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Relationship between *Scyphophorus acupunctatus* Gyllenhal Damage and Nutrient and Sugar Content of *Agave angustifolia* Haw

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Abstract. In Oaxaca, Mexico, *Agave* spp. is produced as the main raw matter of the mezcal (distilled alcoholic beverage) industry. Agave weevil, *Scyphophorus acupunctatus* Gyllenhal, in adult agave plants causes rot and death, which in turn affects production and quality of mezcal. Five amounts of damage by the agave weevil were analyzed in relationship to total reducing sugars and nitrogen-phosphorus-potassium nutrient content of mature *A. angustifolia* Haw plants. A significant ($p \leq 0.05$) relationship was found between the amount of damage to a mature plant and numbers of lesions (r = 0.981), adult insects (r = 0.908), and larvae (r = 0.994). Severely damaged agave plants had more lesions on the leaves (33.2) and more agave weevil larvae (40) and adults (30.5). Likewise, the greater the amount of damage, was reduced 52 % the weight of the plant, and 68% total reducing sugars. Nutrient content (NPK) in severely damaged plants were 0.7, 0.36 y 2.37 % in contrast to healthy plants was 2.8, 0.60 y 3.48 % respectively.

Introduction

In Mexico, maguey (*Agave* spp.) plants have been used as a source of food, medicine, and fiber since the pre-Hispanic era (García-Mendoza and Chiang 2003) and are the raw material for the most famous Mexican distilled beverages, tequila and mezcal. Production of *Agave* spp. has increased in Oaxaca, Mexico. Nevertheless, yield of the plants for mezcal production has decreased because of environmental problems and pests and diseases such as stem rot associated with *Pectobacterium carotovora* (Jones) Waldee (Aquino-Bolaños et al., 2011) and wilting caused by the agave weevil *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae); both are the most aggressive pests of *Agave* spp. (Kelly and Olsen 2006, Rubio-Cortés 2007) and *Yucca* spp. (Brown 2012).

S. acupunctatus ranges from the southern United States to South America, the Caribbean (Cuba and Jamaica), eastern Africa, Hawaii, Java, and Australia (Vaurie 1971). The insect can live as long as 433 days (Valdés-Estrada et al. 2010). Adult females are about 1.3 cm. With their snout, they bore into the outside of the plant and base of the fleshy leaves to deposit eggs (Knox 2010). After the whitish larvae hatch from the eggs, they bore into the core of the plant (piña) and feed on the rotting tissue (Ruiz-Montiel et al. 2003, Knox 2010). The tissues rot because pathogenic microorganisms enter the wound and rot and wilt the plant, killing it. Rot is probably caused by the gram-positive bacterium *Pseudomona* *fluosescens* Flügge biotype I that has been isolated from the surface of *S. acupunctatus* and rotted *A. tequilana* F.A.C. Weber plants (Fucikovsky 2001). Likewise, there is a positive correlation between the number of agave weevils and the degree of rotting of the plant (Solís-Aguilar et al. 2001).

Sap of *Agave* spp. plants contains sugars (García-Curbelo et al. 2009), saponins (García-Mendoza and Chiang, 2003; Debnath et al., 2010), and toxic compounds (oxalate crystals that cause rashes on the skin, mouth, tongue, and throat, De la Cueva et al. 2005; Knox 2010), among others. The high sugar content yields syrup containing vitamins, minerals, and aminoacids (Bautista and García 2001). The amount and type of sugars in agave are essential for distilled alcoholic beverages like mezcal, tequila, bacanora, and stool (García-Mendoza and Chiang, 2003). The content of reducing sugars in the piña is an indicator of quality, and thus its alteration affects the quality of the final product. The content of reducing sugars is 16 to 28% in the piña and 13 to 16% at the base of the leaves (Iñiguez-Covarrubias et al. 2001).

Before flowering, *A. tequilana* plants accumulate concentrated sugars in their sap (Bautista and García 2001). In Oaxaca, *A. angustifolia* Haw flowers after the plant is 7 years old. Agave weevils infest plants mainly during the flowering stage and in abandoned plantations (Ramírez 1993, Ruiz-Montiel et al. 2003). Different regions of the state suffer losses ranging from 1.4 to 26% of *Agave* spp. because of this insect pest (Bravo 2003); however, Aquino-Bolaños et al. (2011) reported damage from 14.4 to 46.4% only for the region of Valles Centrales de Oaxaca (Central Valleys). After flowering, when splitting the agave (mezontle, core of the piña) to extract mezcal, the producers collect the larvae known as “botija”, “chatita”, “black weevil”, or “agave weevil” to broil and use as food (Ramos-Elorduy 2006, Rumpold and Schlüter 2013). The larvae contain 32-26% raw protein, 52% fat, 6% fiber, 1% ash, and 18 aminoacids (Finke 2008). Considering the high fat content of the larvae, Manzano-Agugliaro et al. (2012) believed the species would be very good for production of biofuel.

The nutritional state affects the crassulacean acid metabolic activity of plants such as agave (Nobel 1983). Fertilization and nutrition of the plants have been studied, with greater emphasis on nitrogen, phosphorus, and potassium because these are required for development and growth (Marschner 1995, Bautista and García 2001) and productivity (Nobel et al. 1988, Nobel 1989). In *A. tequilana*, nitrogen fertilization was related to chlorophyll synthesis, development of foliar area, plant growth, production of a greater number of leaves, biomass, and growth of the radicle system (Uvalle and Vélez 2007, Díaz et al. 2011). In *A. potatorum* Zucc, nitrogen fertilization increased foliar area, soluble solid content, and plant growth (Martínez-Ramírez et al. 2012). In *A. lechuguilla* Torr., nitrogen application increased the number of leaves and CO_2 interchange ratio, while potassium improved nighttime assimilation of CO_2 (Nobel et al. 1988). Yield of *A. tequilana* doubled by nitrogen-phosphorus-potassium fertilization. Nobel (1988) found metabolic activity increased when the nitrogen content in the chlorenchyma was close to 2%, which coincides with that of other plants of economic importance. Nitrogen is emphasized as the most influential element in growth of agave and cacti. Other results from the same research showed growth of the root, as well as the aerial part of the plant, increased when the amount of nitrogen in the soil was 0.1% but decreased at greater concentrations. Nobel and Hartsock (1986), when evaluating the response of *A. deserti* