CRISTINA SCHETINO BASTOS

PLANT PHYSIOLOGICAL RESPONSE TO INSECT INJURY AND INSECTICIDE IMPACT OVER SOIL ARTHROPODS.

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós Graduação em Fitotecnia, para obtenção do título de "Doctor Scientiae."

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Prof. Herminia Emília Prieto Martinez (Conselheira)	Prof. Raul Narciso Carvalho Guedes (Conselheiro)
Prof. Germano Leão Demolin Leite	Prof. Paulo Roberto Cecon

Prof. Marcelo Coutinho Picanço
(Orientador)

"*No princípio não havia existência ou inexistência. O mundo era energia não revelada. Ele vivia sem viver, por seu próprio poder. E, nada mais havia.*"

(Hino da Criação – Rig Veda)

(Extraído de "O despertar do Espírito" por Divaldo Franco/Joanna de Ângelis)

Aos meus amados pais Ruth Schetino Bastos e Wantuil Bastos pelo apoio incondicional,

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Ao meu querido Fábio por seu amor em todos os momentos,

eu dedico esta tese.

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RESUMO

BASTOS, Cristina Schetino, DS., Universidade Federal de Viçosa, outubro de 1998. **Resposta fisiológica de plantas à injúria por insetos e impacto de inseticida sobre artrópodos de solo.** Orientador: Marcelo Coutinho Picanço. Conselheiros: Raul Narciso Carvalho Guedes e Herminia Emília Prieto Martinez.

A crescente necessidade por segurança ambiental quando enfrentando o problema advindo do controle de pragas tem levado a melhoria nas técnicas atualmente disponíveis para alcançar-se tal objetivo. Este estudo foi conduzido para avaliar a resposta de batata e soja à injúria ocasionada por insetos fitófagos e o impacto do aldicarbe sobre habitantes do solo em lavoura de café usando a análise por variáveis canônicas. Visando estudar a resposta das plantas à injúria por insetos, dois experimentos foram conduzidos em um campo comercial de batatas localizado no município de Kearney no centro sul do estado de Nebraska, EUA e em um campo experimental de soja localizado na Rosemount Research and Outreach Center, Universidade de Minnesota, Rosemount, MN, EUA. No campo de batata foram realizados experimentos em três épocas diferentes: 12-13/07/2001, 1-2/08/2001, and 14-15/08/2001. O primeiro e o segundo experimentos foram representados por um fatorial de 3 (variedades - Russet Norkotah, Atlantic e Frito-Lays) x 2 (tratamentos – testemunhas com gaiola e folhas infestadas) e o terceiro foi um fatorial de 3 (variedades - Russet Norkotah, Atlantic e Frito-Lays) x 3 (tratamentos – testemunha com gaiola, testemunha sem gaiola e folhas infestadas), em ambos os casos sendo os tratamentos dispostos no delineamento em blocos ao acaso com oito repetições. No campo de soja, o experimento foi composto de três tratamentos os quais foram: níveis de infestação baixo, intermediário e alto, sendo estes analisados como delineamento inteiramente ao acaso com oito repetições. Foram medidas as trocas gasosas das plantas de batata e soja, fluorescência

e proporções de isótopos estáveis de carbono de plantas de soja em resposta á injúria ocasionada pelo gafanhoto de perna vermelha (*Melanoplus femurrubrum* Orthoptera: Acrididae) e pelo pulgão da soja (*Aphis glycines* Homoptera: Aphididae), respectivamente. Os gafanhotos não afetaram as trocas gasosas das plantas de batata (quando as taxas de injúria estavam entre 10-25%). As plantas de soja apresentaram reduções nas trocas gasosas de mais de 50% nos folíolos infestados, porém não alteraram os parâmetros de fluorescência. Visando estudar o impacto do aldicarbe sobre artrópodos do solo de café, conduziu-se um experimento em um cafezal da Universidade Federal de Viçosa, Viçosa, MG, Brasil, o qual foi composto por três tratamentos (controle, uma aplicação de 10 g de Temik 150/planta e duas aplicações de 10 g de Temik 150/planta) e quatro blocos, avaliados uma data antes da aplicação do inseticida e 13 datas após a aplicação do inseticida. Verificou-se que impacto do aldicarb sobre a abundância da comunidade de artrópodos do solo de café visualizada através do padrão diferencial de dispersão adotado pelas parcelas controle quando comparadas com as parcelas tratadas uma e duas vezes com aldicarb. As parcelas que receberam uma e duas aplicações de aldicarb não difereriram entre si. A principal espécie impactada foi Acarinae: morfoespécie 4.

KEY WORDS: Trocas gasosas, interação inseto-planta, ecotoxicologia, técnicas multivariadas, aldicarbe.

ABSTRACT

BASTOS, Cristina Schetino, DS., Universidade Federal de Viçosa, outubro de 1998. **Plant physiological response to insect injury and insecticide impact over soil arthropods.** Advisor: Marcelo Coutinho Picanço. Committee members: Raul Narciso Carvalho Guedes e Herminia Emília Prieto Martinez.

The growing need for environmental safety when dealing with pest control has led to the improvement of the approaches currently available to achieve this goal. In such context, methods that tend to envision pest problem in a more balanced way has gained importance. This study was carried out to evaluate soybean and potato response to the injury of phytophagous insects and the aldicarb impact over the inner coffee soil dwellers. To study plant response to insect injury two experiments were carried out: the first one was run on a potato field at Kearney County in south-central Nebraska, USA, and the second one was done on a soybean field at the University of Minnesota Rosemount Research and Outreach Center, Rosemount, MN, USA. On the potato field, it was carried out experiments in three different times: 07/12-13/2001, 08/1-2/2001, and 08/14-15/2001. The first and second one were represented by a 3 (varieties - Russet Norkotah, Atlantic and a Frito-Lays proprietary cultivar) x 2 (treatments – caged control and infested leaves) factorial and the third one was a 3 (varieties - Russet Norkotah, Atlantic and a Frito-Lays proprietary cultivar) x 3 (treatments – caged control, uncaged control and infested leaves) factorial, in both cases being the treatments arranged in randomized blocks with eight replicates. On the soybean field, the experiment envisioned three treatments which were low, intermediate and high aphid infestation levels which were arranged in randomized blocks with eight replicates. It was measured the potato gas exchange response and the soybean gas exchange, fluorescence, and stable carbon isotope ratio response to the redlegged

grasshopper (*Melanoplus femurrubrum* Orthoptera: Acrididae) and soybean aphid (*Aphis glycines* Homoptera: Aphididae), respectively. With injury rates of about 10-25% the potato gas exchange was not affected by the grasshopper. Soybean plants showed reductions of up to 50% on the gas exchange parameters of infested leaflets but did not alter the fluorescence parameters. To study the aldicarb impact over coffee soil arthropods, it was set up an experiment in a coffee plantation at the Federal University of Viçosa, Viçosa, MG, Brasil, with three treatments (control, one application of 10 g of Temik 150 (aldicarb)/plant and two applications of 10 g of Temik 150/plant) and four blocks, sampled one day before insecticide application and 13 different times after the insecticide application. There was significant effect of aldicarb application on the abundance of coffee soil arthropods based on different dispersion pattern of the control plots on the canonical axis when compared to plots treated once and twice with aldicarb. The plots which received one and two aldicarb applications did not differ significantly. The main species affected was mite morphspecies 4.

KEY WORDS: Gas-exchange, insect-plant relationship, ecotoxicology, multivariate techniques, aldicarb.

General Introduction

The ever growing need for environmental safety when dealing with pest control has led to improvements on the approaches currently available to achieve this goal. In this context the idea of integrated pest control or integrated management, developed at about 30 years ago, has increased in importance. The original idea aggregated to the concept is about reducing pest population to a level where it can be tolerated. In this context, it is implicit that not all organisms associated with a culture should be considered as pests. The pest concept is linked to economic losses, which requires action(s) against such organisms whenever losses take place (Pedigo, 2002). In this case, the most common tactic adopted is the chemical control.

The chemical control can increase the total production cost and it can also lead to problems such as resurgence, outbreaks of secondary pests and insecticide resistance. Ripper (1956) suggests as possible causes of these phenomena 1) reduction of natural enemies, 2) positives influences of the pesticides in the arthropod physiology/behavior (hormoligosis) and 3) elimination of competitive species. As a consequence, added to the possibility of losing many compounds actually available to pest control, the register agencies became tighter concerning the procedure for registration approval. This whole situation demands better approaches to assess the impact of pesticides upon non-target organisms.

The commonly used selectivity tests that aimed to evaluate the effect of insecticides over natural enemies has been gradually replaced for more accurate approaches as the sequence laboratory/semi-field/field strategy proposed by the IOBC (The International Organization for Biological Control) and WPRS (The West Paleartic Regional Section) in these type of studies. These approaches can be considered as a little more accurate since they already input some level of interaction in the studies as predator or parasitoid or as pest/plant interactions. But it still does not account for the interaction among the organisms that encompasses the community living in a specific environment (Hassan, 1989). This is why the ecotoxicology approach emerged aiming to provide a better understanding of how to evaluate pesticide impact upon non-target organisms, since it takes into account all the possible levels of interaction (pest/host/natural enemies/environment).

Moreover, the techniques available for monitoring the ecological effects of toxicants tend to cover the fields of bioaccumulation, biotransformation, biodegradation,

biochemical monitoring, physiology and behavior, population parameters, community parameters and ecosystem effects (Scott & Clarke, 2000). But if one thinks about increasing the level of organization involved in the approach (as changing from population to community parameters), it is important to say that the higher status approach will absorb the lower status approach which finishes saving effort spent in the evaluations and, in many cases, leading to a greater efficacy.

In the same line, approaches that can improve the process of taking actions against insect-pests will improve the techniques actually available. The study of plant response to insect injury rises as a powerful tool in this sense, since beyond the theoretical importance of establishing common injury responses, characterizing photosynthetic responses to injury provide a basis for establishing multiple species economic injury levels (Hutchins *et al.*, 1988; Peterson 2001). It can allow, for example, the utilization of a multiple species Economic Injury Level (EIL) for defoliators or sucking insects that cause the same sort of gas exchange response in the plants, grouping them in functional guildas.

The use of multiple species EILs can contribute to economy in the effort spent in sampling procedures as well as in the number of pesticide applications (Peterson, 2001) what, just like in the ecotoxicology approach, seems to be a more rational technique when dealing with pest problem.

