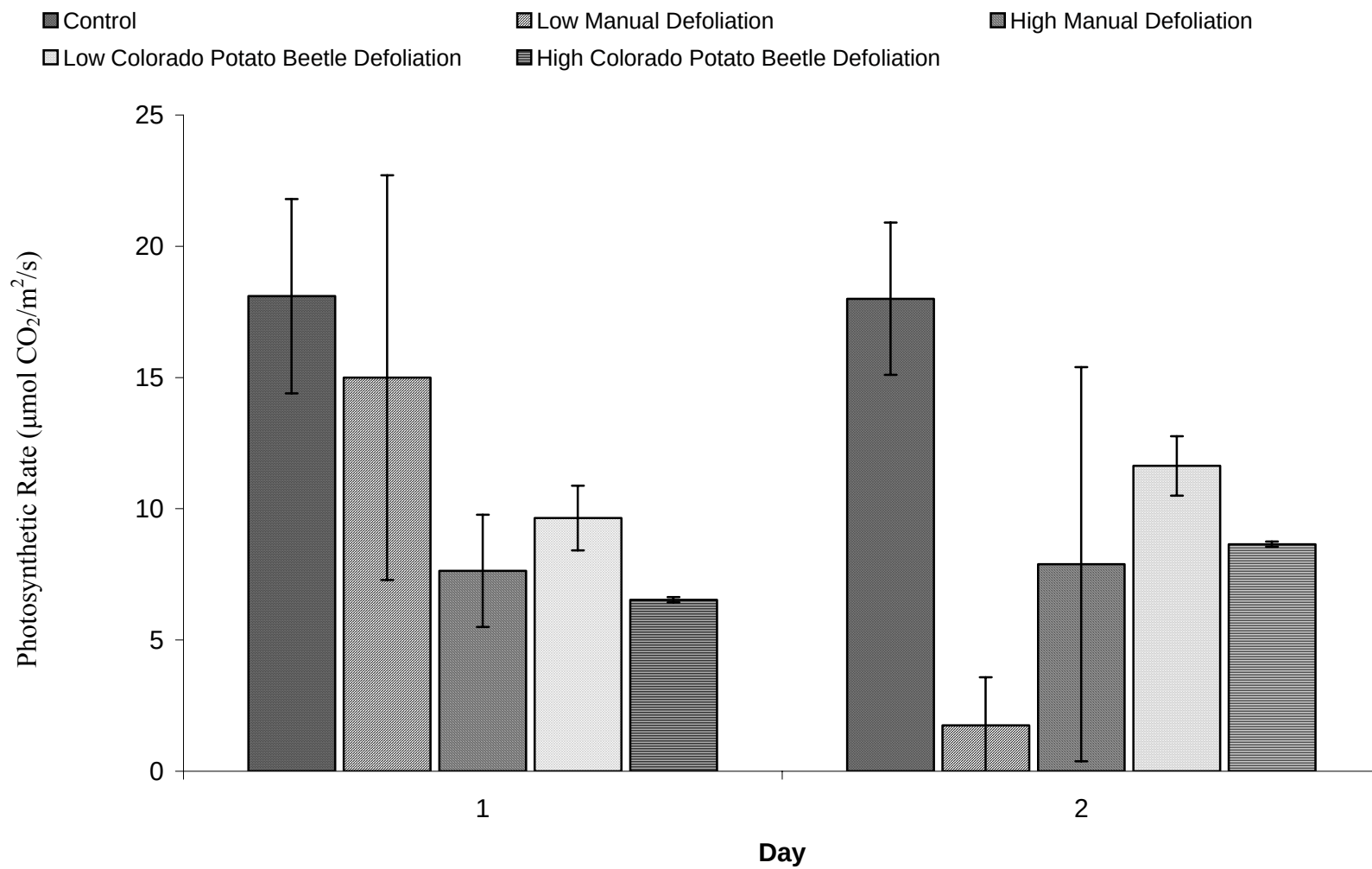


Figure 3. Photosynthetic Rates of the Adult Colorado Potato Beetle Leaf Defoliation Study (2005) Cal Red Plants.

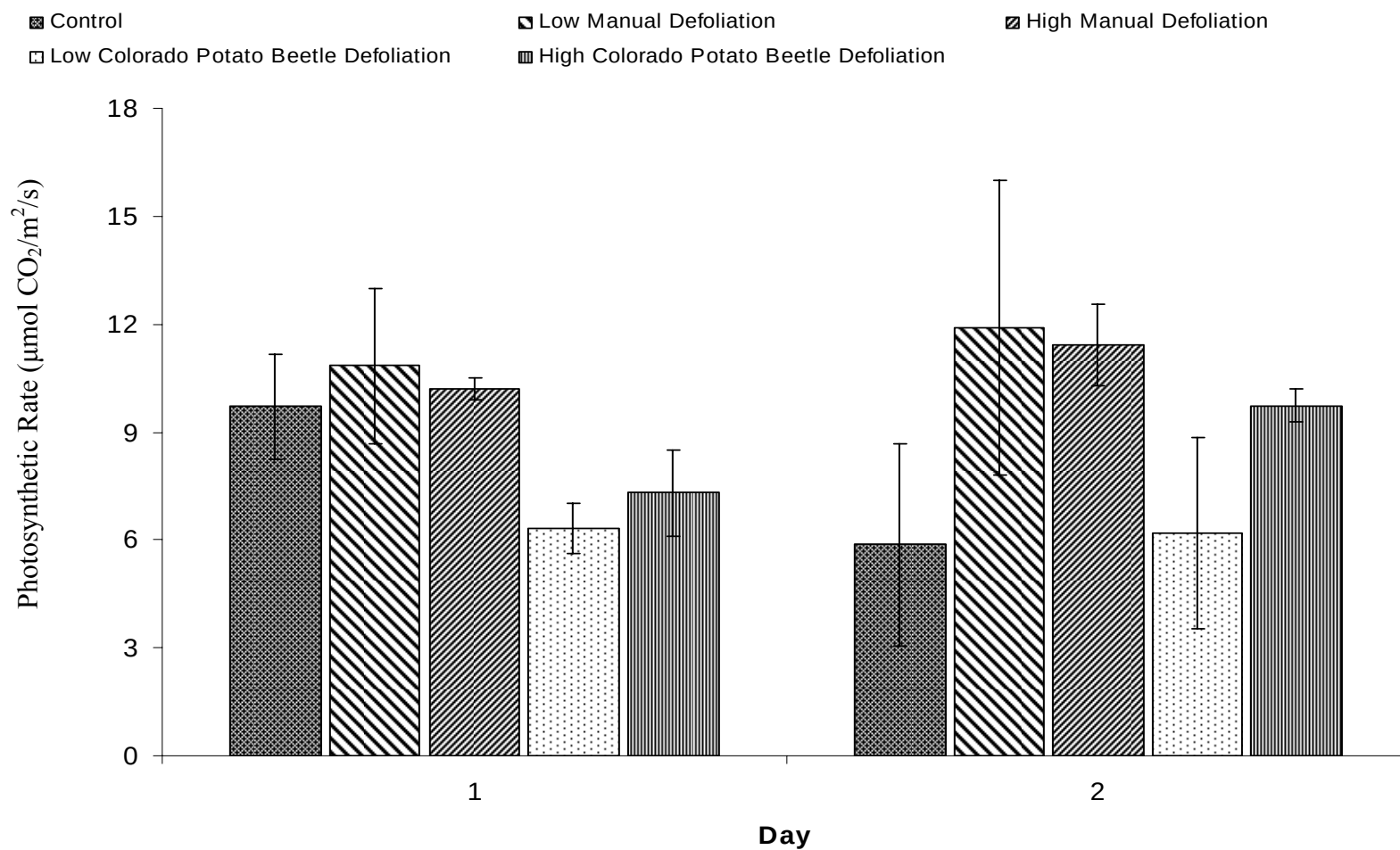


Figure 4. Photosynthetic Rates of the Adult Colorado Potato Beetle Leaf Defoliation Study (2005) on Russet Burbank Plants.

Stomatal conductance was log transformed and variety ($F = 7.32$, $df = 1$, $P = 0.012$) and time ($F = 4.72$, $df = 1$, $P = 0.04$) had a significant effect on conductance (Table 2). The linear relationship between percent defoliation and transpiration rate was not significant. A significant quadratic relationship was measured between percent defoliation and conductance 48 hours post injury ($F = 5.62$, $df = 1$, $P = 0.0279$, $\beta_0 = 0.7501$, $\beta_1^2 = 0.00001$, $r^2 = 0.21932$).

Transpiration was log transformed and variety ($F = 5.464$, $df = 1$, $P = 0.028$) and time ($F = 5.001$, $df = 1$, $P = 0.035$) had a significant effect on transpiration (Table 2). The linear relationship between percent defoliation and transpiration rate was not significant. A significant quadratic relationship was measured between percent defoliation and transpiration 48 hours post injury ($F = 6.24$, $df = 1$, $P = 0.0214$, $\beta_0 = 1.1226$, $\beta_1^2 = 0.00003$, $r^2 = 0.23771$).

Manual Defoliation Study – 2004

Photosynthesis was log transformed and block ($F = 18.36$, $df = 4$, $P < 0.0001$), defoliation ($F = 4.218$, $df = 1$, $P = 0.006$), leaf location ($F = 4.742$, $df = 1$, $P = 0.03$), and time ($F = 134.87$, $df = 9$, $P < 0.0001$) had a significant effect on photosynthetic rate (Table 3). The interaction between leaf and time also had a significant effect on photosynthetic rate ($F = 1.914$, $df = 9$, $P = 0.05$) (Table 3). Photosynthetic rates for upper and lower leaves across all defoliation levels varied considerably throughout the 10 weeks of measurements (Figures 5, 6). Linear and quadratic relationships between percent defoliation and photosynthesis were not significant.

Table 3. ANOVA for Manual Defoliation Experiment (2004).

		Photosynthesis	Stomatal Conductance	Transpiration
	df	p-value	p-value	p-value
Block	4	< 0.0001	0.1905	0.5593
Leaf	1	0.0302	0.0092	0.0697
Defoliation	3	0.0061	0.0097	0.0186
Time	9	< 0.0001	< 0.0001	< 0.0001
Leaf x Defoliation	3	0.5196	0.2337	0.0977
Leaf x Time	9	0.0496	0.2738	0.103
Defoliation x Time	27	0.6196	0.0002	< 0.0001
Leaf x Defoliation x Time	27	0.9919	0.9088	0.7127

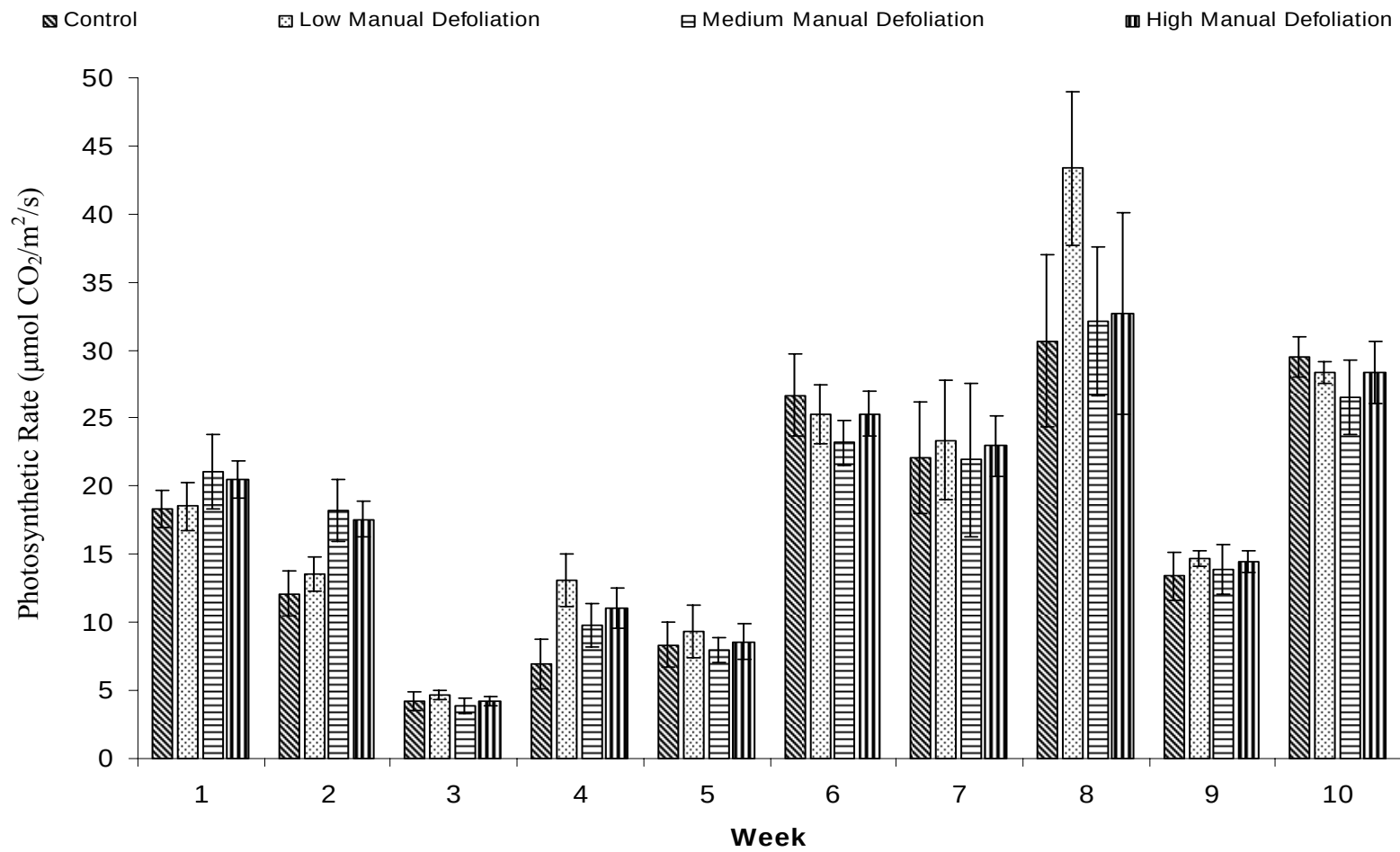


Figure 5. Photosynthetic Rates of the Manual Defoliation Study (2004) on Upper Leaves of Russet Burbank Plants.