The objective of this thesis was to examine if injury caused by a common defoliating-pest, the redlegged grasshopper (*Melanoplus femurrubrum*) and a sucking-insect, soybean aphid (*Aphis glycines*) would influence the gas exchange parameters on potato and soybean, respectively as well as study the impact of aldicarb over the inner soil arthropod dwellers of coffee plantations using multivariate techniques. While doing that, we tried to access the potential adoption of more rational techniques as a tool to be used, later on, in Integrated Pest Management (IPM).

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Photosynthetic Responses of Potato (*Solanum tuberosum*) to Grasshopper (*Melanoplus femurrubrum*) Injury

Abstract

Establishing photosynthetic responses of potato to grasshopper injury is an important first step in developing multiple species EILs. Consequently, the main objective of this study was to examine if injury caused by a common defoliating-pest, the redlegged grasshopper (*Melanoplus femurrubrum*), would influence the gas exchange parameters on remaining tissue. We conducted this examination on three potato varieties (Norkotah, Atlantic, and Frito-Lays proprietary) and at three times during potato growth (all post flowering). With injury rates of about 10-25%, we observed no significant change in photosynthetic rates on remaining (uninjured) leaf tissue in any variety or in any of the three experiments. These results are consistent with the pattern we and other researchers have observed with gross tissue removal by various insects on other plant species. Additionally, this finding indicates grasshopper defoliation will reduce photosynthetic leaf area and may follow mechanisms of yield loss (through reduced canopy light interception) observed in other plant species.

Key words: redlegged grasshopper, gas-exchange, plant-insect interaction

The impact of leaf mass consumers (or defoliators) such as grasshoppers on yield losses are correlated most often with the amount of leaf tissue removed (Peterson and Higley 2001). A second method to quantify plant injury is through physiological measures such as gas exchange (e.g. Alderfelder and Eagles 1976, Poston et al. 1976, Detling et al. 1979, Ingram et al. 1981, Li and Proctor 1984, Peterson et al. 1992, Peterson and Higley 1996, Peterson et al. 1996, Peterson et al. 1998, Haile et al. 1999, Holman and Oosterhuis 1999) which is a way of estimating plant photosynthesis, comparing the CO_2 absorbed by the leaves with a reference of CO_2. However, despite of the large number of studies, contradictory findings remain. For example, Welter (1989) showed that the removal of either partial or entire leaves by insect herbivores increased photosynthetic rates of the remaining leaf tissue, while many other early studies found reduction in photosynthetic rates (Alderfelder and Eagles 1976, Hall and Ferree 1976, Detling et al. 1979, Ingram et al. 1981, Li and Proctor 1984). More recent studies in a number of crops have found no effects of insect feeding on photosynthetic rates of remaining tissues (Burkness et al. 1999, Peterson et al. 1992, Peterson and Higley 1996, Peterson et al. 1996).

The explanation for the variable outcomes of these studies has been the variation in the manner in which the studies have been conducted. While some of these studies assess the question through the evaluation of the whole canopy others evaluate only the local response or measure photosynthesis of the infested leaf, making generalizations difficult (Peterson 2001). The most extensive work to address plant response to insect injury has been conducted on soybean (Poston et al. 1976, Ingram et al. 1981, Hammond and Pedigo1981, Ostlie and Pedigo 1984, Peterson and Higley 1996, Peterson et al. 1998). Other crops including alfalfa (Peterson et al. 1992), apple (Hall and Ferree, 1976, Peterson et al. 1996), cotton (Holman and Oosterhuis 1999), beans

(Peterson et al. 1998), and wheat (Detling et al. 1979, Haile et al. 1999) have also been studied.

A key question is if different plant species respond similarly to the same type of insect injury. For example, alfalfa plants subjected to actual and simulated weevil (Coleoptera: Curculionidae) injury did not show any significant differences in photosynthetic rates (Peterson et al. 1992). The same was true concerning the effects of actual and simulated injury of cecropia moth over apple (3 and 24 hours after the cessation of injury) and crabapple (Peterson et al. 1996) and simulated injury over soybean photosynthesis (Peterson and Higley 1996). Clearly some types of leaf injury (such as sucking injury, leaf mining, and some skeletonizing) cause photosynthetic rate reductions on remaining tissue (Welter 1989), but the situation with insects causing gross tissue removal is not as clear.

Beyond the theoretical importance of establishing common injury responses, characterizing photosynthetic responses to injury provides a basis for establishing multiple species economic injury levels (Hutchins et al. 1988, Peterson and Higley 2001). Grasshoppers (Orthoptera: Acrididae) are occasional pests on potato and frequently occur in conjunction with other defoliators. Consequently, establishing photosynthetic responses of potato to grasshopper injury is an important first step in developing multiple species EILs.

The main objective of this study was to examine if injury caused by a common defoliating-pest, redlegged grasshopper (*Melanoplus femurrubrum*) would influence the gas exchange parameters on remaining tissue. We conducted this examination on three potato varieties and at three times during potato growth (all post flowering). Our results indicate injury does not affect photosynthesis of remaining tissue, which is consistent with the pattern we and other researchers have observed with gross tissue removal by various insects on other plant species.

Methods and Materials

The study was carried out at a commercial potato field in Kearney County in south-central Nebraska. Potatoes were at post-flowering stage and experiments were conducted during July and August, 2001. One table variety (Russet Norkotah) and two chipping varieties (Atlantic and a Frito-Lays proprietary cultivar) of potato were used. The plant density was 4 plants/meter and the soil type was silt loam. Row spacing was 0.914 m. The experiment was settled following a factorial arrangement of 3 (varieties) x 2 (control caged leaf, and infested leaves) in experiment one and two and 3 (varieties) x 3 (control caged leaf, non-caged check, and infested leaves) in experiment 3, in randomized complete blocks, with 8 replicates.

Two adult grasshoppers, *Melanoplus femurrubrum* (Orthoptera: Acrididae), were confined in mesh cages (10 x 10 cm) for 24 h on the top five leaflets in the upper portion of the plant canopy, at three different times. The insects were collected from nearby grass boarders or from the field. Empty cages were placed on leaves for caged control treatments and additional uncaged leaves were marked and served as a control for cage effect. Cages allowed transmission of >90% of photosynthetically active radiation in full sun (based on direct measurements with a quantum sensor inside and outside cages). After feeding, the insects and the cages were removed from the plants and gas exchange measurements were made. Experiments were conducted (with measurements on the second day) on 12-13 July, 2001 (experiment 1), 1-2 Aug., 2001 (experiment 2), and 14-15 Aug., 2001 (experiment 3).

To measure plant photosynthesis we used a portable photosynthesis system (model LI-6400, Li-Cor, Lincoln, NE) with the following settings: blue light source in the flux of 1,500 µmol photon m^{-2} s^{-1}, 500 µmol of CO2 m^{-2} s^{-1}. The potato gas exchange readings (µmol/m^2/s) were taken on the second or third leaflet of each leaf in all plots (control and infested leaves).

Because low levels of injury might not provide an adequate measure of leaf response, grasshoppers were confined to leaves to provide ca. 25% defoliation. The exact percent of defoliation was estimated from pictures of damaged leaves using MatLab software for experiment 1, visually for experiment 2, and by leaf area meter (Model LI-3100, Li-Cor, Lincoln, NE) for experiment 3.

The gas exchange data ($\mu mol/m^2/s$) were analyzed with ANOVA using PROC MIXED (SAS institute 1990).

Results and Discussion

Grasshopper feeding produced mean injuries of 5-10% in experiment 1 and 20-30% in experiments 2 and 3. Figure 1 presents gas exchange results by variety and experiment. Photosynthetic rates estimated through carbon exchange rates in $\mu mol/m^2/s$ did not differ between injured and uninjured leaves ($p=0.0674$, 0.2781 and 0.1386 for experiment one, two and three respectively) for any variety in any experiment. Similarly, no significant effect for variety ($p=0.7061$, 0.8465 and 0.1204 for experiment one, two and three respectively) and for the variety x injury interaction ($p=0.6202$, 0.2348 and 0.9034 for experiment one, two and three respectively) was observed.

The photosynthetic rate means of uninjured leaflets, estimated by the carbon gas exchanges, generally tended to be higher than the comparative ones on injured leaflets, excepting on the variety ‘Norkotah’ in experiment 2 (Fig. 1).

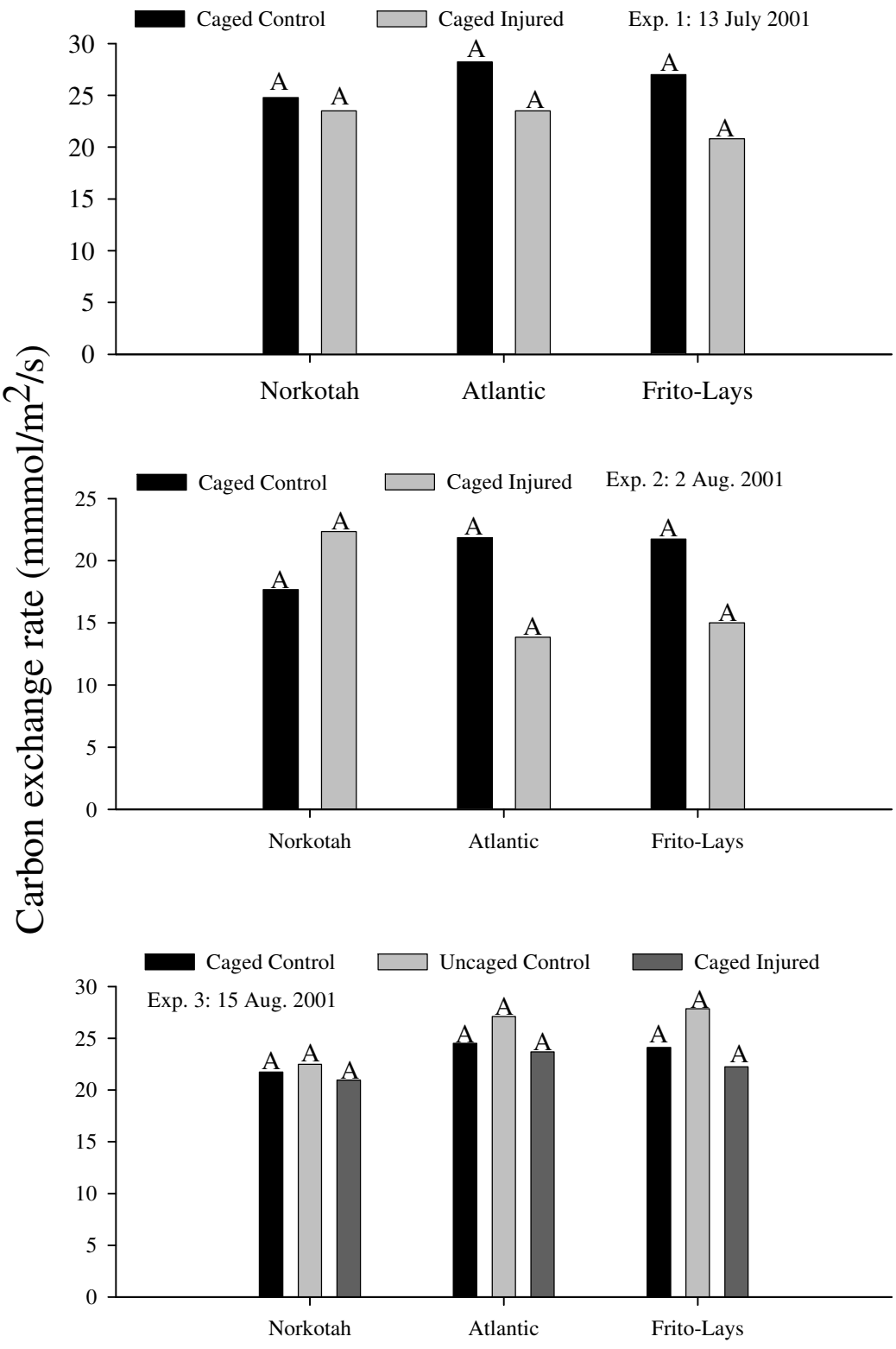

Figure 1. Mean carbon exchange rates of potato leaves injured by *Melanoplus femurrubrum* and controls. Rates determined immediately (< 1h) post injury, after grasshoppers were caged on leaves for 24 h. Measurements reported for three varieties (Norkotah, Atlantic, and Frito-Lays proprietary) in three experiments (20 July, 2 Aug., and 15 Aug., 2001).

Consistent numerical differences across multiple experiments might be taken to suggest a biological difference does exist below the level of statistical significance. Ostlie and Pedigo (1984) observed small, transient (1-4 h) reductions in stomatal conductance in defoliated soybean immediately post injury, which are thought to be associated with wound healing. Transient differences in leaf water potential and stomatal conductance might also cause minor differences in gas exchange. However, in this study no significant effect on gas exchange was observed, and the level of replication and absence of excess variability in measurements both indicate sufficient power was provided to resolve underlying differences if they existed.

Increases in photosynthesis after defoliation are possible, even expected, with increased light penetration through the plant canopy. For example, such an increase was observed by Holman and Oosterhuis (1999) in an evaluation of defoliation injury to cotton. Increases in photosynthesis because of increased light, water, or nutrient availability after injury are called extrinsic responses. As Peterson and Higley (1993) discuss, distinguishing intrinsic versus extrinsic responses to injury is crucial, in part because intrinsic responses are genetic and, therefore, heritable. Intrinsic responses to injury (like tolerance and compensation) are both an evolutionary expression of plants to herbivory and a basis for genetic manipulations of plants to improve tolerance to insects.

The question of intrinsic versus extrinsic factors is important in placing our findings here in the context of other research. The absence of effects in response to grasshopper injury agrees with much previous work on gross tissue removal (rapid loss of leaf tissue) by various insects on various plant species (e.g., Poston et al. 1976, Hammond and Pedigo 1981, Ostlie and Pedigo 1984, Peterson et al. 1992, Peterson et al. 1996, Burkness et al. 1999,). The results reported here agree with findings from our on-going research of grasshopper injury to soybean (Higley, personal communication).

However, Detling et al. (1979) studied the effect of simulated grasshopper damage over the net photosynthetic rate of wheatgrass, and they reported that net photosynthesis was reduced in 31% in injured leaves (with 25% tissue removal). Perhaps this difference represents a species difference between potato and wheat, or perhaps it represents differences in extrinsic conditions under which the two experiments were conducted. For example, Hall and Ferree (1976) reported rate reductions on the gas exchange parameters with defoliation of apple, however, Peterson et al. (1996) observed no such reductions and concluded the differences in response were likely attributable to differences in plant-water status.

Assuming no systemic effects of defoliation (which can occur with injury from some sucking insects), the two potential physiological impacts of defoliation are reductions in leaf photosynthetic rates or reductions in leaf area. In soybean, it is increasingly clear that the main effect of defoliation is to reduce photosynthetic leaf area rather than reduction or enhancement of photosynthetic capacity of remaining tissue of injured leaves (Higley 1992, Peterson and Higley 1996, Haile et al. 1998a,b). Recognizing this mechanism underlying yield loss from defoliation, leads to both improved models for yield loss and an explanation for the inherent (genetically determined) ability of plants to buffer some leaf surface losses without yield loss. For example, in soybeans a leaf area index (LAI) of 3.5 at reproductive stage is considered critical for maximum yield which corresponds to about 90% of canopy light interception. But in optimal growing conditions, soybeans can achieve a LAI as high as 7.0, and could tolerate removal of half of this area without lowering yield significantly (Higley 1992).

It is not yet clear if the light interception hypothesis (Higley 1992) for understanding yield loss from defoliation applies in species other than soybean. However, one expectation for it to be valid is that most defoliators would not

intrinsically affect photosynthetic rates of remaining tissue. Our data here show that grasshopper injury to potato fits these criteria. This represents an important step in our current research to explore the applicability of the light interception hypothesis for defoliation and yield loss in potato and other species.

Because our data here agree with previous responses to defoliators detected in other plant species, these results suggest a general model of response to injury guilds can be developed. Additionally, if other potato defoliators do not affect photosynthetic rates, then multiple-species economic injury levels can be developed for potato leaf-mass consumers.

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Photosynthetic Responses of Soybean to Soybean Aphid Injury

Abstract

The soybean aphid, *Aphis glycines* Matsumara was discovered in the United States in the summer of 2000. Since that initial discovery, the aphid has spread across northern soybean production regions. In 2001 we examined the photosynthetic responses of soybeans to low densities of aphids (fewer than 50 aphids per leaf). This examination included leaf fluorescence responses, carbon isotope ratios, and photosynthetic responses estimated by carbon exchange rates of soybean leaflets. It was observed rate reductions of up to 50% on infested leaflets, including leaflets with no apparent symptoms of aphid injury (such as chlorosis). Fluorescence data indicated that the overall photochemical efficiency of photosystem II and the antennal chlorophyll complexes were not affected by the aphid injury, suggesting that the light capture and electron transfer to the reaction center of PSII was not impaired. Failure to observe any difference in carbon isotope ratios between the aphid infested leaflets and control leaflets, even when we noticed carbon exchange rates reduction, suggests that aphid injury was not sufficiently severe or had not occurred over a sufficiently long period of time to be reflected in tissue samples. These results indicate that substantial physiological impact on soybean is possible even at low aphid densities. Also, the conventional view of aphid injury acting through reductions in light harvesting reactions of photosynthesis is not supported by our findings in this system.

Key words: Insect-plant relations, fluorescence, gas exchange, photosynthesis.

The soybean aphid, *Aphis glycines* Matsumura, is a recently introduced pest into North America. First recognized in Wisconsin, Illinois, and Michigan in the summer of 2000, the aphid rapidly spread across the Midwest over the last two years. Aphid densities of thousands per plant were reported in 2001 with significant yield losses (Ostlie 2001). High aphid densities lead to stunting, incomplete canopy closure, the development of sooty mold from aphid honeydew, and even leaf chlorosis in low phosphorus soils (Ostlie 2001).

How arthropods alter plant physiological processes has been the focus of much research, particularly in soybean, *Glycine max* (L.) Merrill (e.g., Boote 1981, Pedigo et al. 1986, Welter 1989, Peterson et al. 1992, Peterson and Higley 1993, Peterson and Higley 1996, Peterson et al. 1998, Haile et al. 1999). Much of this work addresses photosynthetic responses of plants to injury. Because soybean physiology is well defined and because diverse types of arthropod leaf injury occur on soybean, we have been using soybean as a model system for examining general photosynthetic responses of plants to herbivory . Understanding the mechanisms by which photosynthesis is affected by injury is slowly emerging for various leaf-feeding insects, but responses to phloem-feeding insects (like *A. glycines*) have not been previously examined in soybean. So, examining photosynthetic response of soybean to *A. glycines* is of both considerable practical and theoretical interest.

Numerous studies have shown that aphids are responsible for changes in plant physiology in a number of crops, such as barley (*Hordeum vulgare* L.), sorghum (*Sorghum bicolor* [L.] Moench), oats (*Avena sativa* L.), and wheat (*Triticum aestivum* L.) (Burd & Elliott, 1996). However, little is known about how aphids elicit these changes and the physiological mechanisms associated with injury. Photosynthesis can be limited by a series of factors, and a growing number of techniques are now available to better understand these processes. Chlorophyll *a* fluorescence, for example, has been

used with success as an indicator for plant responses to stressors, such as unfavorable weather conditions (Havaux and Lannoye 1985, DiMarco et al. 1988, Moffat et al. 1990, Flagella et al. 1994, Janda et al. 1994), nutrient deficiency (Sun et al. 1989), salinity (Krishnaraj et al. 1993), herbicides (Harris and Camlin 1988, Ponte-Freitas et al. 1991), and insect injury (Burd and Elliott 1996, Haile et al. 1999). With this technique it is possible to evaluate the impact of insect feeding on light reactions, specifically on photosystems II and I. It happens because plants will tend to overcome imposed stresses by adjusting their photosynthetic apparatus, when facing a stressful condition. While trying to adapt, they can, for example, increase the dissipation of the incoming energy through routes others than those commonly used in normal conditions (photochemical quenching). An example of these routes are the non-photochemical quenchings (qN) and the energy dissipation as initial fluorescence (Fo) (Taiz, 1991). Then, evaluating these characteristics represents a way of assessing potential damage of stressful agents (biotic or abiotic) over plant physiological characteristics.