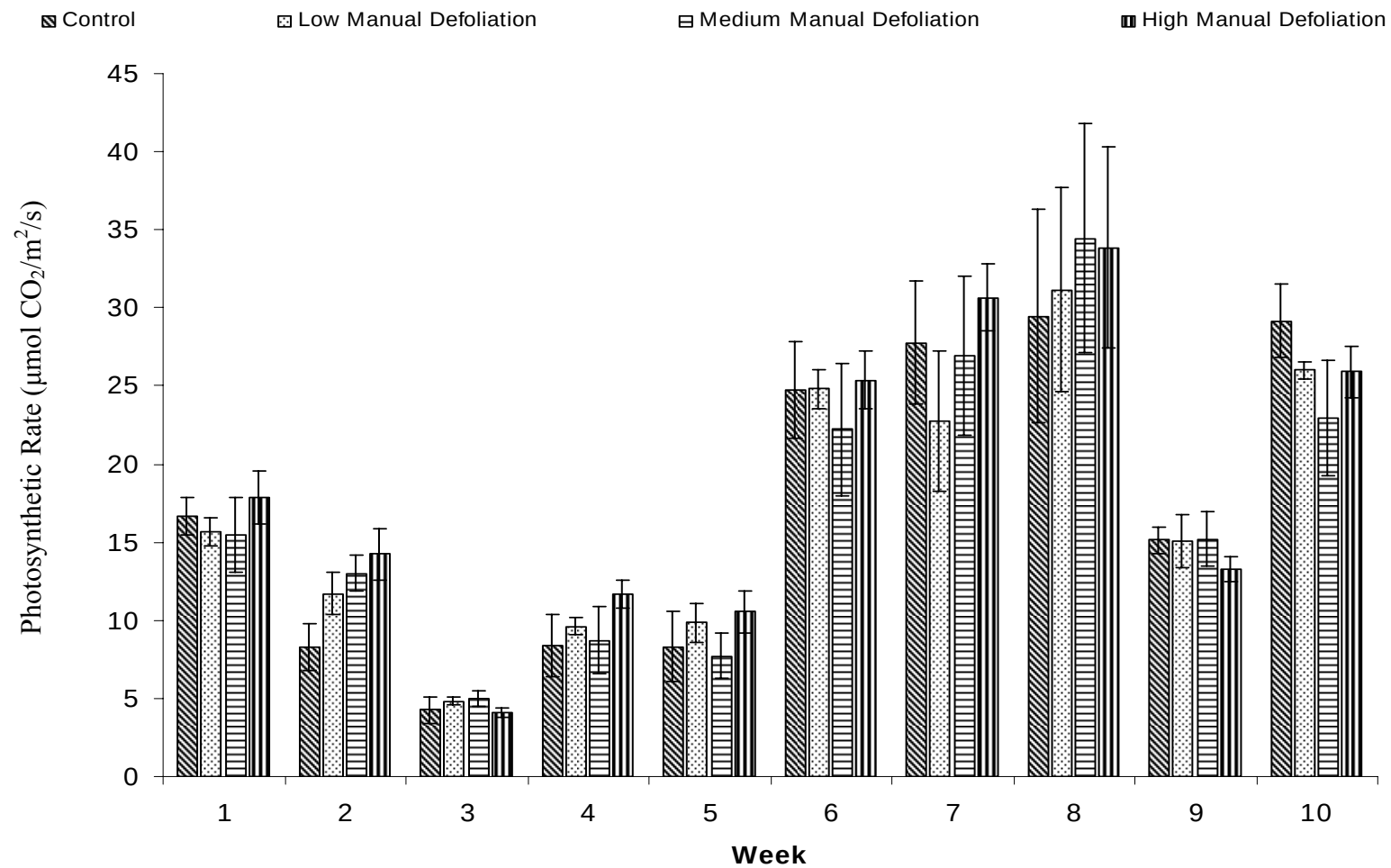


Figure 6. Photosynthetic Rates of the Manual Defoliation Study (2004) on Lower Leaves of Russet Burbank Plants.

Stomatal conductance was log transformed and treatment ($F = 3.878$, $df = 3$, $P = 0.01$), time ($F = 55.749$, $df = 9$, $P < 0.0001$), and leaf location ($F = 6.877$, $df = 1$, $P = 0.0092$) had a significant effect on stomatal conductance (Table 3). The interaction between defoliation level and time ($F = 2.444$, $df = 27$, $P = 0.0002$) also had a significant effect on stomatal conductance.

Transpiration was log transformed and defoliation level ($F = 3.392$, $df = 3$, $P = 0.019$) and time ($F = 27.773$, $df = 9$, $P < 0.0001$) had a significant effect on mean transpiration (Table 3). The interaction between defoliation and level and time had a significant effect on mean transpiration ($F = 2.635$, $df = 27$, $P < 0.0001$) (Table 3).

Manual and Colorado Potato Beetle Defoliation Study – 2005

Block ($F = 6.077$, $df = 4$, $P = 0.0001$), defoliation level ($F = 2.843$, $df = 4$, $P = 0.024$), time ($F = 54.208$, $df = 5$, $P < 0.0001$), and leaf location ($F = 22.483$, $df = 1$, $P < 0.0001$) had a significant effect on photosynthetic rate (Table 4). The interaction between variety and leaf location ($F = 5.453$, $df = 1$, $P = 0.020$) and defoliation level and leaf location ($F = 3.199$, $df = 4$, $P = 0.013$) also were significant (Table 4). Although defoliation treatments had a significant effect on photosynthetic rates, no consistent pattern was evident (Figures 7 to 10). However, there was a trend for upper leaves on defoliated plants to have higher photosynthetic rates than lower leaves (Figures 7 to 10). Linear and quadratic relationships between percent defoliation and photosynthesis were not significant.

Stomatal conductance was log transformed and block ($F = 6.700$, $df = 4$, $P = 0.0001$), defoliation level ($F = 9.190$, $df = 4$, $P = 0.0001$), time ($F = 51.300$, $df = 5$, $P =$

Table 4. ANOVA for Manual and Colorado Potato Beetle Defoliation Experiment (2005).

		Photosynthesis	Stomatal Conductance	Transpiration
	df	p-value	p-value	p-value
Block	4	0.0001	< 0.0001	0.071
Leaf	1	< 0.0001	< 0.0001	< 0.0001
Defoliation	4	0.0238	< 0.0001	0.002
Time	5	< 0.0001	< 0.0001	< 0.0001
Variety	1	0.4574	0.0962	0.4371
Leaf x Defoliation	4	0.0131	0.0001	0.0008
Leaf x Time	5	0.4322	0.8214	0.2014
Defoliation x Time	20	0.981	0.9596	0.9803
Variety x Time	5	0.917	0.2095	0.3491
Variety x Leaf	1	0.02	0.0001	0.0021
Variety x Defoliation	4	0.417	0.2447	0.7925
Defoliation x Time x Leaf	20	0.7981	0.785	0.6947
Variety x Defoliation x Time	20	0.9048	0.9869	0.9722
Variety x Defoliation x Leaf	4	0.3856	0.013	0.1266
Variety x Time x Leaf	5	0.8377	0.7867	0.8033
Variety x Defoliation x Time x Leaf	20	0.611	0.5272	0.6114

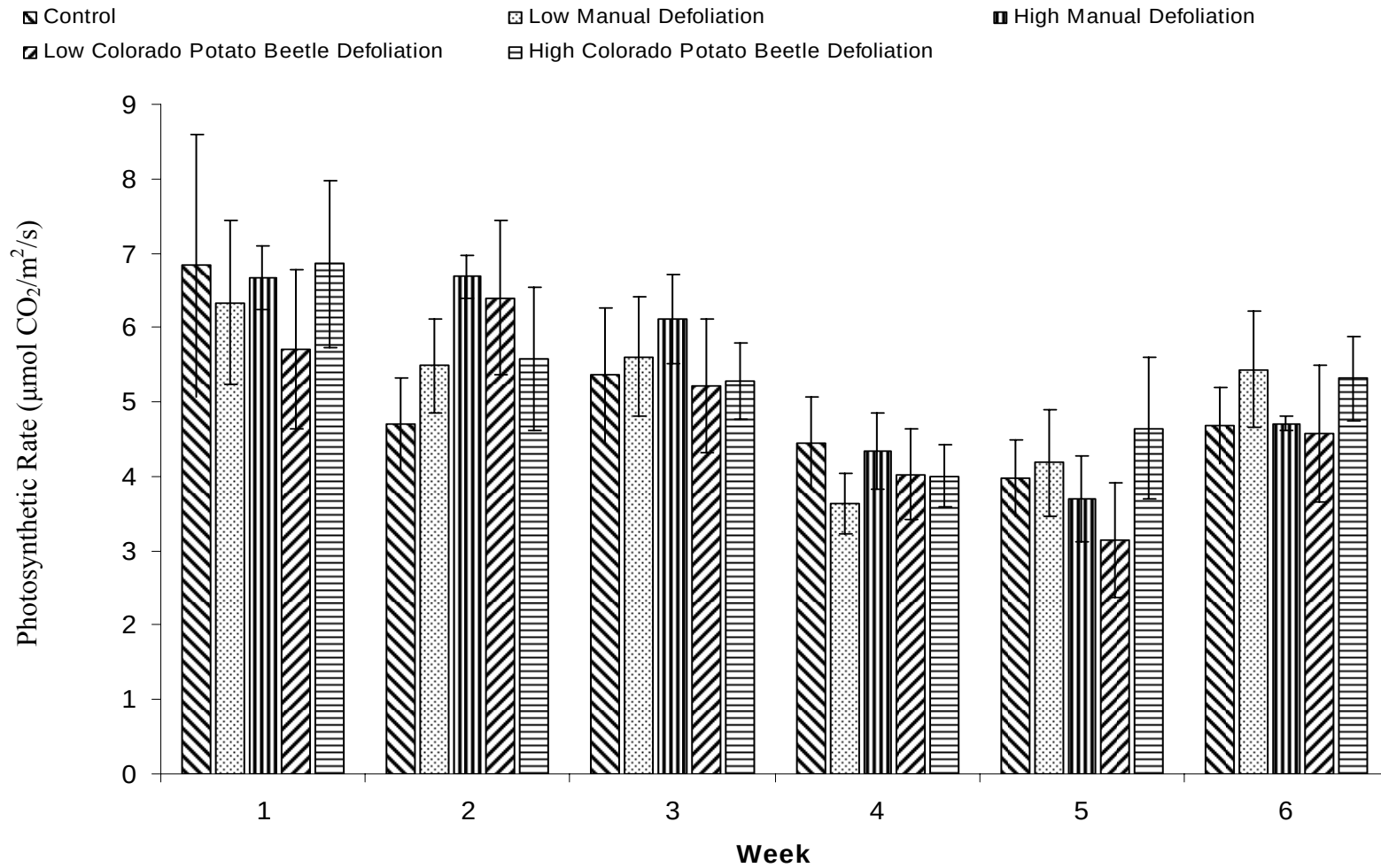


Figure 7. Photosynthetic Rates of the Manual and Colorado Potato Beetle Defoliation Study (2005) on Upper Leaves of Cal Red Plants.

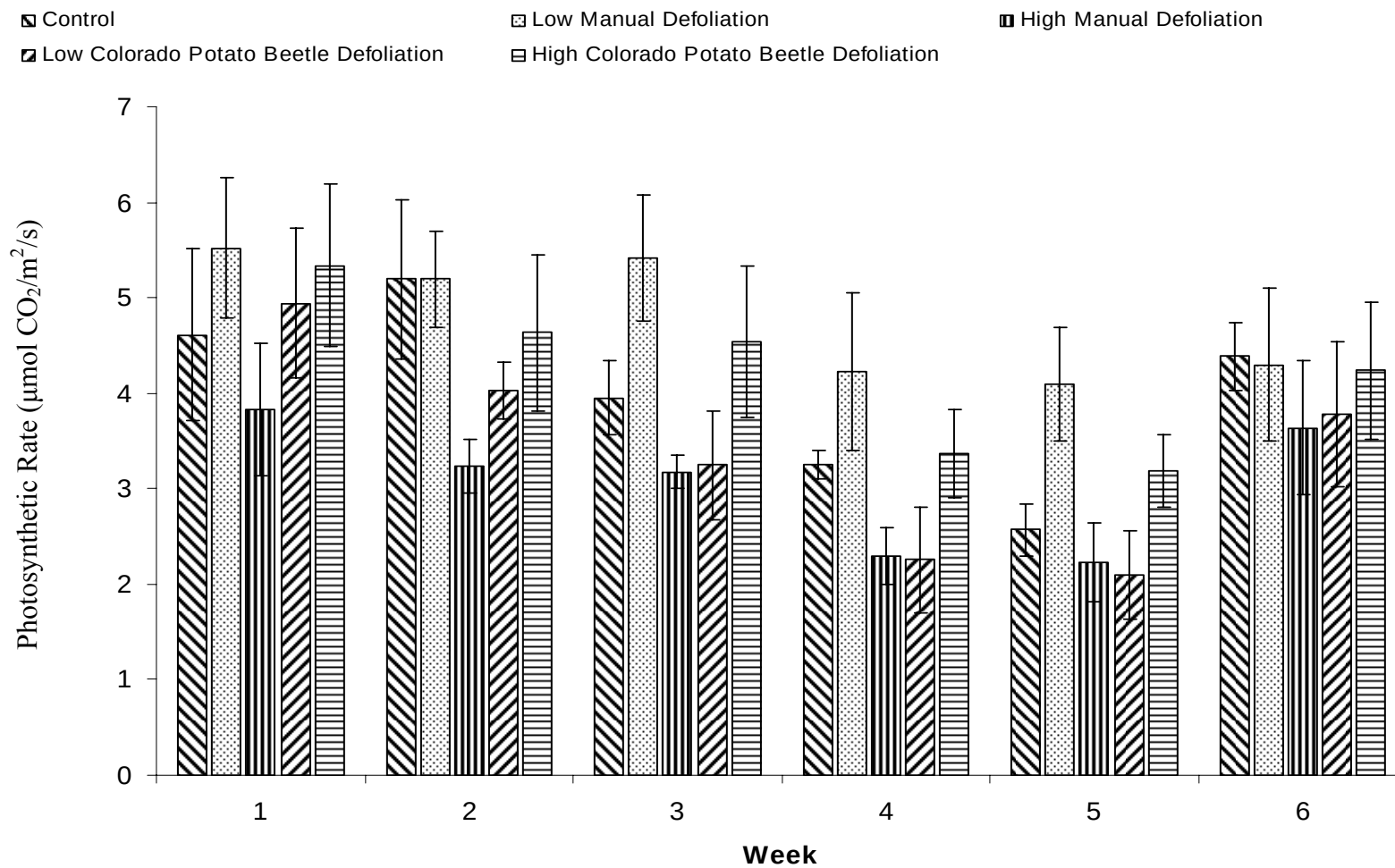


Figure 8. Photosynthetic Rates of the Manual and Colorado Potato Beetle Defoliation Study (2005) on Lower Leaves of Cal Red Plants.

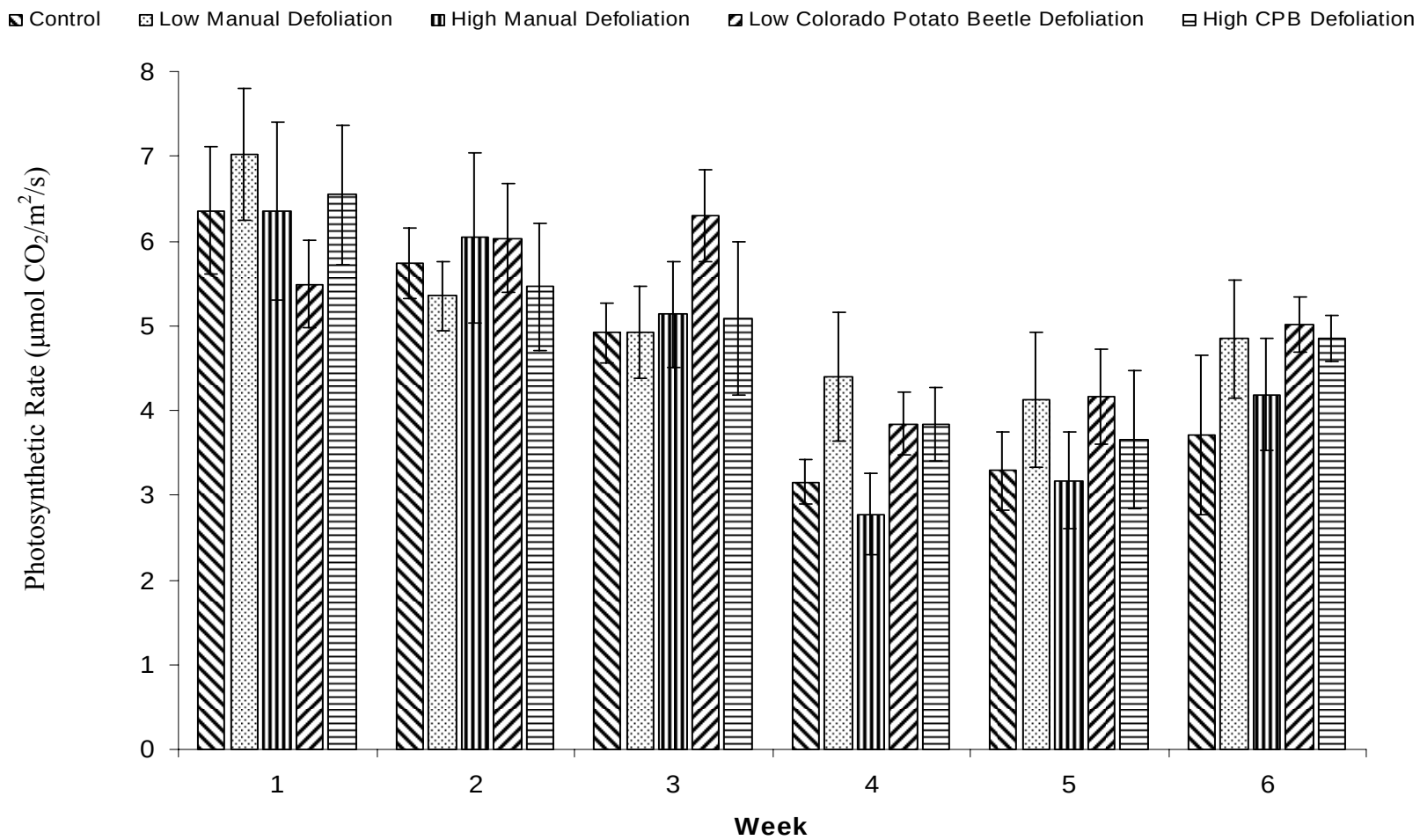


Figure 9. Photosynthetic Rates of the Manual and Colorado Potato Beetle Defoliation Study (2005) on Upper Leaves of Russet Burbank Plants.

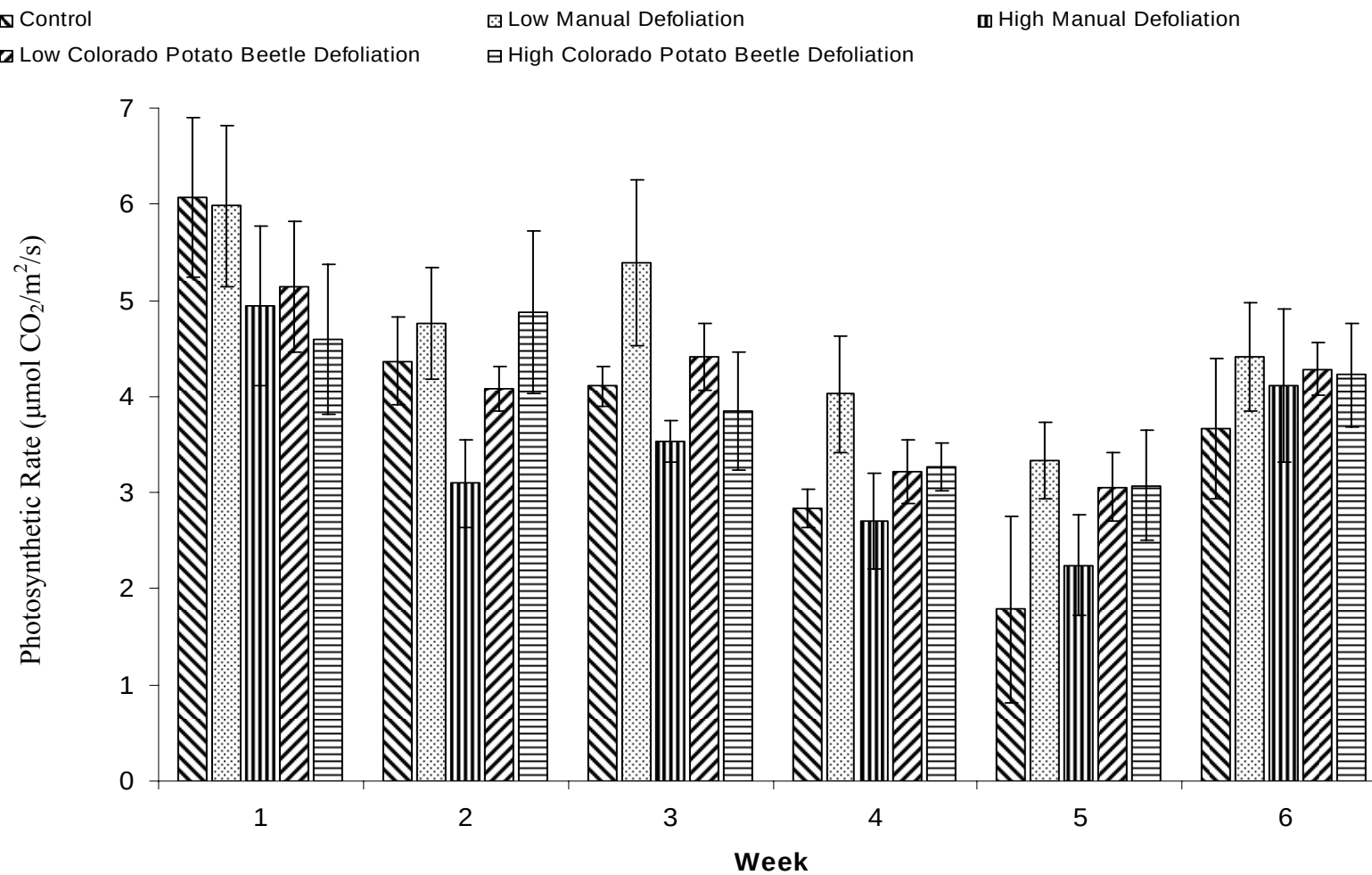


Figure 10. Photosynthetic Rates of the Manual and Colorado Potato Beetle Defoliation Study (2005) on Lower Leaves of Russet Burbank Plants.

0.0001), leaf location ($F = 63.420$, $df = 1$, $P < 0.0001$) and the interaction between defoliation level and leaf location ($F = 5.800$, $df = 4$, $p = 0.0001$) and variety and leaf location ($F = 15.630$, $df = 1$, $p = 0.0001$) had a significant effect on conductance (Table 4). Linear and quadratic relationships between percent defoliation and conductance were not significant.

Transpiration was log transformed and defoliation levels ($F = 4.303$, $df = 4$, $P = 0.002$), time ($F = 26.6176$, $df = 5$, $P < 0.0001$), and leaf location ($F = 43.964$, $df = 1$, $P < 0.0001$) had a significant effect on transpiration (Table 4). The interaction between variety and leaf ($F = 9.607$, $df = 1$, $P = 0.0021$) and defoliation level and leaf ($F = 4.828$, $df = 4$, $P = 0.008$) also had a significant effect on transpiration (Table 4). Linear and quadratic relationships between percent defoliation and transpiration rate were not significant.

Discussion

In both the larval and adult Colorado potato beetle single-leaf defoliation experiments, there were generally no significant differences in photosynthetic rates among defoliation type or levels. This indicates that there was no alteration in photosynthesis of remaining tissue on an injured leaf. This response has been observed in numerous studies with the leaf-mass consumption injury type (Welter, 1991; Higley, 1992; Peterson et al., 1992, 1996; Burkness et al., 1999; Peterson, 2001). There was a significant relationship between Cal Red leaves and defoliation level, but there were fewer replications of defoliation levels with that variety. Additionally, there were no significant differences between actual Colorado potato beetle and manual defoliation,

indicating that artificial defoliation techniques may be appropriate to measure certain responses of potato to Colorado potato beetle injury. Although the photosynthetic responses of larval and adult Colorado potato beetles were not compared in the same experiment, it seems likely that both life stages elicit similar responses and therefore belong to the same injury guild.

For both larval and adult Colorado potato beetle single-leaf defoliation experiments, both variety and time had significantly altered stomatal conductance and transpiration. Although previous studies on other plant species have noted significant, yet transient effects of insect and manual defoliation on those physiological parameters (i.e., typically higher transpiration and lower stomatal conductance until the cut edges of tissues healed) (Poston et al., 1976; Hammond and Pedigo, 1981; Ostlie and Pedigo, 1984; Peterson and Higley, 1996; Aldea et al., 2005), those effects were not observed in these studies 24- or 48-hours after injury.

Because Hlywka et al. (1994) and this research (Chapter 2) found that tubers from plants subjected to Colorado potato beetle defoliation contained higher glycoalkaloid concentrations than tubers from undefoliated plants, but photosynthetic rates among treatments were unaffected, there does not seem to be a tradeoff between a potato plant's natural defense and photosynthesis. This is counter to the observations with common milkweed where defoliation caused a decrease in photosynthetic rate, indicating there may be a tradeoff between milkweed's natural defense (i.e., high cardenolide concentrations) and the ability for the leaf to compensate for insect injury.

In both of the whole-plant defoliation experiments, there were significant defoliation effects on photosynthesis, stomatal conductance, and transpiration when

measured on individual leaves. However, there were no consistent trends to establish a generalized treatment effect. That is, defoliation type or level did not result consistently in increased or decreased gas exchange parameters compared to undefoliated controls. Although not consistent, the undefoliated controls seemed to have lower photosynthetic rates than defoliated treatments over several dates (Figures 7 to 10). This was not unexpected because defoliated plants have altered source-sink relationships, resulting in increased photosynthesis of remaining leaves through increased assimilate demand from sinks. Photosynthetic rates also could have increased from improved water and nutrient availability. According to Taiz and Zeiger (1998), if the source-to-sink ratio is altered so that the number of sources per sink is reduced, an increase in the photosynthetic rate of that single source leaf would be observed. In addition, when sink demand increases, the photosynthetic rate often increases, but when the sink demand decreases, the photosynthetic rate often decreases. Because source leaves transfer their photoassimilates to sink tissue, and sinks store the photoassimilates for use in respiration and to make other critical compounds, it is likely that the source-to-sink ratio was not severely altered and stored assimilates in sinks were adequate to maintain the plant's needs.

In other plant-defoliator systems, defoliation of whole plants or plant canopies results in a delay of normal, progressive leaf senescence (Higley, 1992; Peterson et al., 1992). This has been observed as delayed physical and photosynthetic senescence in remaining leaves, especially lower leaves. Although there were significant differences between gas exchange variables in upper and lower leaves in this study, there was no evidence of delayed senescence in defoliated treatments.

Because there were no direct effects of single-leaf defoliation on leaf photosynthetic rate, photosynthetic measurements were taken on a leaf in which an entire plant was defoliated for the two whole-plant defoliation experiments. A more comprehensive characterization of gas exchange responses of potato to Colorado potato beetle defoliation would include whole-plant gas exchange measurements rather than individual leaf measurements. Temporal, varietal, and potato developmental stage variations seemed to influence gas exchange parameters in this study. Because temporal variations in photosynthetic rates were observed in control plants, it suggests that the variations were not directly related to defoliation levels themselves, but other factors such as sunlight, temperature, atmospheric CO₂ concentrations, developmental stages, and/or plant diel periodicities. Measurements were taken at approximately the same time during the day and suggest that plant diel periodicities are an unlikely contributor to the observed temporal variation. Varietal effects indicate that plant gas exchange responses to insect injury cannot be extrapolated readily between varieties. This also has been observed in tomato and soybean (Welter, 1991; Haile, 2001).

Delaney and Macedo (2001) state that a plant's ability to compensate for herbivore injury depends on the level of injury, the plant's developmental age when injury occurs, the plant organs and tissues injured, and the resources available for new growth. However, in a related context, these same factors could also determine a plant's overall temporal response to injury. For example, Dripps and Smilowitz (1989) found that injury from Colorado potato beetles during the tuber growth or full-bloom phase, caused a greater reduction of leaf, stem, and tuber growth than injury during the vegetative phase. This phenomenon has also been observed in other plant species

(Pedigo et al., 1986). Decreases in vegetative and tuber growth at the flowering stage may be caused by a decrease in leaf area and thus photosynthesis and carbohydrate production in plants with high levels of defoliation. A decrease in the production of carbohydrates at a growth stage where carbohydrate production is critical to maintain growth could decrease total yield. The amount of photoassimilates available to the plant in relation to the plant growth rate after insect injury will affect the level of plant compensation, so that if the growth rate is high (Delaney and Macedo, 2001), the demand for photoassimilates will be high. Insect injury in-and-of-itself may fluctuate, but in a system where insect injury is combined with the plant's natural development and fluctuations in daily sunlight and temperature, the overall result would be temporal variations in photosynthesis.