Carbon isotope ratios of plant tissues signify an intricate interplay between stomatal diffusion and the chemistry of carbon fixation during photosynthesis. Atmospheric carbon dioxide contains approximately 1.1% of the non-radioactive isotope carbon-13 and 98.9% of carbon-12. During photosynthesis plants discriminate against ^{13}C because of small differences in chemical and physical properties imparted by the difference in mass. Then, the analysis of tissue richness in $^{13}CO_2/^{12}CO_2$ (R) can offer an insight about if the factors which are limiting photosynthesis have a diffusional or chemical nature. It may be visualized through the analysis of the tissue $\delta^{13}C$ {[R (sample)/R(standard) – 1] x 1000} using a mass spectrometer. A more negative $\delta^{13}C$ means more ^{12}C in the tissue or lighter in mass and may be associated to limitations on the carboxylation phase of photosynthesis, possibly involving the carboxylase enzime. A more positive $\delta^{13}C$ means more ^{13}C, or heavier in mass and may be associated with

limitations on the diffusion of CO_2 to the cells, associated to stomatal restraint to CO2 acquisition (O'Leary, 1988).

The objective of this study was examine if the injury by the soybean aphid (*A. glycines* Homoptera: Aphididae) would influence the gas exchange rates, fluorescence parameters, and carbon isotope ratios of soybean leaflets. Our results document a severe impact on photosynthesis at low aphid densities, the involvement of aphids in altering chlorophyll quenching (and not the light reactions themselves as might have been expected a priori), and imply that yield losses might be possible even with relatively small populations (if photosynthetic productivity is limiting for yield).

Material and Methods

Plant Material and Insects. The study was conducted in August of 2001 at the University of Minnesota Rosemount Research and Outreach Center, Rosemount, MN. Soybeans (var. Asgrow 0901) were planted on 23 June in 0.76 m row width at 60705 plants/ha in a Waukegan silt loam soil. Plots were maintained with standard agronomic practices for southern MN, including two cultivations and one herbicide treatment of N-(phosphonomethyl)-glycine (Round-Up® Ultra) at 0.383 l/ha plus 1.36 kg ammonium sulfate sprayed on 20 July 2001. Plots were 4 rows by 8 m in length. Experimental design was a completely randomized block design of 4 replicates with three insecticide treatments (two rates of chlorpyrifos and one rate of dimethoate). These treatments provided plots with different *A. glycines* densities, specifically: low aphid density (0-10 aphids per leaflet) in chlorpyrifos (Lorsban® 4EC at 0.367 kg/ha) treated plots, intermediate density (20-30 aphids per leaflet) in dimethoate (Dimethoate 4EC at 0.1835 kg/ha) treated plots, and high aphid density (50-94 aphids per leaflet) in untreated plots. Plots were spatially separated from each other to avoid aphid movement between plots.

Insecticide treatments were applied late afternoon on 2 August 2001 under calm, sunny conditions. Plots were sprayed using a tractor-mounted CO_2 powered sprayer with flat fan nozzles (TeeJet 11003) spaced every 0.381 m and adjusted so that 100% overlap theoretically occurred 0.15 m below the canopy. Spray volume was 280.5 l/ha (30 gal/a) using a pressure of 2.95 kg/cm^2 (42 psi). Pretreatment aphid counts averaged 250/plant. Aphids were mostly found on the uppermost three leaves of the plant (61%). A potential concern in using insecticides in studies on photosynthetic responses to insect injury is that the insecticides might themselves alter photosynthetic processes. However, in previous research, Haile et al. (1999) observed no changes in soybean photosynthesis, stomatal conductance, or transpiration associated with use of carbamates, organophosphates, or pyrethroid insecticides, even at one day post application. Therefore, changes in photosynthesis observed in this study would not be associated with insecticide use.

Photosynthetic measurements and plant samples were taken on 23 Aug., 2001 at soybean stage R3-R4. Within each plot we randomly selected individual leaflets for photosynthesis, fluorescence, and carbon isotope analysis from eight plants, with the restriction that only fully expanded leaflets from the uppermost three nodes were used (to ensure leaves used were of comparable age). Infestation levels of *A. glycines* used were low (0 to 10 aphids per leaflet), intermediate (20-49 aphids per leaflet), and high (50 or more aphids per leaflet).

Photosynthesis. Photosynthetic rates estimated through gas exchange parameters ($\mu molCO_2/m^2/s$) were measured from the same plants and leaflets used in the fluorescence tests. The measurements were made using a LI-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE), with CO_2 injector and light source (to allow for stable light and CO_2 concentrations for all measurements). Photosynthetic

rates were measured at 400 ppm intracellular CO_2 concentration and 1600 ìmol photons m^{-2} s^{-1} light intensity.

Chlorophyll Fluorescence Kinetics. Chlorophyll *a* kinetic transients were measured using a OS5-FL modulated chlorophyll fluorometer (Opti-Sciences, Tyngsboro, MA). The readings were taken from the adaxial leaf surface, in the third trifoliate from the top of the plant.

Three basic tests were performed on plants: dark-adapted test (modulation intensity = 40 ìmols electrons $m^{-2}s^{-1}$; saturation intensity = 190 ìmols electrons $m^{-2}s^{-1}$; duration = 0.8 s; and detector gain = 80), light-adapted test (modulation intensity = 200 ìmols electrons $m^{-2}s^{-1}$; saturation intensity = 230 ìmols electrons $m^{-2}s^{-1}$, duration = 0.8 s; detector gain = 80; default PAR value = 1100 ìmols electrons $m^{-2}s^{-1}$), and kinetic test (modulation intensity = 180 ìmols electrons $m^{-2}s^{-1}$; saturation intensity = 200 ìmols electrons $m^{-2}s^{-1}$, duration = 0.8 s; actinic intensity = 120; far red intensity = 120 ìmols electrons $m^{-2}s^{-1}$; duration = 4.0 s; detector gain = 70; default PAR = 200 ìmols electrons $m^{-2}s^{-1}$; auto pulse interval = 20 s; auto pulse count = 10). The primary objectives of all these tests were to determine the following characteristics: F_0 (non-variable fluorescence), F_m (maximal fluorescence), F_v (variable fluorescence), qP (photochemical fluorescence quenching), qN (non-photochemical fluorescence quenching), Y (yield of photochemical efficiency of photosystem II), ETR (eletron transfer ration).

Carbon Isotope Ratios (ä$^{13}CO_2$). Soybean leaflets used for chlorophyll fluorescence kinetics and gas exchange measurements were harvested and stored in a -80° C freezer for further analyses. Carbon isotope ratio measurements in leaflets were determined following the procedures of Madhavan et al. (1991). Soybean leaflets stored at -80° C were later dried in an oven at 60° C for 48h. The dried leaflets were ground to a

homogenous powder. Finely ground leaf tissue (1-3 mg) was combusted in an on-line elemental analyzer (Heraeus, CHN-O Rapid) at 1000° C. The resulting CO_2 was cryogenically purified through a trapping box system. The purified CO_2 was analyzed by a Finnigan Delta-S isotope ratio mass spectrometer at the laboratory for isotope biodynamic, Department of Biochemistry, University of Nebraska, Lincoln, NE.

For samples measured via continuous flow, samples were compared to an acetanilide reference (-29.9‰ versus PDB). Reference gas used during the analysis had been previously calibrated against a Pee Dee Belemnite (PDB) standard. The precision of the continuous flow is about 0.3‰.

Data Analysis. The experimental protocol followed a complete randomized design (CRD) with eight test plants for each of 3 infestation levels of *A. glycines*. Data were analyzed using ANOVA and the means were compared by t-test (á=0.05) [PROC MIXED procedure of the SAS program (SAS Institute, 2001)]. Means were compared by t test.

Results

Photosynthetic rates were significantly affected by *A. glycines*. Infestation significantly reduced gas exchanges in infested plants ($F = 7.93$; df = 2, 21; $P = 0.0027$) (Table 1). With aphid densities over 20/leaflet, photosynthetic rates were considerably lower than rates of uninfested leaves.

A. glycines infestations had no significant effect on the carbon isotope ratio ($ä^{13}CO_2$) of leaflets analyzed ($F = 0.07$; df = 2, 12; $P = 0.9306$) (Table 1). Proportions of $ä^{13}CO_2$ relative to total CO_2 were consistent with observed values from other C_3 plants (O'Leary 1988). Results indicate reductions in photosynthesis were not a consequence of diffusion (stomatal) limitations.

The impact of different *A. glycines* infestations on the chlorophyll fluorescence for each test is shown in the Table 1. *A. glycines* had no significant effect on the photochemical efficiency of the photosystem II (F_v/F_m), when comparing the three infestation levels ($F = 0.89$; df = 2, 21; $P = 0.4254$). However, differences in *A. glycines* densities were associated with significant differences in the non-variable fluorescence (F_o) ($F = 14.44$; df = 2, 21; $P = 0.0001$). The moderate and high infestation levels had greater Fo values. Maximal fluorescence (F_m) differed among treatments ($F = 10.20$; df =2, 21; $P = 0.0008$), with high and intermediate infestation levels having significantly higher F_m values as compared with low infestation level. No significant differences were observed between high and intermediate infestation levels ($t = -2.02$; df = 21; $P = 0.0567$). Aphid infestations also were responsible for significant differences in the variable fluorescence (F_v) ($F = 16.44$; df = 2, 21; $P < 0.0001$). F_v increases also were observed when comparing high and intermediate against low infestation levels.

Both quenching coefficients were significantly affected by *A. glycines* feeding. The coefficient of photochemical fluorescence quenching (qP) was significantly affected by the aphid infestations ($F = 4.57$; df = 2, 27; $P = 0.0195$). However, the difference was only observed when comparing the high against the intermediate infestation levels ($t = 2.99$; df = 27; $P = 0.0059$). Non-photochemical fluorescence quenching (qN) also was affected by aphid density ($F = 613.4$; df = 2, 27; $P < 0.0001$), with low aphid density leaves having lower qN's than other treatments.

The yield of photochemical efficiency of photosystem II (Y) was not significantly affected by infestation level ($F = 1.28$; df = 2, 21; $P = 0.2985$), nor was electron transfer ratio (ETR) ($F = 2.53$; df = 2, 21; $P = 0.1041$).