QUANTITATIVE HUMAN DIETARY RISK ASSESSMENT ASSOCIATED
WITH GLYCOALKALOID RESPONSES OF POTATO TO
COLORADO POTATO BEETLE DEFOLIATION

Abstract

A quantitative human dietary risk assessment was conducted using the glycoalkaloid concentrations measured for tubers from plants manually defoliated and defoliated by Colorado potato beetles. Two experiments were conducted measuring the glycoalkaloid concentrations in response to Colorado potato beetle and manual defoliation. For plants defoliated by Colorado potato beetles, there was a significantly greater production of glycoalkaloids than in control plants and manually defoliated plants for both skin and inner tissue of tubers in experiment one. In experiment one, there was a 32.6% and a 36.8% increase in glycoalkaloids in skin and inner tissue of tubers from plants defoliated at high (~ 90% of total leafmass) levels by Colorado potato beetles in comparison to control plants. In experiment two, although a significant difference in glycoalkaloid concentration was not observed among the treatments, the skin and inner tissue of tubers from plants defoliated at high levels by Colorado potato beetles increased glycoalkaloid concentration by 18.9% and 12.7% in comparison to tubers from control plants. For experiment one, there was 90.2% higher concentration of glycoalkaloids in the skin than the inner tissue, and for experiment two there was a 73.1% higher concentration of glycoalkaloids in the skin than the inner tissue. In experiment one, the concentration of tuber extract required to reduce Chinese hamster ovary (CHO) cellular proliferation by 50% was 10 times less for the skin versus the inner tissue, indicating that

skin tissue is more toxic. The dietary risk posed to different human subgroups associated with the consumption of potatoes was determined for the 50th, 95th, and 99.9th percentile U.S. national consumption values. The population percentile that the EPA uses to determine risk from dietary exposure to pesticides is 99.9, and at a toxic dose of 1 mg/kg body weight (BW), glycoalkaloid concentrations within the inner tissue of tubers exceeded the toxic endpoint for all human subgroups at less than the 99.9th percentile of exposure. This is both a function of the use of extremely high consumption percentiles as exposure endpoints and the use of 1 mg/kg BW as a toxic endpoint. Regardless, the dietary risk assessments presented here support the arguments that current potato safety levels for glycoalkaloids are not sufficiently protective of public health.

Introduction

An extensive amount of research has identified the physiology, behavior, ecology, and life history of pest insects and how their numbers affect plant yield, but relatively little is known about how the plant host responds to insect injury (Peterson and Higley, 2001). Physiological and biochemical responses of plants to insect herbivory have documented effects on both primary and secondary metabolic processes. Primary metabolites are compounds involved in photosynthesis, respiration, solute transport, translocation, nutrient assimilation, or differentiation (Taiz and Zeiger, 1998). Unlike primary metabolites, secondary metabolites have no known direct function in growth and development, but have been observed to function as biochemical defenses against injury and stress (Taiz and Zeiger, 1998). Secondary metabolites are highly diverse among plant taxa and their presence has led to extensive research on the origin, diversity, and

role that these chemicals play in plant-insect interactions (Theis and Ler dau, 2003). Through heritable mutations in metabolic pathways and through natural selection, secondary metabolites are thought to have evolved with low metabolic costs and toxicity to the plants themselves (Theis and Ler dau, 2003).

Many of these secondary compounds serve as natural pesticides within plants, and there is increasing interest to enhance these natural pesticides for commercial use (Fenwick et al., 1990). Plants are being bred to contain not only a greater diversity of natural compounds, but also greater quantities (Hlywka et al., 1994). However, at certain concentrations, these compounds can be toxic to humans and other animals (Theis and Ler dau, 2003).

There are three types of secondary compounds: terpenes, phenolics, and nitrogen-containing alkaloids. Although terpenes are the largest class of secondary metabolites (Thies and Ler dau, 2003), glycoalkaloids are thought to be the most highly consumed natural toxin in North America (Hall, 1992). Little is known about the human dietary risks associated with consumption of these chemicals or how the dietary risks change in response to insect herbivory.

In potatoes (*Solanum tuberosum* L.), glycoalkaloids serve as natural defense mechanisms against pathogens and insects (Lachman et al., 2001). Because naturally occurring pesticides are synthesized when plants are under stress, it is expected that injury to plant tissue would instigate synthesis of higher concentrations of these compounds in the injured versus uninjured plant tissue. Hlywka et al. (1994) found that tubers from plants subjected to Colorado potato beetle (*Leptinotarsa decemlineata* Say)

defoliation contained higher glycoalkaloid concentrations than tubers from undefoliated plants.

Glycoalkaloids

In the potato plant, glycoalkaloids are found in high concentrations in the leaves, stems, and sprouts. Lower concentrations of glycoalkaloids can be found in the skin of tubers and areas where sprouts emerge (Lachman et al., 2001). Friedman and Dao (1992) found that leaves had a concentration of glycoalkaloids 10 times greater than the tubers and a sprout glycoalkaloid concentration nearly 68 times greater than the tubers. Phillips et al. (1996) observed a greater concentration of glycoalkaloids in the leaves compared to tubers from the same plants; however, there was a great deal of variability among leaf glycoalkaloid concentrations within the same variety of plants. In tubers, the greatest concentration of glycoalkaloids was found in the skin (Bejarano et al., 2000), and the greater the concentration of glycoalkaloids present in tubers, the more bitter the taste (Lachman et al., 2001).

Although there are many glycoalkaloids, α -chaconine and α -solanine make up 95% of the total glycoalkaloids present (Friedman and McDonald, 1997); α -solanine is found in greater concentrations than α -chaconine, and α -solanine has only half as much specific activity as α -chaconine (Lachman et. al., 2001). Other glycoalkaloids that are present, but in much lower concentration, are β - and γ - solanines and chaconines, α - and β -solamarines, and aglycones demissidine and 5- β -solanidan-3-a-ol (Friedman and McDonald, 1997). In wild potato, leptines, commersonine, demissine, and tomatine are also present (Friedman and McDonald, 1997).

Factors that increase glycoalkaloid levels in tubers include tuber exposure to light, bruising, cutting, rotting by fungi or bacteria, and other forms of mechanical damage (Lachman et al., 2001). Lachman et al. (2001) found that between damaged tubers and undamaged tubers, damaged tubers had 89% higher glycoalkaloid content and in 1994, when weather conditions were unfavorable and dry, the glycoalkaloid content was 71% higher than the content in 1995. In a greenhouse study, tubers harvested from a “hot” chamber versus a “cool” chamber contained a greater concentration of glycoalkaloids (Lachman et al., 2001). In addition to growing conditions, handling, and storage, tubers exposed to light often turn green and can have especially high glycoalkaloid concentrations (Friedman and McDonald, 1997).

Health Risks

The world produces approximately 350 million tons of potatoes per year with a U.S. annual consumption per capita of 61 kg fresh weight (FW) (Lachman et al., 2001), resulting in an average daily consumption of 167 g FW. Potato is the fourth largest crop grown in the world and is exceeded in total hectares only by rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and corn (*Zea mays* L.) (Petroff, 2002). They are grown primarily for human consumption, providing a major source of energy and protein (Lachman et al., 2001).

Tablestock tubers typically have about 75 mg/kg FW or 500 mg/kg dry weight (DW) total of α -chaconine and α -solanine (Zeiger, 1998). Neither α -solanine nor α -chaconine are regulated in the US. The U.S. Department of Agriculture (U.S.D.A.) has recommended a food-safety level for glycoalkaloids of 200 mg/kg FW or 1000 mg/kg

DW (Bejarano et al., 2000; Zeiger, 1998). Most commercial tablestock tubers contain between 20 and 130 mg/kg FW (Zeiger, 1998) or 133 and 867 mg/kg DW. Levels of α -solanine greater than 140 mg/kg FW or 933 mg/kg DW taste bitter, and levels greater than 200 mg/kg FW or 1333 mg/kg DW cause a burning sensation in the throat and mouth (Lachman et al., 2001). Dry weights of potatoes typically are calculated by assuming a fresh weight reduction of 85% (Eborn, 2000).

In humans, the acute oral toxic dose for glycoalkaloids is 2 to 5 mg/kg of body weight (BW) and the fatal dose is 3 to 6 mg/kg BW (Morris and Lee, 1984). According to Friedman and McDonald (1997), the minimum acute toxic effect level seems to be about 1.0 mg/kg BW or less, but there are few human toxicity studies.

Although the potential hazard associated with human consumption of potato injured by insects has been recognized (Hlwyka et al., 1994), there has not been an analytical consideration of the potential human dietary risks associated with increased production of glycoalkaloids as a result of Colorado potato beetle injury.

Risk assessment is a science-based framework for the objective evaluation of risk (NRC 1983). It involves three major steps: problem formulation, data analysis, and risk characterization (USOSTP, 1999). Problem formulation establishes the objective and scope of the risk assessment, whereas the data analysis phase incorporates two steps: hazard identification and exposure assessment. In terms of human dietary risk assessment, hazard identification identifies the inherent ability for a substance to cause harm to a human subgroup and may either be an estimate or based on a direct measurement. Hazard identification often utilizes dose-response relationships to

determine the dose required to elicit a harmful response. Exposure assessment identifies the human subgroups at greatest risk for adverse health effects, the route of exposure (dermal, oral, or inhalation), the type of exposure (acute, subchronic, or chronic), and the duration of exposure; it also is the phase where exposure levels are determined based on consumption data and potential changes in the exposure level due to food preparation and storage practices. Risk characterization is the phase where the estimate of risk to the human subgroups is determined by integrating the information gathered in the data analysis phase; this involves the identification of areas where assumptions were made, areas of uncertainties, and areas where more information is required to refine the estimates of risk.

Objective

Using the early-maturing potato cultivar 'Superior', Hlwyka et al. (1994) showed that Colorado potato beetle injury of leaves increased glycoalkaloid concentrations in tubers. However, they did not examine toxicological responses or assess human dietary risk. Therefore, the overall objective of this study was to assess the potential human dietary risk associated with consumption of potatoes from plants defoliated by Colorado potato beetles by determining the glycoalkaloid responses of potatoes to Colorado potato beetle and manual defoliation. Responses were measured by determining the concentration of glycoalkaloid production and the percentage of glycoalkaloid extract that caused a 50% inhibition in mammalian cellular proliferation for the skin and innermost flesh of tubers from Russet Burbank potato plants at different levels of Colorado potato beetle and manual defoliation.

Materials and Methods

Plant Material

All plants were grown in a greenhouse (Montana State University, Bozeman, MT) and planted in 50:50 MSU:Sunshine #1 soil mix, and fertilized weekly with Scott's Peter Professional Peat-Lite Special 20-20-20. The Sunshine Mix #1 consisted of Canadian Sphagnum Peat Moss, perlite, vermiculite, starter nutrient charge, wetting agent, and Dolomitic lime (Sun Gro Horticulture, Inc., Bellevue, WA). The MSU soil mix consisted of equal parts of Bozeman Silt Loam Soil, washed concrete sand, and Canadian Sphagnum Peat Moss in addition to AquaGro 2000 G wetting agent blended in at 593 g/m³ of soil mix. The MSU soil mix was aerated-steam pasteurized to 80°C for 45 min.

Plants were grown at 21 ± 2°C with a photoperiod of 14:10 (Light:Dark). Two cultivars were used, Cal Red and Russet Burbank, and were obtained from VenHuizen Seed Potatoes, Inc., Belgrade, MT. Each seed tuber was cut, ensuring at least one eye per cut, and placed cut-side down in a 20-L pot filled approximately with 12 to 13 cm of pre-moistened 50:50 MSU:Sunshine soil mix and covered five to nine cm with pre-moistened 50:50 MSU:Sunshine soil mix.

Plants were watered four to five times weekly and fertilized with Scott's Peter Professional Peat-Lite Special 20-20-20 bi-weekly. Once plants reached approximately 9 cm tall, in a process called "hilling," soil was added to the pots weekly until pots were full.

In a greenhouse, a two-by-five factorial design was arranged within a randomized complete block design (RCBD). Metal halide lamps (1000 W) served as the blocking

factors. The following factors were used: potato cultivar (Cal Red and Russet Burbank) and defoliation level (control [no defoliation], low, medium, and high Colorado potato beetle and manual defoliation). The treatment factors were replicated five times and the experiment was replicated twice (2004 and 2005).

At the early vegetative stage, all plants were individually covered with nets approximately 91 cm x 40 cm x 40 cm (h x w x d) made of white tulle and Colorado potato beetle eggs were obtained from the Phillip Alampi Beneficial Insect Lab, New Jersey Department of Agriculture, Trenton, New Jersey. Approximately 120 egg masses were placed on approximately 10 to 15 extra potato plants and allowed to hatch and feed until approximately the third instar. At approximately the third instar, just before flowering, approximately 15, 20, and 25 third instars were applied to the low, medium, and high treatment plants and allowed to defoliate. Once the low, medium, and high defoliation plant leaf area was reduced by approximately 30% (low), 60% (medium), and 90% (high) the Colorado potato beetles were removed. The manually defoliated plants were defoliated with scissors weekly to simulate the percentage and patterns of leaf mass removed by the Colorado potato beetles.

Upon senescence, stems were cut and potatoes were harvested two weeks later. The tubers were washed, placed in brown paper bags, and the bags were stored in a dark cold room held at 4°C.

Glycoalkaloid Analysis

Sample Preparation. Stored Russet Burbank tubers of approximately the same size across treatments were separated into three tissue samples: the skin, the outer flesh,

and the inner core. Tubers were skinned approximately 2 mm from the surface using a vegetable peeler and approximately 50% of the remaining tissue was separated to consist of 50% of outer flesh and the other half the inner core. These three tissue samples were cut into smaller pieces, dipped into liquid nitrogen, placed into 50-ml centrifuge tubes with a cotton cap, immediately placed in a 1200-ml fast freeze flask and freeze dried for approximately 60 hours using a freeze dryer (LABCONCO Freeze Dry System/Freezone 4.5, Labconco Corporation, Kansas City, MO).

After 60 hours, the samples were removed and ground using a Black & Decker HandyChopper Plus™ (Model HC3000, Towson, MD) until the consistency of a fine powder was achieved. The powder was placed into 50-ml centrifuge tubes and stored in a -60°C refrigerator.

Once all samples were freeze dried and ground, blocks I, II, III, and IV in the spring of 2005 and blocks I, III, IV, and V in the spring of 2006 for the following treatments: control (no defoliation), high manual defoliation, and high Colorado potato beetle defoliation (24 total samples), were coded and sent to Eurofins Scientific (Petaluma, CA) for glycoalkaloid quantification using high performance liquid chromatography (HPLC). Because of cost considerations, only Russet Burbank samples from inner and skin locations were analyzed.

Eurofins' Preparation of Standards. The diluent was prepared by combining water, acetonitrile, and 85% phosphoric acid (80:20:0.1 (v/v/v)). In a 25-ml volumetric flask, the standard stock solution was prepared by using approximately 3.5 mg of α -chaconine and 4.5 mg of α -solanine diluted to volume using the diluent. Standard 1 was

prepared by using 2 ml of the stock standard solution diluted to volume using the diluent in a 100-ml volumetric flask. Standard 2 was prepared by combining 2 ml of the standard stock solution in a 50-ml volumetric flask diluted to volume using the diluent. In a 50-ml flask, standard 3 was prepared by diluting 3 ml of the standard stock solution to volume using the diluent. Standard 4 was prepared using a 25-ml volumetric flask with 2 ml of the stock standard solution diluted to volume using the diluent. Standard 5 was prepared by diluting 2 ml of the standard stock solution to volume using the diluent in a 10-ml volumetric flask.

The stock standard solution and each standard solution were injected twice prior to actual sample injections, after every 16th sample injection, and upon completion of all sample injections. The standard curve was created using exponential curve fitting and the y-intercept, correlation coefficient, and percent relative standard deviation (RSD) of the standard curves for α -solanine and α -chaconine were calculated. The concentration of α -solanine and α -chaconine per sample were quantified using the standard calibration curves. High RSDs of the standard curves are typical for Evaporative Light Scanning Detector (ELSD) detection when calculated more than two orders of magnitude in concentration.