Table 1. Summary of mean values for photosynthetic capacity (ìmolCO_2 $m^{-2}s^{-1}$), Carbon Isotope Ratios (ä$^{13}CO_2$), and chlorophyll *a* fluorescence induction transients measured at three infestation levels (high, intermediate, and low) of *A. glycines* on soybean leaves. Means in the same row followed by the same letter are not significantly different by Student's t test at $P>0.05$.

	Infestation Levels		
	High	Intermediate	Low
	Photosynthetic Capacity		
ìmolCO_2 $m^{-2}s^{-1}$	10.5 b	11.5 b	19.4 a
	Carbon Isotope Ratios (ä$^{13}CO_2$)		
‰ (per mil)	-28.723 a	-28.806 a	-28.701 a
	Chlorophyll *a* Fluorescence		
F_o [1]	91.875 b	104.63 a	75.25 c
F_m [2]	599 a	616.38 a	487.25 b
F_v [3]	467.13 b	511.75 a	412 c
F_v/F_m [4]	0.8339 a	0.83 a	0.844 a
qP [5]	0.996 a	0.951 b	0.9797 ab
qN [6]	0.3069 a	0.1199 b	0.025 c
Y [7]	0.7541 a	0.724 a	0.7137 a
ETR [8]	55.155 a	58.418 a	59.62 a

[1]non-variable fluorescence; [2]maximal fluorescence; [3]variable fluorescence; [4]variable fluorescence/maximal fluorescence ratio; [5]photochemical fluorescence quenching; [6]non-photochemical fluorescence quenching; [6]yield of photochemical efficiency of photosystem II; [5]eletron transfer ration.

Discussion

The observed reductions in gas exchange associated with aphid feeding (about 50% at aphid densities over 20/leaflet) were surprising. Experimental leaves were asymptomatic (without visible chlorosis or sooty mold), and aphid densities in this study were at least an order of magnitude (tens/leaflet versus hundreds/leaflet) lower than densities reported in severe infestations. Carbon isotope ratios of plants reflect an integrated balance between stomatal diffusion and carboxylation. Reduction in gas exchange of leaflets as a consequence of aphid injury would have altered the carbon isotope ratios of those leaflets. Failure to observe any difference in carbon isotope ratios between the aphid infested leaflets and control leaflets suggests that aphid injury here was not sufficiently severe or had not occurred over a sufficiently long period of time to be reflected in tissue samples. Given the relatively low aphid densities observed in this study, too short a duration of aphid infestation seems the most likely explanation for the lack of differences in carbon isotope ratios between infested and control leaves.

Previous work on how aphids alter plant photosynthesis has indicated that inhibitions occur prior to CO_2 assimilation with rubisco (ribulose-1,5-bisphosphate carboxylase). For example, Haile et al. (1999) reported that limitations in photosynthetic efficiency of wheat injured by Russian wheat aphid, *Diuraphis noxia* (Mordvilko), were not related to the CO_2 assimilation phase. Similar results were also reported for greenbug, *Schizaphis graminum* (Rondani), by Ryan et al. (1987) when A-Ci curves from injured and non-injured wheat plants were compared. Increasingly, attention is focused on aspects of photoelectron transport (e.g., Burd and Elliot 1996). Our examination of leaf fluorescence in aphid-injured soybeans provides a means of illuminating the mechanism behind this drop in photosynthetic rates.

The negative impact of *A. glycines* on gas exchange, as demonstrated in Table 1, led us to questions which mechanism or group of events would be involved in triggering

this phenomenon. In evaluating potential causes underlying reductions in photosynthesis, an initial distinction must be determined between stomatal limitations in CO_2 availability versus mesophyll limitations. The two broad possibilities in mesophyll limitations are reductions associated with light harvesting complexes (light reactions) and/or CO_2 fixation (dark reactions). Regarding light harvesting, factors to be considered are the integrity of reaction centers (PSI and/or PSII), in which light is captured and electrons are transferred along the photosystems yielding energy and substrates for CO_2 fixation. The amount and activities of rubisco normally regulate photosynthetic carbon fixation. Fluorescence measurements provide an indication of function of light harvesting and photoelectron transport. Measurements of photosynthesis under varying internal CO_2 concentrations and measures of stable carbon isotope ratios can both be used to differentiate stomatal from mesophyll limitations and to indicate impaired CO_2 fixation.

Non-variable fluorescence is indicative of the integrity of the photosynthetic reaction centers; an increase in non-variable fluorescence indicates that light energy is being lost, rather than passed through the photoelectron transport chain. In this study, increases in non-variable fluorescence (F_o) at high and intermediate aphid densities suggest that *A. glycines* injury may cause photo-inhibitory damage in the photosystem II reaction centers. However, the overall photochemical efficiency of photosystem II was not affected by aphid injury, so at our observed aphid densities the injury to photosynthetic reaction centers does not seem to be sufficiently severe to measurably alter photoelectron transport.

Burd and Elliott (1996) reported that stressed, susceptible varieties of wheat, *Triticum aestivum* L. by Russian wheat aphids showed increased F_o, indicating an interruption in electron transfer through photosystem II and a possible reduction in D1 protein synthesis. Limited capacity in synthesis of protein D1 can lead to an over-

reduction on the electron acceptor (Q_A) raising the activity of oxygen species, which may damage the thylakoid membrane (Mishra and Singhal 1992), and bleach chlorophyll and carotenoids through generation of chlorophyll triplets in the pigment-protein complex (Barry et al. 1990). This chlorosis is observed in wheat infested with *D. noxia*, but we did not observe chlorosis in *A. glycines* infested plants in this study, suggesting that the primary site of damage for *A. glycines* is not related to D1 protein site as occurs with *D. noxia* on wheat.

F_v/F_m ratios indicate the efficiency of the photochemical system; specifically, how much light energy captured is being used by the reaction center and propagated through the photoelectron transport chain. F_v/F_m ratios were not significantly affected by *A. glycines* injury because of proportional increases in both maximal fluorescence (F_m) and variable fluorescence (F_v) of the test plants. These findings indicate that *A. glycines* injury does not affect the antennal chlorophyll complexes, thus the light capture and electron transfer to the reaction center of PSII is not a limiting factor.

The overall photochemical quantum yield of PSII (Y) is a good indication of the efficiency in light utilization, i.e., how efficiently absorbed photons are converted into chemical products (Malkin and Niyogi 2000). In photosynthetic systems under optimal conditions, the measured quantum yield of photochemistry is approximately 1, indicating that other decay routes do not occur to any substantial extent and that almost all absorbed photons are used for photochemical charge separation. However, in general this value is around 0.8, which is closer to our values. We did not observe significant effects of aphid feeding on Y, suggesting that aphids were not responsible for loss in efficiency in charge separation.

Another parameter to be considered when looking at the photochemical efficiency of PSII is the electron transfer ratio (ETR). The ETR represents the apparent photosynthetic electron transport rate in ìmols electrons $m^{-2}s^{-1}$, which is calculated

based on the values of yield (Y) and PAR (ìmols quanta $m^{-2}s^{-1}$). Our data indicate that the ETR values for the tested plants were not affected by the aphid injury.

Changes in the quenching coefficients, qP and qN, suggest that aphid feeding may influence the photoprotective xanthophylls cycle altering the thylakoid membrane pH gradient. This cycle plays an important role protecting photosystem II under excess light conditions during abiotic and biotic stresses by dissipating excess excitation as heat (Horton et al. 1994, Gilmore et al. 1995, Demming-Adams et al. 1996, Yamamoto and Bassi 1996). Changes in trans-thylakoid pH might compromise synthesis of zeaxathin by the xanthophylls deepoxidase enzyme, which could lead to increased formation of triplet state chlorophyll and singlet state oxygen, thereby decreasing the efficiency of photosynthesis (Malkin and Niyogi 2000).

In total, fluorescence data indicate that aspects of photoelectron transport, mainly those related to non-photochemical quenching, may be a limiting factor for photosynthetic efficiency in plants injured by *A. glycines*. Because chlorosis can occur from *A. glycines* injury at higher aphid densities on low potassium soils (Ostlie 2001), additional secondary effects of injury at higher aphid densities would be expected. However, our data demonstrate that significant reductions in photosynthesis from *A. glycines* injury can occur before leaves are symptomatic and do not seem to involve impaired light reactions.

Results reported here illustrate that injury by *A. glycines* can profoundly impact soybean physiology, even at low densities. The combination of gas exchange, $ä^{13}CO_2$, and fluorescence data offer important insights into the proximate impact of *A. glycines* on soybean physiology. Our evidence suggests that biochemical mechanisms for restoring chlorophyll to a low energy, light receptive state (quenching) may be more immediately impaired by aphid feeding. This finding is valuable in focusing additional

research efforts and may provide a physiological target for developing aphid resistant soybeans.

Clearly additional research on mechanisms underlying photosynthetic responses to soybean to *A. glycines* injury is needed. Beyond further work regarding the role of aphid injury on chlorophyll quenching, studies detailing soybean responses across soybean phenological stages and at different aphid intensities are needed. Also, the remarkable reduction in photosynthesis observed here at low aphid densities implies that yield losses from *A. glycines* might occur at lower aphid densities than might otherwise be anticipated, at least under those circumstances in which photosynthetic productivity is limiting for yield.

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To submit:

Agricultural and Forest Entomology

Ecological stress of aldicarb on soil arthropods associated with coffee plantations.

Abstract 1. Soil arthropods have been considered by various authors as a good indicators of environmental effect of pesticides.

2. This study aimed to evaluate the impact of a systemic insecticide (aldicarb) on soil arthropods associated with coffee plantations.

3. The experiment encompassed three treatments (control, 1 application – 10 g of Temik 150 (aldicarb)/plant applied in 16/12/1999 and two applications - 10 g/plant applied in 16/12/1999 and 10g/plant applied in 01/03/2000) and four blocks, evaluated once before insecticide application and at 13 different days after the insecticide application. It was measured the soil arthropod densities found in berleze traps and the data was submitted to multivariate techniques. The days of evaluation were considered as treatments replicates through time and the generated data set was analyzed by canonical variate analyses and multivariate repeated analysis.

4. Aldicarb effect on the assemblage of soil arthropods was significant and the main specie impacted was mite MfSp4.

5. As the variation on the abundance of the coffee soil arthropods community could not be explained by the variation in weather conditions, it is suggested that the insecticide was the major factor causing the effects described previously

Keywords soil inner arthropods, canonical variate analysis, multivariate repeated analysis, systemic insecticide.