Eurofins' Sample Preparation. Approximately 500 mg of sample was weighed, transferred to a 15-ml centrifuge tube, combined with 10 ml of diluent, and shaken for approximately 10 minutes on a wristaction shaker. The tube was then sonicated for 15 minutes, allowed to cool to room temperature, centrifuged for 10 minutes, and the supernatant was filtered through a 0.45- μ m PTFE filter.

Eurofins HPLC-ELSD. Each sample was run twice through a Dionex Summit HPLC with ELSD and Dionex Chromeleon software. The Dionex Summit HPLC was fitted with an Alltech Altima HP C-18 Amide (150-mm x 4.6-mm, 5- μ m) column at 25°C. The mobile phase consisted of A- 0.1% Trifluoroacetic Acid in water and B- Acetonitrile with a pump program of 10% B to 34% B over a 36-minute period. The flow rate was set at 1.0 ml/minute with an injection volume of 50 μ L. The detection was ELSD. The drift tube temp was set at 110°C, the gas flow was set at 3.0 L/min, and the impactor was off.

Cellular Proliferation Analysis

Inhibition of Cellular Proliferation. The protocol was based on Sayer et al. (2006) and evaluated the ability of glycoalkaloids found in the skin and inner tissue of potatoes from plants with varying levels of Colorado potato beetle defoliation to inhibit Chinese hamster ovary (CHO-K1-BH4) cell proliferation. Inhibition of cell proliferation by the potato samples was evaluated by comparing the number of cells in each treatment to the untreated control.

Sample Preparation. This procedure has been described above.

Once all potato samples were freeze dried and ground, four replicates (blocks I, II, III, and IV) of the control (no defoliation), high manual defoliation, and high Colorado potato beetle defoliation treatments (24 total samples) were coded and sent to Professor J. E. Gibson (East Carolina University, Greenville, NC) in 2005 for a blind analysis.

Cell Maintenance. CHO-K1-BH4 cells were obtained from G. D. Charles (Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, MI). CHO-K1-BH4 cells were grown in 25-cm² plastic culture flasks containing 5 ml of F-12 nutrient media (Ham) (Invitrogen, Carlsbad, CA) combined with 5% fetal bovine serum (Hyclon, Logan, UT), 100 units/ml penicillin-streptomycin (Invitrogen, Carlsbad, CA), and 2.5 µg/ml of Fungizone Amphotericin B (Invitrogen, Carlsbad, CA). The cells were allowed to grow for approximately four days at 37°C at 5% CO₂ in a humidified incubator (Sanyo Model MCO-18 AIC (UV), Chatsworth, CA).

Treatment Preparation. Dry potato samples from the control, high manual defoliated, and high Colorado potato beetle defoliated treatments were extracted in the F-12 nutrient media (Ham) at a ratio of 0.05-g sample/ml of nutrient media for 24 hours, followed by centrifugation and sterilization of the supernatant using a 0.2-µm Whatman sterile syringe filter, CA filter media fitted with polypropylene hosing. The supernatant for each treatment (control, high manual defoliation, and high Colorado potato beetle defoliation) was diluted to 0, 0.4, 0.8, 1.2, 1.6, 2, 4, 6, 8, and 10% using the nutrient media. The positive control consisted of 0, 1, 2, 3, 4, and 5% ethanol.

In six-well plates, each well consisted of 2 ml of culture media combined with each dilution percentage and 100,000 CHO-K1-BH4 cells. The plates were returned to the incubator for 48 hours under the same conditions as above. Two plates (subsamples) per dilution were used.

Evaluation of Cellular Proliferation. After 48 hours in the incubator, the plates were removed, the media was aspirated, and the cells were trypsinized and re-suspended

in 1 ml of the culture media. Each well was counted for CHO-K1-BH4 cells using a coulter counter (Z2 Duel Threshold Coulter Counter, Beckman Coulter, Fullerton, CA). Treatment cell counts were compared to the untreated control cell counts from the wells on the same plate. It was assumed that 100% of the cells from the untreated control were considered capable of proliferating. The Inhibitory Concentration (IC₅₀)—the dilution necessary to cause a 50% reduction in cellular proliferation—was determined by using a probit regression.

Statistical Analyses. Control and treated groups were compared ($\alpha = 0.05$) using analysis of variance (ANOVA), interaction plots fitted with standard error, and treatment means were separated by TukeyHSD multiple comparison of means using 95% family-wise confidence levels. Data were analyzed using R 2.0.1 (R: A Language and Environment for Statistical Computing; R Development Core Team, The R Foundation for Statistical Computing, 2004, Vienna, Austria). Linear and non-linear regression analyses were conducted to determine the relationship between defoliation level and glycoalkaloid concentrations and IC₅₀s using SAS system for Windows V8 (SAS Institute, Inc. ©1999-2001, Cary, NC).

Risk Assessment

Problem Formulation. The tier-1 quantitative human dietary risk assessment focused on the human acute oral exposure to glycoalkaloids, α -solanine and α -chaconine, in the inner tissue of tubers from plants with no defoliation, high manual defoliation, and high Colorado potato beetle defoliation. The initial symptoms of glycoalkaloid toxicity

occur within 30 minutes of consumption and last for approximately seven hours (Friedman and McDonald, 1997). Therefore, in this assessment acute exposure was defined as a single-day exposure after consumption of skinless tubers. Sub-chronic (< 180 days) and chronic risks (> 180 days) were not addressed in this study because consumption of potatoes injured by Colorado potato beetles was assumed to be short term and represent less than a single bag of potatoes and therefore represent an acute exposure scenario. To account for the potential age and size differences related to exposure, the risk to different population subgroups in the US were estimated (e.g., infants, children 7 to 12 years, and women 13 years and older who are pregnant).

Hazard Identification and Dose-Response Relationships. Human dietary risks associated with the consumption of α -solanine and α -chaconine, the two glycoalkaloids that comprise approximately 95% of the total glycoalkaloids in potato (Friedman and McDonald, 1997), were assessed for potatoes from plants with no defoliation (control), high manual defoliation, and high Colorado potato beetle defoliation.

Tablestock tubers (including the skin) typically have about 75 mg/kg FW or 500 mg/kg DW total of α -chaconine and α -solanine (Zeiger, 1998). Neither α -solanine nor α -chaconine are regulated in the United States. The U.S.D.A. has recommended a food-safety level for glycoalkaloids of 200 mg/kg FW or 1000 mg/kg DW (Bejarano et al., 2000; Zeiger, 1998). Most commercial tablestock tubers contain between 20 and 130 mg/kg FW (Zeiger, 1998) or 133 and 867 mg/kg DW. Levels of α -solanine greater than 140 mg/kg FW or 933 mg/kg DW taste bitter, and levels greater than 200 mg/kg FW or 1333 mg/kg DW cause a burning sensation in the throat and mouth (Lachman et al.,

2001). Dry weights of potatoes typically are calculated by assuming a fresh weight reduction of 85% (Eborn, 2000).

Hellanäs et al. (1992) used seven volunteers who abstained from eating potatoes for two days and were then given potatoes containing glycoalkaloids at a dose of 1 mg/kg BW per individual. Six out of the seven subjects experienced burning sensation of the mouth and light to severe nausea and one of the six experienced diarrhea; the first initial symptoms were observed 30 minutes after consumption and lasted for approximately four hours. Animal studies have shown that in mouse, rat, and hamster tissue, α -chaconine and α -solanine reached their highest concentrations within 6 to 14 hours of ingestion and in less than 35 hours, peak concentrations in the blood were reached (Zeiger, 1998).

The symptoms of “solanine” poisoning include nausea, vomiting, diarrhea, stomach and abdominal cramps, headache, fever, rapid and weak pulse, rapid breathing, hallucinations, delirium, and coma (Friedman and McDonald, 1997). Effects on the nervous system include increased heart, pulse, and respiratory rates, sedation, and coma (Zeiger, 1998). Effects from cell membrane disruption include internal hemorrhaging, edema, diarrhea, constriction of the abdominal muscles, and lesions in the stomach and duodenum of the large intestines. Teratogenic effects were observed mainly in the central nervous system and included exencephaly, cranial bleb, encephalocele, and anophthalmia (Zeiger, 1998). Alpha-chaconine seems to exert teratogenic effects at lower concentrations than α -solanine (Zeiger, 1998).

Tissues that were observed to accumulate α -chaconine and α -solanine were abdominal fat, adrenals, blood, brain, heart, kidney, liver, lungs, muscle, pancreas,

spleen, testis, thymus, and thyroid. Alpha-chaconine and α -solanine remained unchanged or as solanidine when excreted in urine and feces (Zeiger, 1998).

Peeling reduces the quantity of glycoalkaloids in tubers 30 to 80% (Zeiger, 1998). Alpha-chaconine and α -solanine are not broken down from cooking or frying because they are heat stable and only begin to break down between 230 to 280°C (Bejarano et al., 2000).

In humans, the toxic dose for glycoalkaloids is 2 to 5 mg/kg BW and the fatal dose is approximately 3 to 6 mg/kg BW (Morris and Lee, 1984). According to Friedman and McDonald (1997), the minimal acute toxic effect level most likely is 1.0 mg/kg BW or less, but there are few human toxicity studies to determine what a toxic or fatal dose would be. An oral LD₅₀ for mice is greater than 1000 mg/kg BW (Nishie et al., 1971) and is approximately 590 mg/kg BW in rats (Gull et al., 1970). Although animal studies have shown similar effects when ingesting α -solanine, α -chaconine, and plant material containing these two glycoalkaloids, α -solanine and α -chaconine have been shown to be poorly absorbed (Zeiger, 1998) and to elicit a similar response as observed in humans requires a much greater concentration to be administered.

Selection of Toxic Endpoint. Many synthetic chemicals have an acute and/or chronic regulatory threshold (acceptable daily intake) that is typically based on a no-observed-adverse-effect-level (NOAEL) from the required toxicity study that generates the lowest dosage necessary to produce the lowest-observed-adverse-effect-level (LOAEL). For potatoes, no acute and chronic regulatory thresholds exist for humans and the consumption of glycoalkaloids in potatoes because although these compounds are

very toxic and insecticidal, they are naturally occurring. As stated above, in humans, the toxic dose for glycoalkaloids has been observed between 2 to 5 mg/kg BW, but there are few human toxicity studies to determine what a toxic or fatal dose would be. The data available indicate that humans are more sensitive to α -chaconine and α -solanine than test animals and because no human oral LD₅₀'s or NOAEL's are available for α -solanine and α -chaconine, the human toxic endpoint of 1 mg/kg BW was used based on Friedman and McDonald (1997).

Exposure Assessment. A human's or other receptor's exposure to a hazard is affected by quantity (dose), route, duration, and frequency of exposure. The quantity subjects may be exposed to either may be known from direct measurements or may need to be estimated.

Because peeling potatoes reduces the quantity of glycoalkaloids in potatoes 30 to 80% (Zeiger, 1998), only the risks for consuming the inner tissue of potatoes were assessed. Alpha-chaconine and α -solanine are found within the tuber, are heat stable, and are not degraded from cooking or frying as they begin to degrade between 230 and 280°C (Bejarano et al., 2000). Therefore, risks associated with different types of preparation were not assessed.

The human subgroups included the entire U.S. population, all infants, children 1 to 6 years, children 7 to 12 years, youth 13 to 19 years, women 13 years and older who were pregnant, but not nursing, women 20 years and older not pregnant nor nursing, and men 20 years and older. All exposure and risk estimates were determined using the Dietary Exposure Evaluation Model™ (DEEM-FCID™, Ver. 2.03, Durango Software,

LLC, Exponent, Inc., Washington D.C.) based on the USDA's Continuing Surveys of Food Intakes by Individuals (CSFII) food consumption data for 1994-1996, 1998. Food translations within the program to convert foods-as-eaten to commodities were based on EPA, USDA Food Commodity Ingredient Data (FCID) recipe set as of August, 2002.

The acute (one day) food consumption patterns for each of the subgroups listed above were evaluated using the 50th, 95th, and 99.9th percentile U.S. national consumption values for skinless potatoes only. DEEM calculates the acute oral exposure, the risk quotient (see below), and exposure as a percentage of the toxic endpoint at different percentiles of U.S. national consumption.

Risk Characterization. The assumptions associated with the dietary risk assessment of glycoalkaloid consumption to all the different human subgroups were: (1) only skinless potatoes were consumed, (2) the only route of exposure was orally through ingestion of skinless tubers, (3) the toxic endpoint for a human was 1 mg/kg BW, and (4) the toxic endpoint was the same for all ages and sizes within the different human population subgroups.

To determine the risk posed to a subgroup in a population, the estimated glycoalkaloid exposure level was divided by the toxic endpoint. The resulting ratio is known as the risk quotient (RQ). An RQ equal to or greater than 1.0 often indicates a level of concern because the estimated exposure would be equal to or greater than the toxic endpoint.

Results

Glycoalkaloids

The mean glycoalkaloid concentration or residue level for tubers from control plants (no defoliation), high manual defoliation plants, and high Colorado potato beetle plants is listed in Table 5. For experiment one, treatment ($F = 6.8$; $df = 2$; $P = 0.008$), tissue type ($F = 271.62$; $df = 1$; $P < 0.0001$), and the interaction between treatment and tissue type ($F = 4.67$; $df = 2$; $P = 0.027$) all had a significant effect on glycoalkaloid concentrations (Table 6). Tuber glycoalkaloid concentrations from plants with high Colorado potato beetle defoliation had a significantly greater glycoalkaloid concentration (2442.5 (skin) mg/kg DW and 258.75 (inner) mg/kg DW) than control plants (1545 (skin) mg/kg DW and 174.5 (inner) mg/kg DW). Glycoalkaloid concentrations were significantly greater within the skin versus the inner tissue. Linear and quadratic relationships between defoliation level and glycoalkaloid concentration were not significant.

Unlike experiment one, tissue type ($F = 88.72$, $df = 1$, $P < 0.00001$) was the only factor to have a significant effect on glycoalkaloid concentrations in experiment two (Table 7). Glycoalkaloid concentrations between treatments were not significantly different. Glycoalkaloid concentrations within the skin were significantly greater than within the inner tissue. Linear and quadratic relationships between defoliation level and glycoalkaloid concentration were not significant.

For experiment one, the skin glycoalkaloid concentrations from control, high manually defoliated, and high Colorado potato beetle defoliated plants were 1545 mg/kg

Table 5. Mean Glycoalkaloid Concentrations in Potatoes from Experiment 1.