Introduction

The coffee plants are attacked by a great number of insect-pests which leads to the adoption of control tactics. Chemical control is the most used and widespread tactic, due to its fast effect. Aldicarb is among the most used insecticides due to its effects over many coffee pests as the coffee leafminer, the coffee cicadas and the coffee nematodes (Andrei, 1999). The product has systemic activity and it is commercialized as granules that once applied to the soil are taken up by the roots moving across the nutrients route (through xylem) throughout the plant and exerting its insecticidal activity in the biomodified form (Matsumura, 1985a). Once in the insect body, it couples to acetylcholinesterase which can not break acetylcholine anymore leading to an accumulation of the latter in the synapses which causes the nervous system to break down (Matsumura, 1985b).

Aldicarb granules are used applied after the first showers and once in the soil, it may give rise to many degradation products. Among these, two oxidation metabolites (aldicarb sulfoxide and aldicarb sulfone) are as potent inhibitors of acetylcholinesterase activity as aldicarb itself (Fava *et al.*, 2001).

A single application of the insecticide provides a good protection against the the pests for which it is registered for control (the coffee leafminer, cicadas, and nematodes) (Rigitano *et al.*, 1989).

Aldicarb is one of the pesticides with the highest toxicity to mammals (Rigitano *et al.*, 1989), but little is known about its effect on the non-target arthropods also subjected to its effects, as soil arthropods.

In the last decades, quite a body of literature has been developed on the use of community parameters to assess effects of anthropogenic impact on ecosystems, especially that of pollutants (Koehler, 1992). These approaches have been rising in terms of importance due to environmental concerns which, in addition to the possibility

of losing many compounds presently available to pest control, has lead the register agencies to become tighter concerning the registration approval procedures.

These renewed registration requirements led to the improvement of the approaches currently available to assess the impact of chemical control agents on non-target organisms. In this sense, the commonly used selectivity tests that aimed to evaluate the effect of insecticides on natural enemies has been gradually replaced by more accurate approaches as the sequence laboratory/semi-field/field proposed by the IOBC (International Organization for Biological Control) and the WPRS (West Paleartic Regional Section). These approaches can be considered more accurate since they already input some level of interaction in the studies as predator or parasitoid or pest/plant interactions. However, it still does not account for the interaction among the organisms that constitutes the community target with insecticide application (Hassan, 1989). This is why the ecotoxicology approach emerged as a better way to assess pesticide impact upon non-target organisms, since it takes into account all the possible interactions level (pest/host/natural enemies/environment).

The techniques available for monitoring the ecological effects of toxicants tend to cover the fields of bioaccumulation, biotransformation, biodegradation, biochemical monitoring, physiology and behavior, population parameters, community parameters and ecosystem effects (Scott & Clarke, 2000). But if one thinks about increasing the level of organization covered by the approach (as changing from population to community parameters), it is right to say that the higher status approach will absorb the lower status approach which ends saving effort spent in the evaluations and, in many cases, achieving greater accuracy.

When thinking about increasing the level of complexity to study a phenomenon, we still need to come up with a good tool to analyze the large data set that will be generated. In the case of ecotoxicology, the sort of response one is going to get are some

response variables or effects on the ecological community that are caused by the explanatory variables or environmental effect. The distinguishing feature of all such multi-attribute surveys is that they record more than one response variable. Each response variable could be analyzed separately using the statistical procedures for univariate responses. However, if there are a large number of attributes, with each analyzed separately, the summary of results is often repetitive and difficult to synthesize. Moreover, variables can be missed by analyzing each variable in turn. Analyzing all responses together can often lead to more powerful statistical tests for the combined effects of pollutants and other environmental factors. What would be a limitation for the usage of multivariate techniques before, is not anymore: suitable software and hardware development made possible the manipulation of large data matrices, allowing the spread and usage of the techniques in applied research (Kennedy *et al.*, 1999; Maund *et al.*, 1999; Clarke, 1999; Sparks *et al.*, 1999; Kedwards *et al.*, 1999a,b).

The main goal of this study was to study the impact of Aldicarb over the inner soil arthropod dwellers of coffee plantation using multivariate techniques to evaluate it.

Materials and Methods

The study was carried out in a coffee field of the Federal University of Viçosa, Agriculture Department, placed in Viçosa County, State of Minas Gerais, Brasil, between 12/20/1999 to 05/31/2000.

The treatments were control, 10 g of Temik 150 (aldicarb)/plant and 20 g of Temik 150 (aldicarb)/plant, established in a completely randomized block design with four replicates. The dose of 20 g/plant was applied at two different times: 10 g/plant were applied in the same day the other treatments were set up (16/12/1999) and 10 g/plant was applied 77 days after the first application (04/03/2000). The application was

carried out using a seed spreader regulated to drop 2.5 g of insecticide/point. Two points were placed on the east side of the rows and two points placed on the west side of the rows. Each plot was constituted by 200 coffee plants, variety 'Catuaí Vermelho', aged between 7 to 8 years, spaced by 0.90 m between rows and 2.0 m between plants.

Before the experiment was established, one soil sample of each plot was taken out and the soil characteristics analyzed being the data subjected to comparisons by ANOVA and Tukey test at 0.05. The arthropod community was also sampled from all the plots before the experiment was established. After the insecticide application, the plots were sampled in the following dates: 20/12/99, 22/12/99, 04/01/00, 26/01/00, 22/02/00, 03/03/00, 08/03/00, 15/03/00, 05/04/00, 19/04/00, 03/05/00, 17/05/00 and 31/05/00.

During all the experimentation, the weather conditions (temperature - maximum, minimum and average, relative humidity and precipitation) were daily assessed through a Meteorological Experimental Station owned by Federal University of Viçosa.

The arthropod community was sampled using Berleze traps which were taken out using a scoop tool. We delimited the soil sampled in order to accomplish an area of 20 cm (width) x 20 cm (height) x 20 cm (depth) which would constitute a composed sample that we would keep a fraction of approximately 2 Kg. The samples were stored in plastic bags properly identified and brought to the laboratory where they were placed in funnels containing a screen at the bottom and a cup filled with ethanol (70%) placed at the end of the funnel. The samples were left there for 24 hours after the placement and by the end of this period they were taken away and stored in the laboratory for future evaluation.

In order to evaluate the diversity of the arthropod community, the samples in alcohool solution were poured over Petri dishes and the number of morphological species was quantified under a stereomicroscope (32x of enlargement). As we

quantified the species, we kept some of them to be, later on, sent to taxonomists for an accurate identification. The relative frequencies of the arthropods were calculated.

The data was analyzed considering the days of sampling as a replicate of treatments through time (resulting in the comparison of three treatments). The data set were first submitted to the SAS (2001) STEPDISC PROCEDURE in order to get a group of species that would account for the maximum explained variance, and then it was subjected to the canonical variate analysis. The species selected by STEPDISC PROCEDURE and sampled before aldicarb application were divided in two groups (mites and insects) and they were submitted to multivariate analysis of variance in order to study if the different plots behaved similarly considering the arthropods abundance. The species selected by the SAS (2001) STEPDISC PROCEDURE and sampled four (20/12/99), 19 (04/01/2000), 41 (26/01/2000), 77 (03/03/2000), 89 (15/03/2000), 124 (19/04/2000), and 152 (17/05/2000) days after aldicarb first application were subjected to repeated measures (multivariate) analysis of variance because the arthropod samplings were carried out on the same replicates (fields) at several times (Green, 1993; Paine, 1996), avoiding the problems of "pseudoreplication" in time (Hulbert, 1984; Stewart-Oaten *et al.*, 1986; Green, 1993). These analyses were carried out using the procedure PROC ANOVA from SAS (2001) with the PROFILE statement, as suggested by Von Ende (1993). Figures on the mean abundance ± standard error/treatment of arthropods that most contributed to the canonical axis which was significant were built. Additionally, canonical correlations were run between the weather variables and the mite Mfsp4 densities including just the sampling days after insecticide application in the analysis.

Results

Table 1 shows the results of soil analysis of the control and treated plots before the insecticide application. It was found significant differences among the plots for the levels of organic matter. The control plots and the plots treated once did not differ between their levels of organic matter, however the plots treated twice showed the lowest content of organic. It was not detected significant differences among the plots for the remaining soil characteristics.

Table 1. Means ± standard error of the soil characteristics as a function of plots that composed the experimental area. Viçosa, MG. 1999/2000.

Treatment	pH[1]	O.M.[2]	BS[3]	t[4]	T[5]	V[6]
Control	6.12 A	4.67 A	4.8 A	5.87 A	8.72 A	63.02 A
1 application	6.07 A	4.82 A	5.52 A	5.4 A	10.07 A	55.47 A
2 applications	6.30 A	3.6 B	4.35 A	4.5 A	8.92 A	55.47 A

[1]hydrogenionic potential (water); [2]organic matter (dag/kg); [3]basis sum ($cmol_c/dm^3$); [4]effective cationic exchange capacity ($cmol_c/dm^3$); [5]total cationic exchange capacity ($cmol_c/dm^3$); [6]basis saturation (%).

* The means followed by the same letter do not differ among them according to Tukey test at $p \geq 0.95$.

It was not detected any significant difference ($p>0.05$) among treatments on the multivariate analysis of variance run for mites (Wilk's Lambda value = 0.44126762, F value = 0.76, df den/num = 12, 8, and p value = 0.6444) and insects (Wilk's Lambda value = 0.02217025, F value = 1.43, df den/num = 4, 16, and p value = 0.3967) sampled before aldicarb application. Table 2 shows the frequencies of arthropods found in berleze traps throughout the experiment. The most frequent arthropods (frequency > 50%) on the area were Acarinae: morphospecies (Mfsp) 1, Mfsp2, Mfsp3, Collembola: Isotomidae and Formicidae: *Hypoponera* spp., while the less frequent (frequency > 10%) were Formicidae: Mfsp4 and Mfsp5. Table 3 presents a summary of the STEPDISC PROCEDURE run to recognize the arthropods species which would constitute the data set used in the canonical variate analysis. According to this procedure, variables are chosen to enter or leave the model according to one of two criteria: a) the significance level of an F test from an analysis of covariance, where the variables already chosen act as covariates and the variable under consideration is the dependent variable, or b) the squared partial correlation for predicting the variable under consideration from the CLASS variable, controlling for the effects of the variables already selected for the model (SAS, 1994). In our case, the species chosen to stay on the model were selected based on the significance of the squared partial correlation ($p\leq0.01$).

Table 2. Relatives frequencies (%) of the major arthropod species found in soil traps (berleze funel) set up in coffee plots non-treated, treated once and treated twice with aldicarb and sampled in 13 different days. Viçosa, Minas Gerais, 1999/2000.

Taxa	Captures Frequency (%)[1]			Frequency on the areas (%)[2]
	Control	Treated area (Once)	Treated area (Twice)	
Acarinae				
Mfsp1*[3]	84.62	92.31	92.31	89.74
Mfsp2*	78.85	84.62	84.62	82.69
Mfsp3*	23.08	23.08	30.77	25.64
Mfsp4*	15.38	15.38	15.38	15.38
Mfsp5*	26.92	30.77	30.77	29.49
Mfsp6	15.38	17.31	23.08	18.59
Mfsp7	53.85	55.77	61.54	57.05
Mfsp8	11.54	15.38	15.38	14.10
Mfsp9	9.62	15.38	15.38	13.46
Collembola				
Isotomidae*	53.85	57.69	61.54	57.69
Entomobryidae*	23.08	23.08	30.77	25.64
Mfsp1	46.15	46.15	51.92	48.07
Mfsp2	7.69	15.38	15.38	12.82
Coleoptera				
Staphylinidae*	30.77	30.77	34.62	32.05
Mfsp1	13.46	15.38	15.38	14.74
Mfsp2	21.15	23.08	23.08	22.44
Mfsp3	19.23	23.08	23.08	21.79
Diptera				
Sciaridae*	23.08	23.08	30.77	25.64
Formicidae				
Hypoponera spp.*	53.85	53.85	61.54	56.41
Solenopsis sp., *Hylomyrma* sp. and *Pheidole* sp.*	30.77	34.62	38.46	34.61
Mfsp1	15.38	15.38	17.31	16.02
Mfsp2	15.38	15.38	15.38	15.38
Mfsp3	53.85	53.85	57.69	55.12
Mfsp4	7.69	7.69	13.46	9.62
Mfsp5	5.77	7.69	7.69	7.05
Larvae				
Diptera: Stratiomyidae*	7.69	9.62	15.38	10.90
Coleoptera: Nitidulidae*	23.08	23.08	25.00	23.72
Mfsp1	7.69	7.69	15.38	10.26
Mfsp2	15.38	23.08	23.08	20.51
Mfsp3	15.38	15.38	19.23	16.67
Mfsp4	23.08	28.85	30.77	27.56
Heteroptera				
Mfsp1	7.69	11.54	15.38	11.54

[1]Number of times that the species occured on the traps within the area/total traps on the area x 100.

[2]# of times that the species occured on the area /total number of sampling x 100.

[3]MfSp – Morphspecies.

*Species included in the analysis.

Table 3. Summary of the ordination procedure run to select arthropod species found in berleze traps that would be included in the canonical variate analysis. Viçosa, Minas Gerais, 1999/2000.

Step	Entered	Removed	Correlation	Prob > Average Squared Canonical Correlation
1	Acarinae: Mfsp4[1]		0.01379191	<0.0001
2	Acarinae: Mfsp2		0.02412385	<0.0001
3	Collembola: Entomobryidae		0.03402311	<0.0001
4	Diptera: Sciaridae		0.04279737	<0.0001
5	Acarinae: Mfsp3		0.05247161	<0.0001
6	Acarinae: Mfsp5		0.06161929	<0.0001
7	*Solenopsis* sp., *Hylomyrma* sp. and *Pheidole* sp.		0.07064199	<0.0001
8	Acarinae: Mfsp1		0.07867988	<0.0001
9	Coleoptera: Staphylinidae		0.08484792	<0.0001
10	Collembola: Isotomidae		0.09307826	<0.0001
11	Diptera: Stratiomyidae		0.10061114	<0.0001
12	*Hypoponera* spp.		0.10764168	<0.0001
13	Coleoptera: Nitidulidae		0.11550484	<0.0001

[1]Mfsp – morphspecies

The combination of treatments with days of sampling (as represented in Fig. 1) resulted in only one axis significant at $p \leq 0.05$, which explained approximately 74% of the total variance of the data set (Table 4). The species which most contribute to the axis composition, based on the higher absolute value of the raw canonical coefficient, were mite Mfsp4, mite Mfsp5, Diptera: Sciaridae, and Coleoptera: Staphylinidae (Table 4).

Table 4. Estimative of the eigenvalues, cumulative explained variance, significance of canonical axes, and raw canonical functions of axis 1 for arthropods in control and treated plots with Aldicarb as represented in Figure 1. Viçosa, Minas Gerais, Brazil. 1999/2000.

Species	Raw Canonical Coefficients	
	1st axis	2nd axis
Acarinae: Mfsp1	0.00230098	-0.00000779
Acarinae: Mfsp2	-0.02986482	0.14614259
Acarinae: Mfsp3	0.02876913	0.00388610
Acarinae: Mfsp4	0.62440236	-0.14662481
Acarinae: Mfsp5	0.37850543	0.19999794
Collembola: Isotomidae	-0.07446021	0.07253921
Collembola: Entomobryidae	-0.05146952	0.01581355
Diptera: Sciaridae	-0.31284257	0.40833829
Hypoponera spp.	-0.06835745	-0.10394076
Solenopsis sp., *Hylomyrma* sp. and *Pheidole* sp.	0.05096869	-0.03893431
Diptera: Stratiomyidae	0.17037868	0.10850321
Coleoptera: Nitidulidae	-0.11918138	0.12862483
Coleoptera: Staphylinidae	0.36804091	-0.07243852
Eigenvalues	0.2937	0.1053
Cumulative Explained Variance	0.7362	1.000
P	0.0021	0.2758

[3]Based on the approximate F test

Figures 1 shows the dispersion of the treatments achieved by the 1st canonical axis composed by scores of the arthropods density. According to the dispersion of treatments and superposition of confidence limits, the control plots differed from plots that received one and two applications, but the later two did not differ between them.

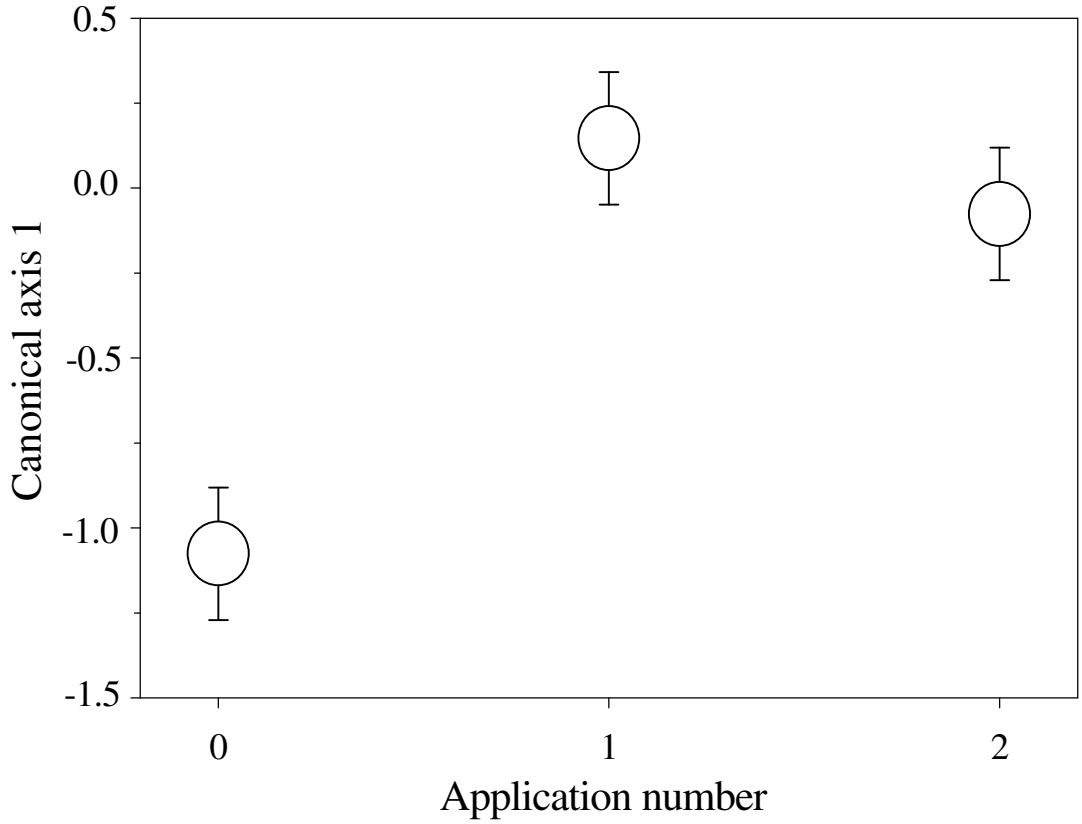

Figure 1. Canonical variates plot of arthropod density data grouped by treatment (control, one application of Aldicarb and two applications of Aldicarb/plant). Viçosa, MG, 1999/200.

The repeated-measures analysis of the main four species contributing to the first canonical axis shows significance only for the Acarinae: Mfsp4 and for Coleoptera: MfSp1 (Table 5). Considering the between-subject factor there was insecticide effect on Acarinae: Mfsp4 ($p<0.05$) and block effect on the Coleoptera: Staphylinidae. On the within-subject factor, there was significant effect of time ($p<0.05$) and time x insecticide interaction for Acarinae: Mfsp4.

Table 5 – Multivariate analysis of variance with repeated measurements on the abundance of the taxa with the higher raw canonical functions associated with the 1st canonical axis of the canonical variate analysis. Effects are computed either (a) between subjects or (b) within subjects.