Treatment	Mean Glycoalkaloid Concentration in Inner Tissue (mg/kg DW)	
	Mean	Standard Error
Control – No Defoliation	174.50	8.59
High Manual Defoliation	149.40	29.28
High Colorado Potato Beetle Defoliation	258.80	23.30
Store-bought Potato ^a	206.00	N/A

^a(Zeiger, 1998)

Table 6. ANOVA for Glycoalkaloid Concentrations of Potatoes from Experiment 1.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Block	3	1004217	334739	1.1726	0.353135
Defoliation Level	2	3884125	1942062	6.8029	0.007894
Tissue Type	1	77539836	77539836	271.6158	< 0.00001
Defoliation x Tissue Type	2	2665331	1332666	4.6682	0.026531
Residuals	15	4282142	285476		

Table 7. ANOVA for Glycoalkaloid Concentrations of Potatoes from Experiment 2.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Block	3	3323910	1107970	3.0826	0.05943
Defoliation Level	2	660620	330310	0.919	0.42025
Tissue Type	1	31887371	31887371	88.7169	< 0.00001
Defoliation x Tissue Type	2	567080	283540	0.7889	0.47233
Residuals	15	5391425	359428		

DW, 1987.5 mg/kg DW, and 2442.5 mg/kg DW, respectively (Figure 11). For experiment two, the skin glycoalkaloid concentrations from control, high manual defoliated, high Colorado potato beetle defoliated plants were 1349.75 mg/kg DW, 1695.5 mg/kg DW, and 1665 mg/kg DW, respectively (Figure 11). For experiment one, the inner tissue glycoalkaloid concentrations from control, high manual defoliated, high Colorado potato beetle defoliated plants were 174.5 mg/kg DW, 149.4 mg/kg DW, and 258.75 mg/kg DW, respectively (Figure 12). For experiment two, the inner tissue glycoalkaloid concentrations from control, high manual defoliated, and high Colorado potato beetle defoliated plants were 404.88 mg/kg DW, 383.63 mg/kg DW, and 463.75 mg/kg DW, respectively (Figure 12).

Toxicity

Inhibitory concentration (IC_{50}) was the concentration of potato extract that caused 50% reduction in proliferation of CHO cells. Because only experiment one was observed to have a significant treatment effect, and because of considerations of costs, only experiment one was used to evaluate the IC_{50} s. Tissue type ($F = 25.786$; $df = 1$; $P = 0.0004$) was the only factor to have a significant effect on IC_{50} ; the inner tissue required a significantly greater amount of extract to elicit a 50% reduction in cellular proliferation (12.523 μ g/ml) than the skin (1.411 μ g/ml) (Table 8). Linear and quadratic relationships between defoliation level and IC_{50} were not significant. Mean IC_{50} concentrations are presented in Figure 13 and Table 9.

Table 8. ANOVA for IC₅₀s of Potatoes from Experiment 1.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Block	3	168.42	56.14	2.5618	0.1081547
Defoliation Level	2	107.8	53.9	2.4597	0.1309381
Tissue Type	1	565.07	565.07	25.7863	0.0003561
Defoliation x Tissue Type	2	102.42	51.21	2.337	0.1426213
Residuals	11	241.05	21.91		

Table 9. Mean IC₅₀ Concentrations of Potatoes from Experiment 1.

	Inner tuber tissue (µg/ml)		Skin (µg/ml)	
	Mean	Standard Error	Mean	Standard Error
Control – No Defoliation	15.13	3.973	1.30	0.608
Manual Defoliation	6.95	4.13	1.88	0.711
High Colorado Potato Beetle Defoliation	15.49	4.253	1.06	0.271

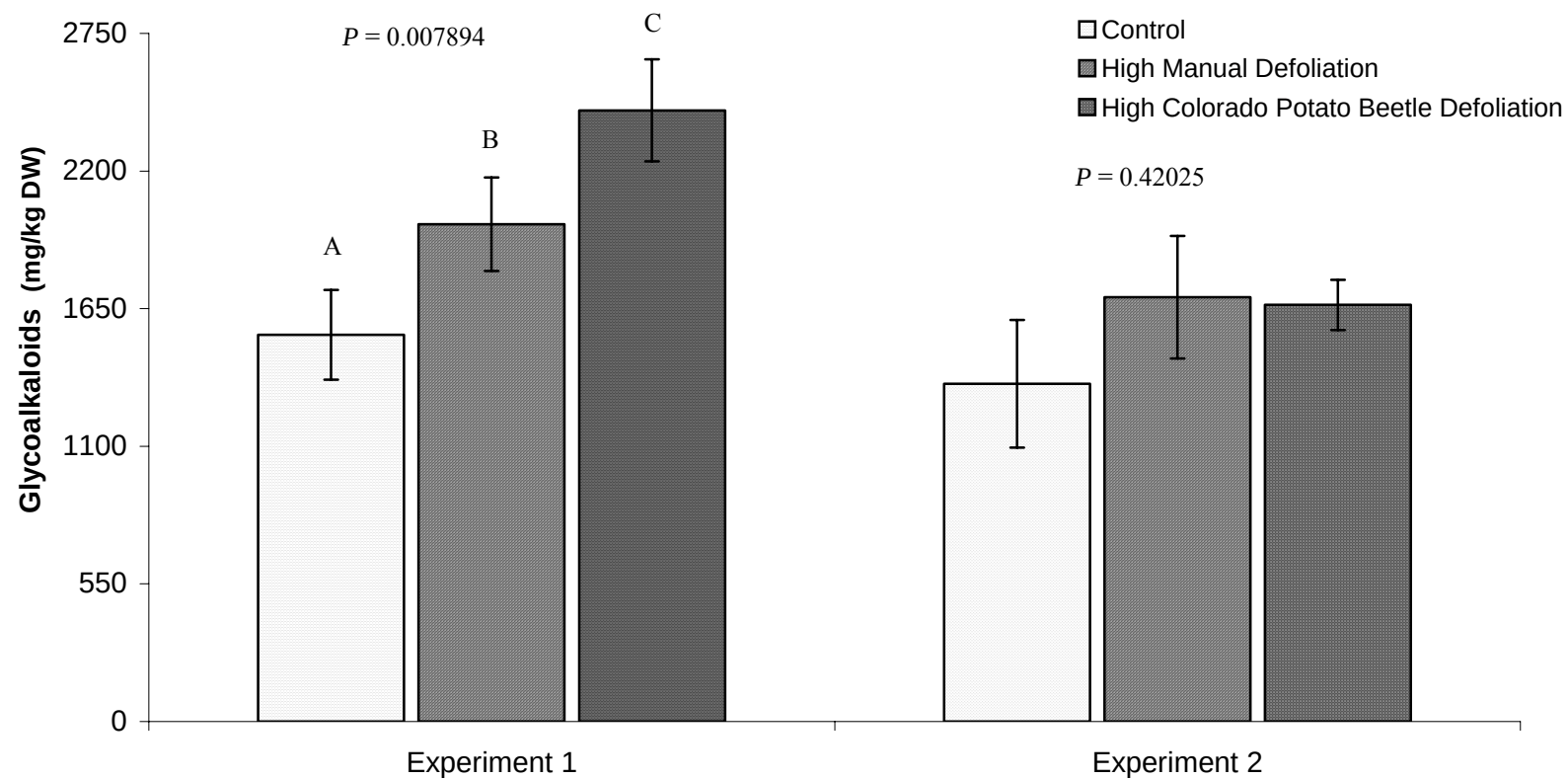


Figure 11. Glycoalkaloid Concentrations for the Skin of Potatoes Harvested from Control Plants (0% defoliation), High Manual Defoliation (~90% defoliation), and High Colorado Potato Beetle Defoliation (~90% defoliation) for Experiments 1 and 2.

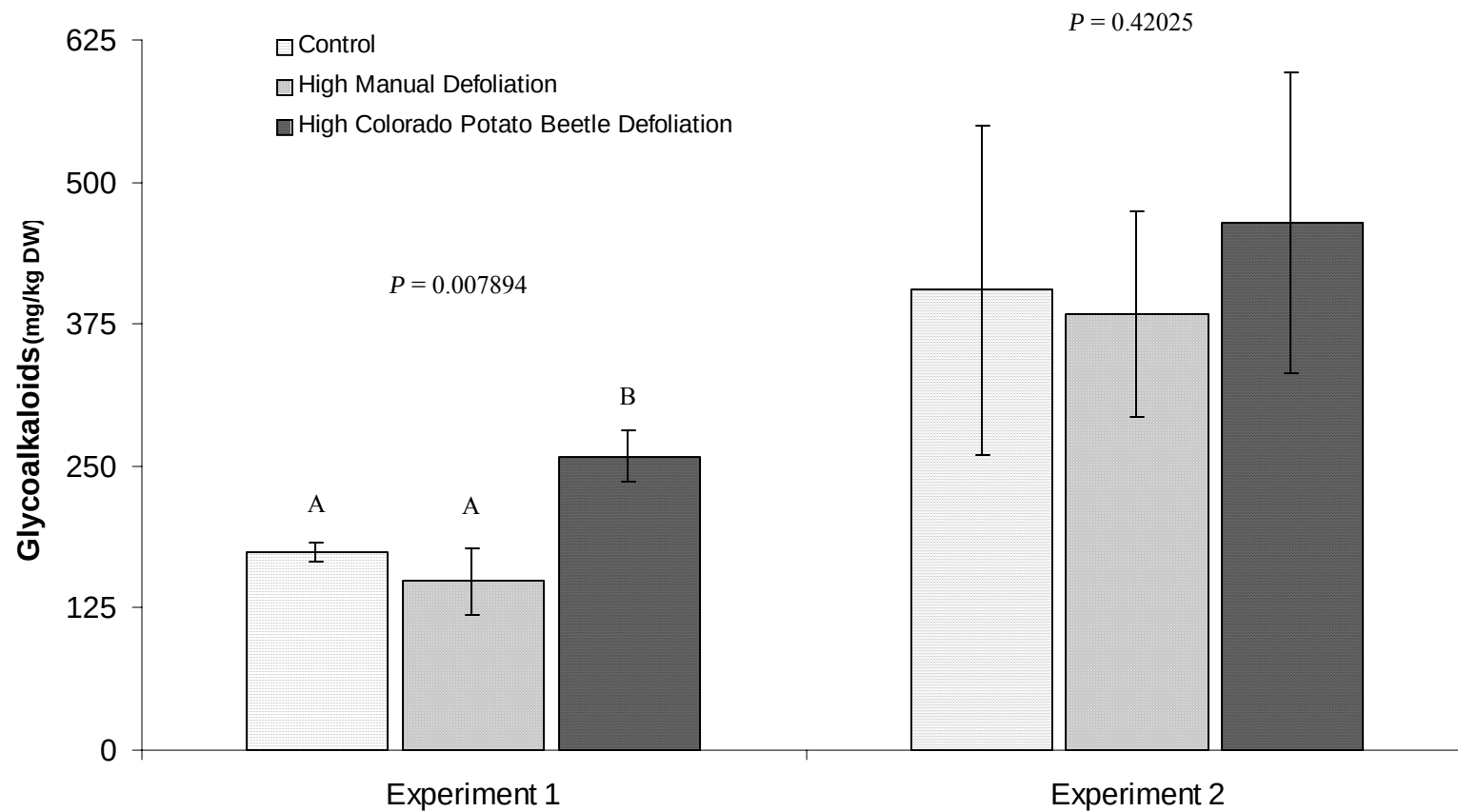


Figure 12. Glycoalkaloid Concentrations for the Inner Tissue of Potatoes Harvested from Control Plants (0% defoliation), High Manual Defoliation (~90% defoliation), and High Colorado Potato Beetle Defoliation (~90% defoliation) for Experiments 1 and 2.

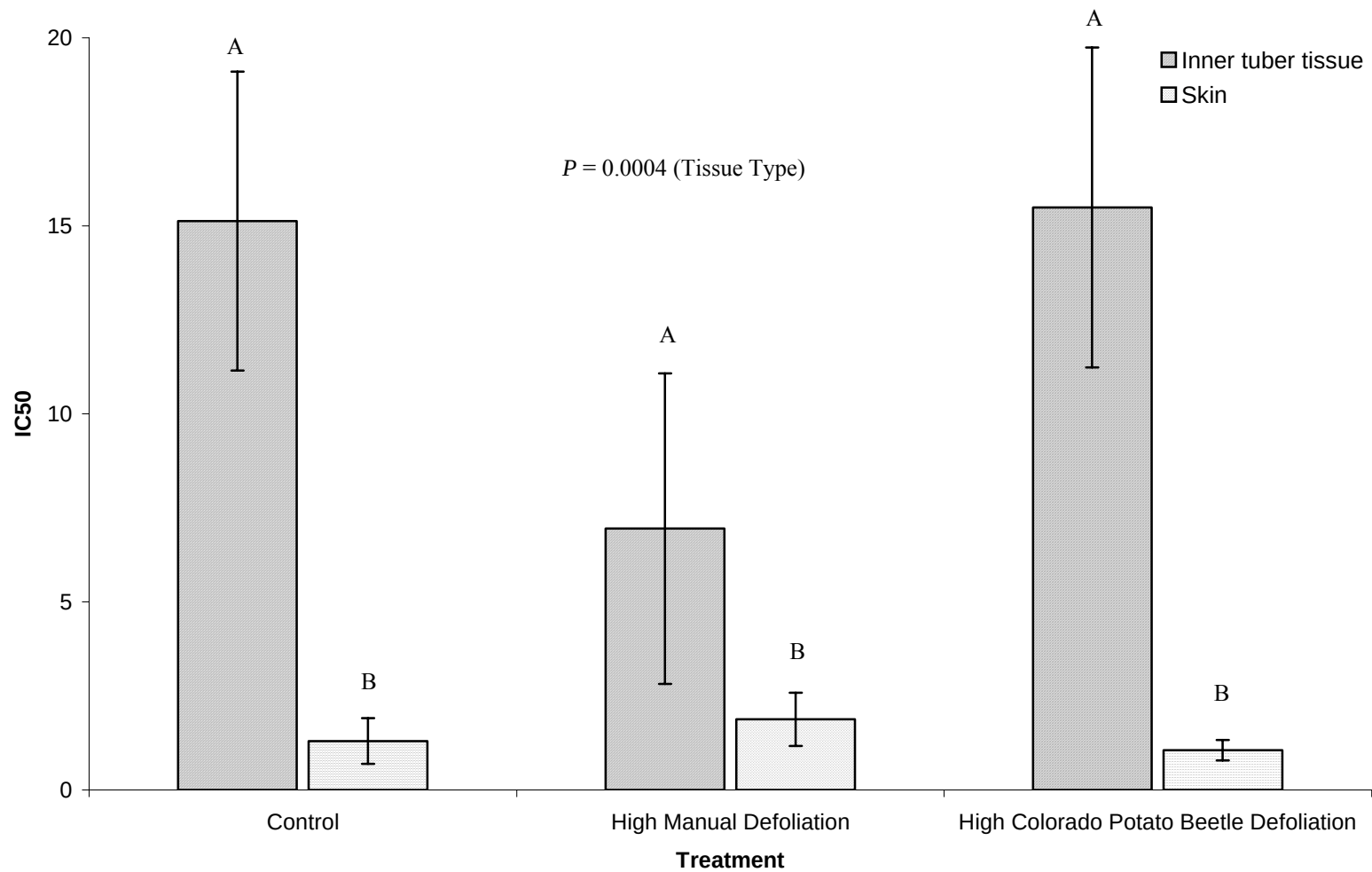


Figure 13. Mean IC₅₀s (Inhibitory Concentration, 50%) from Potatoes of Control, High Manual Defoliated, and High Colorado Potato Beetle Plants Experiment 1.