Acarinae: Mfsp4					
(a) Between subject					
Sources of variation		F-values	df		p
Blocks		2.83	3		0.1290
Insecticide		7.96	2		0.0205*
Error		-	6		-
(b) Within subjects					
Sources of variation	Wilks' Lambda	F-values	Num df	Den df	p
Time	0.05072283	14.04	4	3	0.0277*
Time x Blocks	0.04787789	1.48	12	8.2288	0.2928
Time x Insecticide	0.00710531	8.15	8	6	0.0099**
Acarinae: Mfsp5					
(a) Between subject					
Sources of variation		F-values	df		p
Blocks		0.22	3		0.8820
Insecticide		0.83	2		0.4788
Error		-	6		-
(b) Within subjects					
Sources of variation	Wilks' Lambda	F-values	Num df	Den df	p
Time	0.13838353	2.49	5	2	0.3109
Time x Blocks	0.04852232	0.79	15	5.9225	0.6725
Time x Insecticide	0.08291271	0.99	10	4	0.5534
Diptera: Sciaridae					
(a) Between subject					
Sources of variation		F-values	df		p
Blocks		0.62	3		0.6274
Insecticide		0.61	2		0.5760
Error		-	6		-
(b) Within subjects					
Sources of variation	Wilks' Lambda	F-values	Num df	Den df	p
Time	0.07978723	1.92	6	1	0.5021
Time x Blocks	0.03552887	0.41	18	3.3137	0.9040
Time x Insecticide	0.07054284	0.46	12	2	0.8431
Coleoptera: Staphylinidae					
(a) Between subject					
Sources of variation		F-values	df		p
Blocks		6.33	3		0.0274*
Insecticide		2.78	2		0.1400
Error		-	6		-
(b) Within subjects					
Sources of variation	Wilks' Lambda	F-values	Num df	Den df	p
Time	0.07733277	1.99	6	1	0.4952
Time x Blocks	0.02539083	0.49	18	3.3137	0.8597
Time x Insecticide	0.00609224	1.97	12	2	0.3859

Figure two shows the mean density of Acarinae: Mfsp4/trap ± mean standard error (SEM) as a function of days after aldicarb application. There was a significant effect of treatments on the abundance of mite Mfsp4 four and 152 days after aldicarb first application ($p < 0.05$) (Figure 2).

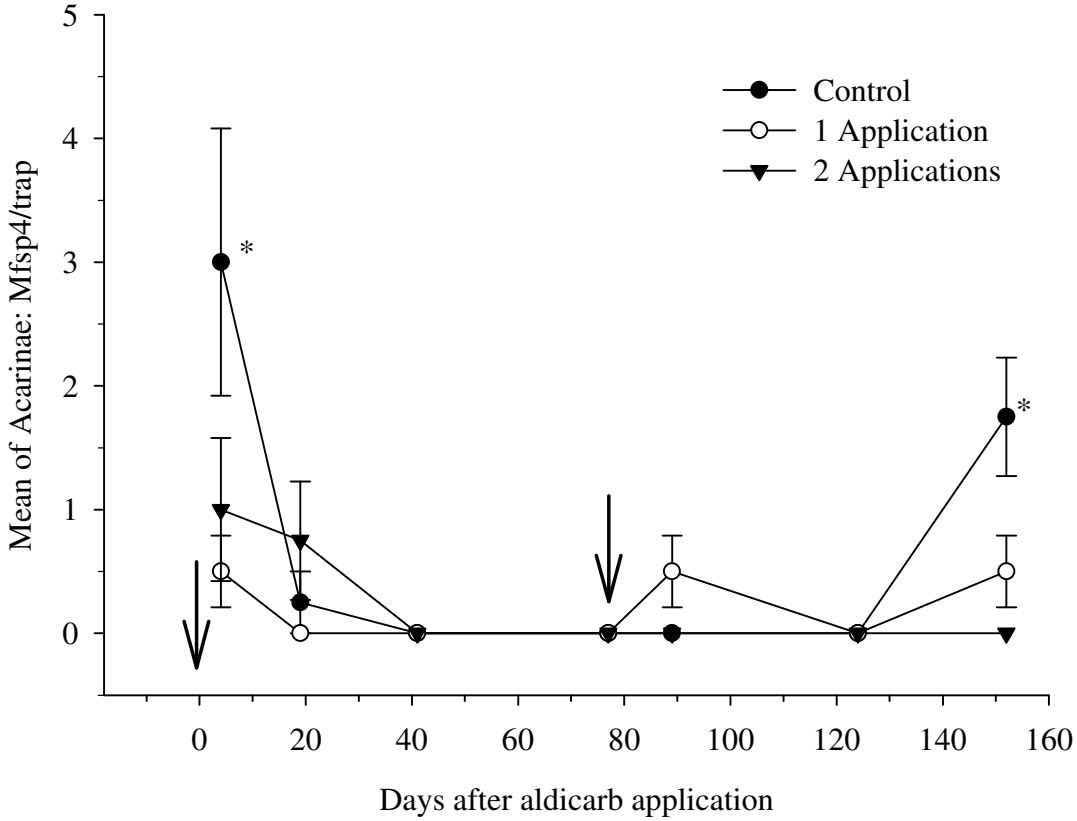

Figure 2 – Variation of Acarinae: Mfsp4 abundance associated to soil cultivated with coffee subjected to aldicarb application or not. Symbols represent the average results of four replicate and the vertical bars indicate the standard errors of the means. Arrows mean where 1st and 2nd Temik 150 application were done.

The (partial) canonical correlation analysis run between the Acarinae: Mfsp4 densities and the weather variable was not significant at $p \leq 0.05$ ($p=0.1165$). Despite the absence of significance, the highest standardized canonical coefficient associated to the canonical axis was for relative humidity (-1.4571). Besides, it was the weather variable that showed the highest canonical correlation coefficient when correlated with Acarinae: Mfsp4 abundance (-0.5091).

Discussion

Some authors have used the frequency information to choose the species which will compose the data set to be statistically analyzed, choosing the higher frequents (Michereff Filho *et al.*, 2002). But we considered the step disc procedure as a better approach, since it keeps variables that accounts for the maximum variance, using statistical tests (F test) and the squared partial correlation to include or discard variables from the data set.

Comparing the two tables it can be seen that choosing the species based on higher frequency would lead to the selection of another set of data. But it would not guarantee that the new data set would account for the variances (high and low) existed in the original data set, biasing the analysis. The selection based on the stepdisc procedure otherwise, included species with frequencies in the area as low as 10.9% and as high as 89.74% (Table 3 and 4) what summarized better the variances of the original data set.

We chose to use the squared partial correlation as a criterion to select species which would compose the data set to be analyzed because there are some errors associated with the selection of variables based on the significance of F test. One of them is that increasing the sample size can lead to increase the number of variables selected by this criteria while it has little effect on the number selected using squared partial correlations (SAS, 1994).

The dispersion of treatments on the first canonical axis suggested a significant effect of aldicarb application over the community of coffee soil arthropods. However, unless we could detect such impact when we analyzed the most important species as suggested by the absolute raw canonical functions, we could find significant effect of aldicarb application only over one species (Acarinae: Mfsp4) what may suggest that the effect was subtle. Koeler (1992) stated that unless the common dosages used in most

studies of this nature are in accordance with the agricultural practice (Edwards & Lofty, 1971; Jones *et al.*, 1987), the high dosage use would be preferable in ecological investigations of long-term effects of a single chemical impact. If we think about aldicarb, this recommendation is even more adequate, considering that among carbamates and other systemic insecticides Aldicarb is the compound that presents the highest solubility in water (3.2×10^{-2} M) (Wei *et al.*, 2001). Moreover, aldicarb and its degradation products (aldicarb sulfoxide and aldicarb sulfone) have a low Koc (carbon/water ratio concentration) and DT50 (persistence) indicating that they are very mobile in soil and degrade easily (Fava *et al.*, 2001). In an experiment established in an artificial area and not subjected to the effects of rainfall, it was found that more than 2/3 of the aldicarb is metabolized within a week and beyond detectability within 10 weeks. Its metabolites (aldicarb sulfoxide and aldicarb sulfone) appear 1 week after contamination and reach their maximal concentrations after 4 weeks (Koehler, 1992).

In addition, the interval that encompassed the first product application (December, 16, 1999 to February, 28, 2000) was subjected to 484.4 mm of precipitation and 148.6 mm more after the second application (March, 1st, 200 to March, 15th, 2000), showing that the experiment was carried out at a high precipitation regime.

Unless the results of soil analysis demonstrated that the plots which received two insecticide applications had a relatively lower content of organic matter than the control plots and those ones that received one insecticide application, the magnitude of such difference was not very expressive . A good content of organic matter balanced with a pH close to neutrality is known to lead to a higher microbial activity increasing the chances of degradation (Kazumi & Capone, 1995; Fava *et al.*, 2001; Wei *et al.*, 2001). All these facts together might have contributed to faster product degradation leading to an impact of the chemical but not in the magnitude as describe by other authors (Koeler,

1992) who described aldicarb impact over other species than mites and longer persistence of such impact.

As the variations observed in the mite abundance could not be explained by weather variations (verified by the canonical correlations analysis), it can be inferred that the insecticide was the major reason to cause the effects described above.

But, it should not be forgotten that the sensitivity of soil arthropods towards an insecticide is influenced by complex ecological factors like successional status and type of vegetation. Soil fauna data provide good arguments for an indication of an anthropogenic impact, but do not allow an ecological judgment. To achieve such judgment, interdisciplinary research on the interactions of indicator compartment (s) with other compartments of the ecosystem is necessary.

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General Conclusions

- The potato gas exchange parameters were not affected by the grasshopper injury when this injury varied from 10 to 25%, indicationg that this level of injury was not high enough to affect intrinsically photosyntetic rates. This result suggested that the main effect of the injury caused was reduction in leaf area;
- It was observed soybean gas exchange reductions of up to 50% on low aphid infested leaflets, including leaflets with no apparent symptoms of aphid injury (such as chlorosis). However, the fluorescence data indicated that the overall photochemical efficiency of photosystem II and the antennal chlorophyll complexes were not affected what suggested that the light capture and electron transfer to the reaction center of PSII was not impaired. Otherwise, it seems that process involved in the non-photochemical quenching (as the photoprotective xanthophylls cycle) are primarily impacted;
- There was aldicarb impact over abundance of the coffee soil arthropods community, with the control plots being differently dispersed on the significant canonical axis when compared to the plots treated once and twice which did not differ between them. The intensity of the impact was not as high as that described by other authors since it was found significant effect of insecticide application only over the abundance of Acarinae: Mfsp4. We believe that these differences noticed were due to high precipitation regime after the product application associated to soil and insecticide characteristics.