Risk Characterization

The acute oral exposures for the eight human subgroups exposed to glycoalkaloid concentrations in the inner tissue of tubers from control plants at the 50th percentile of exposure ranged from 0.19 to 0.58 mg/kg BW/day, and the risks ranged from 19% to 58.3% of the toxic endpoint (Table 10). At the 95th percentile, the acute oral exposures for the eight human subgroups ranged from 0.41 to 1.13 mg/kg BW/day, and the risks ranged from 40.6% to 113.1% of the toxic endpoint. At the 99.9th percentile, the acute oral exposures for the eight human subgroups ranged from 1.07 to 4.81 mg/kg BW/day, and the risk ranged from 107% to 481.2% of the toxic endpoint (Table 10).

The acute oral exposures for the eight human subgroups exposed to glycoalkaloid concentrations in the inner tissue of tubers from manually defoliated plants at the 50th percentile ranged from 0.16 to 0.5 mg/kg BW/day, and the risk ranged from 16.3% to 49.9% of the toxic endpoint (Table 11). At the 95th percentile, the acute oral exposures for the eight human subgroups ranged from 0.35 to 0.97 mg/kg BW/day, and the risk ranged from 34.8% to 96.8% of the toxic endpoint. At the 99.9th percentile, the acute oral exposures for the eight human subgroups ranged from 0.92 to 4.12 mg/kg BW/day, and the risk ranged from 91.6% to 412% of the toxic endpoint.

The acute oral exposures for the eight human subgroups exposed to glycoalkaloid concentrations in the inner tissue of tubers from plants defoliated by Colorado potato beetles at the 50th percentile ranged from 0.28 to 0.86 mg/kg BW/day, and the risk ranged from 28.2% to 86.5% of the toxic endpoint (Table 12). At the 95th percentile, the acute oral exposure for the eight human subgroups ranged from 0.6 to 1.68 mg/kg BW/day, and

Table 10. Exposure, Percentage of Toxic Dose, and Risk Quotient for the Acute Exposure of Human Subgroups to Glycoalkaloids in the Inner Tissue of Tubers from Control Plants.

Control									
Demographic	50 th percentile			95 th percentile			99.9 th percentile		
	Exposure (mg/kg BW/day)	% toxic dose	RQ*	Exposure (mg/kg BW/day)	% toxic dose	RQ*	Exposure (mg/kg BW/day)	% toxic dose	RQ*
U.S. Population	0.2389	23.89	0.24	0.551	55.1	0.55	2.4883	248.83	2.49
All Infants	0.5831	58.31	0.58	0.5531	55.31	0.55	4.812	481.2	4.81
Children 1-6	0.5149	51.49	0.51	1.1305	113.05	1.13	3.7531	375.31	3.75
Children 7-12	0.3301	33.01	0.33	0.7314	73.14	0.73	2.5966	259.66	2.6
Youth 13-19	0.2451	24.5	0.25	0.5263	52.63	0.53	2.4068	240.68	2.41
Women 13+ (pregnant/not nursing)	0.1903	19.03	0.19	0.4064	40.64	0.41	1.1467	114.67	1.15
Women 20+ (not preg./not nurs.)	0.2057	20.57	0.21	0.4283	42.83	0.43	1.0698	106.98	1.07
Males 20+	0.2161	21.61	0.22	0.4876	48.76	0.49	1.4218	142.18	1.42

*RQ = Risk Quotient

∞

Table 11. Exposure, Percentage of Toxic Dose, and Risk Quotient for the Acute Exposure of Human Subgroups to Glycoalkaloids in the Inner Tissue of Tubers from Manually Defoliated Plants.

Manually Defoliated									
Demographic	50 th percentile			95 th percentile			99.9 th percentile		
	Exposure (mg/kg BW/day)	% toxic dose	RQ*	Exposure (mg/kg BW/day)	% toxic dose	RQ*	Exposure (mg/kg BW/day)	% toxic dose	RQ*
U.S. Population	0.204541	20.45	0.2045	0.471745	47.17	0.4717	2.130388	213.04	2.1304
All Infants	0.499249	49.92	0.4992	0.473504	47.35	0.4735	4.119855	411.99	4.1199
Children 1-6	0.440828	44.08	0.4408	0.967926	96.79	0.9679	3.213248	321.32	3.2132
Children 7-12	0.282629	28.26	0.2826	0.626214	62.62	0.6262	2.223111	222.31	2.2231
Youth 13-19	0.209802	20.98	0.2098	0.450560	45.06	0.4506	2.060605	206.06	2.0606
Women 13+ (pregnant/not nursing)	0.162910	16.29	0.1629	0.347967	34.80	0.3480	0.981771	98.18	0.9818
Women 20+ (not preg./not nurs.)	0.176106	17.61	0.1761	0.366653	36.67	0.3667	0.915912	91.59	0.9159
Males 20+	0.185045	18.50	0.1850	0.417482	41.75	0.4175	1.217316	121.73	1.2173

*RQ = Risk Quotient

Table 12. Exposure, Percentage of Toxic Dose, and Risk Quotient for the Acute Exposure of Human Subgroups to Glycoalkaloids in the Inner Tissue of Tubers from High Colorado Potato Beetle Defoliated Plants.

High Colorado Potato Beetle Defoliated									
Demographic	50 th percentile			95 th percentile			99.9 th percentile		
	Exposure (mg/kg BW/day)	% toxic dose	RQ*	Exposure (mg/kg BW/day)	% toxic dose	RQ*	Exposure (mg/kg BW/day)	% toxic dose	RQ*
U.S. Population	0.354250	35.42	0.3542	0.817029	81.70	0.8170	3.689679	368.97	3.6897
All Infants	0.864663	86.47	0.8647	0.820075	82.01	0.8201	7.135293	713.53	7.1353
Children 1-6	0.763482	76.35	0.7635	1.676378	167.64	1.6764	5.565113	556.51	5.5651
Children 7-12	0.489493	48.95	0.4895	1.084558	108.46	1.0846	3.850268	385.03	3.8503
Youth 13-19	0.363362	36.34	0.3534	0.780337	78.03	0.7803	3.568820	356.88	3.5688
Women 13+ (pregnant/not nursing)	0.282148	28.21	0.2821	0.602653	60.27	0.6027	1.700356	170.04	1.7004
Women 20+ (not preg./not nurs.)	0.305003	30.50	0.3050	0.635017	63.50	0.6350	1.586293	158.63	1.5863
Males 20+	0.320484	32.05	0.3205	0.723049	72.30	0.7230	2.108304	210.83	2.1083

*RQ = Risk Quotient

the risk ranged from 60.3% to 167.6% of the toxic endpoint. At the 99.9th percentile, the acute oral exposure for the eight human subgroups ranged from 1.58 to 7.14 mg/kg BW/day, and the risk ranged from 158.6% to 713.5% of the toxic endpoint.

Table 13 compares the RQ's for all eight human subgroups for tubers from control plants, manually defoliated plants, and high Colorado potato beetle defoliated plants at the 50th, 95th, and 99.9th percentiles. At the 50th percentile, the RQ's were greatest for all infants and least for women 13+ who were pregnant but not nursing. At the 95th percentile, the RQ's were greatest for children between the ages of 1 and 6 years and least for women 13+ who were pregnant but not nursing. At the 99.9th percentile, the risk quotients were greatest for all infants and least for women 20+ who were neither pregnant nor nursing. At the 50th, 95th, and 99.9th percentiles, RQ's were greatest for all human subgroups exposed to tubers from high Colorado potato beetle defoliated plants (approximately 33% greater than control tubers).

Discussion

For plants defoliated by Colorado potato beetles, there was a significantly greater production of glycoalkaloids than in control plants and manually defoliated plants for both skin and inner tissue in experiment one. In field experiments, Hlywka et al. (1994) observed a 37.5% increase in glycoalkaloid concentration of tubers from 'Superior' plants defoliated at high levels by Colorado potato beetles in comparison to control plants. However, there were no significant increases associated with manual defoliation. In the greenhouse experiments presented here, using a different cultivar, 'Russet

Table 13. Acute Risk Quotients (RQ) for Human Subgroups Exposed to Glycoalkaloids in Tubers.

Acute Risk Quotients for Human Subgroups									
Demographic	<i>50th percentile</i>			<i>95th percentile</i>			<i>99.9th percentile</i>		
	Control	Manual Defoliated	High CPB Defoliated	Control	Manual Defoliated	High CPB Defoliated	Control	Manual Defoliated	High CPB Defoliated
U.S. Population	0.2389	0.2045	0.3542	0.5510	0.4717	0.8170	2.4883	2.1304	3.6897
All Infants	0.5831	0.4992	0.8647	0.5531	0.4735	0.8201	4.8120	4.1199	7.1353
Children 1-6	0.5149	0.4408	0.7635	1.1305	0.9679	1.6764	3.7531	3.2132	5.5651
Children 7-12	0.3301	0.2826	0.4895	0.7314	0.6262	1.0846	2.5966	2.2231	3.8503
Youth 13-19	0.2450	0.2098	0.3534	0.5263	0.4506	0.7803	2.4068	2.0606	3.5688
Women 13+ (pregnant/not nursing)	0.1903	0.1629	0.2821	0.4064	0.3480	0.6027	1.1467	0.9818	1.7004
Women 20+ (not preg./not nurs.)	0.2057	0.1761	0.3050	0.4283	0.3667	0.6350	1.0698	0.9159	1.5863
Males 20+	0.2161	0.3205	0.3205	0.4876	0.4175	0.7230	1.4218	1.2173	2.1083

Burbank', similar results were observed. In experiment one, there was a 32.6% and a 36.8% increase in skin and inner tissue of tubers from plants defoliated at high levels by

Colorado potato beetles in comparison to control plants. In experiment two, although a significant difference in glycoalkaloid concentration was not observed among the treatments, the skin and inner tissue of tubers from plants defoliated at high levels by Colorado potato beetles increased by 18.9% and 12.7% in comparison to tubers from control plants. The lack of significance in experiment two may be a result of differences in storage length; experiment one was stored for a greater period of time than experiment two. Because significant differences were observed in experiment one and a trend was observed in experiment two, increases in tuber glycoalkaloids, overall, seem to be a general response of potato to Colorado potato beetle defoliation.

Hlywka et al. (1994) observed that manually defoliated plants and injury by potato leafhoppers (*Empoasca fabae* Harris) did not elicit increases in glycoalkaloid production in tubers whereas tubers from plants injured by Colorado potato beetles had increases in tuber glycoalkaloid concentrations. Because of the similar response in glycoalkaloid production observed between control plants, manually defoliated plants, and plants injured by potato leafhoppers, it is illustrative of a specific interaction occurring between the potato plant and the Colorado potato beetle; this response may be either specific to Colorado potato beetles or specific to the type of injury, defoliation, that Colorado potato beetles impose.

Although the mechanism for the initiation of glycoalkaloid synthesis has yet to be determined, glycoalkaloids cannot be translocated within plants (Roddick, 1982) and any increases observed in the tuber as a result of injury to the plant have to be elicited by a

signal from the plant and received by the tuber. Therefore, a signal-response mechanism most likely exists. In the case of the Colorado potato beetle, because injury is occurring strictly to the leaves, it seems that it is a plant signal-response and as a result, the tuber initiates increased synthesis of glycoalkaloids. The Colorado potato beetle may initiate the signal either through interactions between plant cells and saliva or because Colorado potato beetles defoliate leaves by lysing cells little by little.

Hlywka et al. (1994) hypothesized that the application of various phytohormones to plant foliage and/or directly on the tuber itself may elicit increases in glycoalkaloid concentrations. Alternatively, they hypothesized that because action potentials typically occur during plant injury and stress and are capable of “long-distance signal transduction from foliage to tubers”, they too may elicit increases in glycoalkaloid concentrations. The systemic expression of a proteinase inhibitor gene via a phytohormone signal involving abscisic acid can result from defoliation of plant leaf mass (Peña-Cortés et al., 1989). Bergenstråhle et al. (1992) did not observe that abscisic acid added to tuber disks affected the induction of increased levels of glycoalkaloids. Because of this, Hlywka et al. (1994) suggested that action potentials most likely were responsible for producing increases in glycoalkaloids in potatoes.

In addition to a significantly greater concentration of glycoalkaloids found in tubers from plants defoliated by Colorado potato beetles, in the first experiment there was a significantly greater concentration of glycoalkaloids found in the skin than the inner tissue of a tuber among all treatments. For experiment one, there was 90.2% higher concentration of glycoalkaloids in the skin than the inner tissue, and for experiment two there was a 73.1% higher concentration of glycoalkaloids in the skin than the inner tissue.

According to Zeiger (1998), peeling may reduce the quantity of glycoalkaloids in potatoes 30 to 80%, but according to our results, peeling potentially would reduce the quantity of glycoalkaloids between 73.1 and 90.2%.

The concentration of tuber extract required to reduce CHO cellular proliferation by 50% was 10 times less for the skin versus the inner tissue, indicating that skin tissue is much more toxic. This most likely is because tuber skin contains greater concentrations of glycoalkaloids than inner tissue. However, glycoalkaloids were not quantified from the extracts used in this study.

In experiment one, tubers from plants defoliated by Colorado potato beetles resulted in a 32.6% increase in glycoalkaloid production compared to tubers from control plants. To get a conservative idea of how this translates in terms of potato consumption, the average tablestock tuber glycoalkaloid concentration is between 133 and 867 mg/kg DW (including both inner and skin tissue). By taking the average tablestock tuber glycoalkaloid concentration, 500 mg/kg DW, a person weighing 60 kg would need to consume 120 g DW or 800 g FW of potatoes to reach the 1 mg/kg BW toxic endpoint. An average bagged potato weighs 193.33 grams, this translates to 4.1 potatoes. If the glycoalkaloid concentrations within the average tablestock tuber were to increase 32.6%, as observed in this experiment, a person weighing 60 kg would need to eat only 90.5 g DW or 603 g FW; this translates to 3.1 potatoes.

The population percentile that the U.S. EPA uses to determine and regulate risk from dietary exposure to pesticides is the 99.9th percentile. Therefore, to get a perspective on when the EPA would implement regulatory actions, at the 99.9th percentile all RQ's that equal or exceed 1.0 would be of concern. At the glycoalkaloid concentrations

measured in this study, regulatory actions most likely would be implemented for tubers from control plants, and these levels are for the inner tissue only; they do not include the levels measured in the skins of tubers.

Using the exposure levels for adult males 20 years or older, a male weighing 70 kg would need to consume approximately 7 and 20 skinless potatoes, respectively, to equal the RQ's determined by DEEM at the 95th and 99.9th percentiles, respectively. Approximately 14 skinless tubers of typical store-bought size from uninjured plants would need to be consumed for the exposure of glycoalkaloids to reach an RQ of 1.0. Nine skinless tubers from plants defoliated by Colorado potato beetle would need to be consumed to reach an RQ of 1.0. Again, this reflects the increase in dietary risk from the injury.

The primary uncertainty associated with this human dietary risk assessment is centered on the toxic endpoint. Toxic endpoints (e.g., acute LD₅₀ and acute, sub-chronic, and chronic NOAEL's or LOAEL's) for glycoalkaloids either are not available or are not sufficiently robust to set threshold levels. It is unlikely that the 1 mg/kg BW endpoint used here is sufficiently conservative because the value has not been established experimentally and is only 50% less than an acute dose known to cause clinical signs of toxicity in humans. Indeed, Friedman and McDonald (1997) and Essers et al. (1998) argue that the current U.S.D.A. recommended food-safety levels are not sufficiently protective of public health. It is likely, therefore, that an acute NOAEL would be much lower, and a chronic NOAEL (which typically forms the basis for the acceptable daily intake for pesticides) would be even lower. Consequently, it is possible that the toxic endpoint could be orders of magnitude less than the value used here (especially given that

safety factors of as much as 1000-fold are typically applied to the NOAEL to establish the acceptable daily intake).

A complicating factor associated with the uncertainty in toxic endpoints is that animal models (which are used to determine most human toxic endpoints) may not be useful for glycoalkaloids. Both mice and rats are much less sensitive to these toxins than humans.

Alpha-chaconine and α -solanine are excreted in the urine and feces as either solanidine or unchanged (Zeiger, 1998); therefore, it would be beneficial to determine if the toxic response of the glycoalkaloids is a function of the concentration of glycoalkaloids, if the concentration of glycoalkaloids that causes a response is the same concentration as consumed, if there is in fact a causal relationship between the consumption of glycoalkaloids and the toxic response observed, and if there is a degree of synergism occurring between α -solanine and α -chaconine and other molecules within the biochemical pathway in relation to the toxic response.

It is disconcerting that the glycoalkaloid concentrations within the inner tissue of tubers exceed the toxic endpoint for all human subgroups at less than the 99.9th percentile of exposure. This is both a function of the use of extremely high consumption percentiles as exposure endpoints and the use of 1 mg/kg BW as a toxic endpoint. Regardless, the dietary risk assessments presented here support the arguments of Friedman and McDonald (1997) and Essers et al. (1998) that current potato safety levels for glycoalkaloids are not sufficiently protective of public health. If potatoes contained 1000

mg/kg DW of glycoalkaloids, the current U.S.D.A. potato safety threshold, 65% of the U.S. population would exceed an RQ of 1.0.

By determining the human dietary risks associated with consumption of potatoes from plants defoliated by Colorado potato beetles, awareness of the potential risks may lead to action by consumers, farmers, scientists, and other individuals who might be involved in activities to reduce the risks. When potatoes are exposed to light, bruised, cut, rot by fungi or bacteria, and experience other forms of mechanical damage, glycoalkaloid concentrations increase (Lachman et al., 2001). Unlike exposure to light, bruising, and rot by fungi or bacteria, high Colorado potato beetle infestations are not visible on the tuber. If the dietary risk is already increased for tubers from plants exposed to defoliation by Colorado potato beetles, what are the risks if these same tubers are bruised or become infected with a pathogen?

Many secondary compounds, like glycoalkaloids, serve as natural pesticides within plants, and there is increasing interest to enhance these natural pesticides for commercial use (Fenwick et al., 1990). Because plants are being bred to contain not only a greater diversity of natural compounds, but also greater quantities (Hlywka et al., 1994), breeders should be aware that if a plant is bred to have higher glycoalkaloid levels, tubers could exceed dietary risk levels of concern regardless of Colorado potato beetle infestations or other forms of mechanical damage.

CONCLUSION

Photosynthetic and glycoalkaloid responses of potatoes (*Solanum tuberosum* L.) to varying levels of Colorado potato beetle (*Leptinotarsa decemlineata* Say) and manual defoliation were examined and characterized for 'Cal Red' and 'Russet Burbank' plants.

For larval and adult Colorado potato beetle single-leaf defoliation experiments, no alteration in photosynthesis was observed on the remaining tissue of an injured leaf for Cal Red and Russet Burbank leaves defoliated by larval Colorado potato beetles nor for Russet Burbank leaves defoliated by adult Colorado potato beetles. There was a significant relationship between Cal Red leaves and defoliation level, but there were fewer replication of defoliation levels with that variety. No significant differences were observed between actual Colorado potato beetle and manual defoliation for both varieties. This indicates that artificial defoliation techniques may be appropriate to measure plant gas exchange of potato to Colorado potato beetle injury and adult and larvae Colorado potato beetles may be assigned to the same injury guild.

In both of the whole-plant defoliation studies, defoliation type or level consistently did not result in increased or decreased gas exchange parameters of individual leaves compared to undefoliated controls. Although there were significant differences between gas exchange variables in upper and lower leaves in this study, there was no evidence of delayed senescence in defoliated treatments. Therefore, Colorado potato beetle injury on Cal Red and Russet Burbank potato plants may be classified in the leaf-mass consumption injury guild.

Because temporal variations in photosynthetic rates were observed in control plants, this suggests that the variations were not directly related to defoliation levels themselves, but other factors such as sunlight, temperature, atmospheric CO₂ concentrations, developmental stages, and/or plant diel periodicities. Measurements were taken at approximately the same time during the day and suggest that plant diel periodicities are an unlikely contributor to the observed temporal variation. Varietal effects indicate that plant gas exchange responses to insect injury cannot be extrapolated between varieties.

Two experiments were conducted that measured the glycoalkaloid concentrations in tubers in response to manual and Colorado potato beetle defoliation. Tubers from plants defoliated by Colorado potato beetles in the initial experiment experienced a significantly greater production of glycoalkaloids than control plants and manually defoliated plants for both skin and inner tissue. In addition, for the initial experiment, there was a 32.6% and a 36.8% glycoalkaloid increase in skin and inner tissue of tubers from plants defoliated at high levels by Colorado potato beetles in comparison to control plants. In the second experiment, although a significant difference in glycoalkaloid concentration was not observed among the treatments, the skin and inner tissue of tubers from plants defoliated at high levels by Colorado potato beetles increased by 18.9% and 12.7% in comparison to tubers from control plants. For the initial experiment, there was 90.2% higher concentration of glycoalkaloids in the skin than the inner tissue, and for the second experiment there was a 73.1% higher concentration of glycoalkaloids in the skin than the inner tissue and peeling potentially would reduce the quantity of glycoalkaloids between 73.1% and 90.2%.

In the initial glycoalkaloid concentration experiment, the concentration of tuber extract required to reduce Chinese hamster ovary (CHO) cellular proliferation by 50% was 10 times less for the skin versus the inner tissue, indicating that skin tissue is much more toxic. This most likely is because tuber skin contains greater concentrations of glycoalkaloids than inner tissue. Therefore, glycoalkaloids within tubers may lyse CHO cells. However, glycoalkaloids were not quantified from the extracts used in this study.

Because this study and Hlywka et al. (1994) found that tubers from plants subjected to Colorado potato beetle defoliation contained higher glycolalkaloid concentrations than tubers from undefoliated plants, but photosynthetic rates among treatments were unaffected, there does not seem to be a tradeoff between a potato plants natural defense and photosynthesis.

The dietary risk posed to different human subgroups associated with the consumption of potatoes was determined for the 50th, 95th, and 99.9th percentile U.S. national consumption values. The population percentile that the EPA uses to determine risk from dietary exposure to pesticides is the 99.9th percentile, and at a toxic dose of 1 mg/kg BW, glycoalkaloid concentrations within the inner tissue of tubers exceeded the toxic endpoint for all human subgroups at less than the 99.9th percentile of exposure. This is both a function of the use of extremely high consumption percentiles as exposure endpoints and the use of 1 mg/kg BW as a toxic endpoint. Regardless, the dietary risk assessments presented here support the arguments that current potato safety levels for glycoalkaloids are not sufficiently protective of public health.

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APPENDIX A

TUBER YIELD OF MANUAL AND COLORADO POTATO BEETLE
DEFOLIATION STUDY 2004 AND 2005

Yield of Russet Burbank and Cal Red tubers was determined at varying levels of Colorado potato beetle defoliation and manual defoliation. The experiment was performed in a greenhouse and replicated (2004 and 2005). All tubers were harvested and averaged per pot. Five replicates for each cultivar were arranged in a two-by-five repeated measures factorial design within a RCBD using the following treatments: control (no defoliation), low, medium, and high Colorado potato beetle and manual defoliation. At the mid-vegetative stage, just before flowering, approximately 15, 20, and 25 third instars were applied to the low, medium and high Colorado potato beetle treatment plants and allowed to defoliate. Once the low, medium, and high treatment plant leaf area was reduced by approximately 30% (low), 60% (medium), and 90% (high) the Colorado potato beetles were removed. The manual defoliated plants were defoliated with scissors throughout the same period to simulate the percentage and patterns of leaf mass removed by the Colorado potato beetles.

Control and treated groups were compared using analysis of variance (ANOVA) ($\alpha = 0.05$). Data were analyzed using R 2.0.1 (R: A Language and Environment for Statistical Computing; R Development Core Team, The R Foundation for Statistical Computing, 2004, Vienna, Austria). To meet normality, necessary data was log transformed. Linear and non-linear regression analyses were conducted to determine the relationship between percent defoliation and yield using SAS system for Windows V8 (SAS Institute, Inc. ©1999-2001, Cary, NC).

Manual and Colorado Potato Beetle Defoliation Study – 2004

There was no significant block, defoliation level, or interaction effect between defoliation and variety on yield of potatoes, but there was a significant effect of variety ($F = 20.679$, $df = 1$, $P < 0.00006$) on yield (Table 14, Figure 14). Linear and quadratic relationships between percent defoliation and yield were not significant.

Manual and Colorado Potato Beetle Defoliation Study – 2005

There was no significant block or defoliation level effect, but variety ($F = 15.329$, $df = 1$, $P < 0.0004$) and the interaction between defoliation and variety ($F = 3.588$, $df = 4$, $P = 0.015$) had a significant effect on yield of potatoes (Table 15, Figure 14). Linear and quadratic relationships between percent defoliation and yield were not significant.

Table 14. ANOVA Table for Tuber Yield of Manual and Colorado Potato Beetle Defoliation Study - 2004

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Block	4	2.46E-06	6.14E-07	1.6086	0.1939
Defoliation Level	4	2.64E-06	6.60E-07	1.7286	0.1658
Variety	1	7.89E-06	7.89E-06	20.679	< 0.00006
Defoliation x Variety	4	6.24E-07	1.56E-07	0.4088	0.8011
Residuals	35	1.34E-05	3.82E-07		

Table 15. ANOVA Table for Tuber Yield of Manual and Colorado Potato Beetle Defoliation Study - 2005

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Block	4	453	113.3	0.853	0.5013
Defoliation Level	4	1239.4	309.9	2.3339	0.0742
Variety	1	2035.2	2035.2	15.3299	< 0.0004
Defoliation x Variety	4	1905.5	476.4	3.5882	0.0146
Residuals	36	4779.4	132.8		

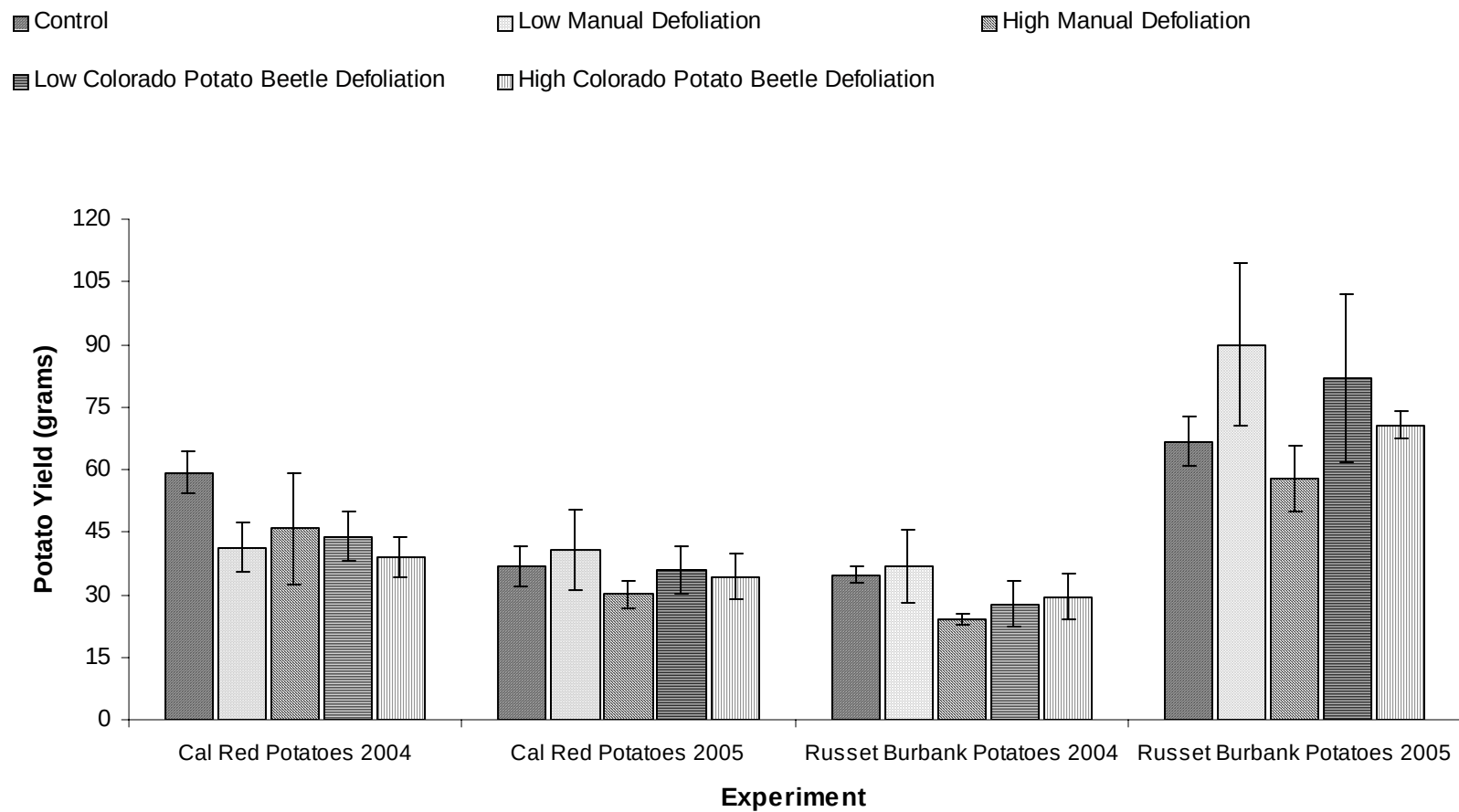


Figure 14. Manual and Colorado Potato Beetle Defoliation Study- 2004 and 2005: Yield of Tubers from Cal Red and Russet Burbank Pplants Across all Defoliation Levels.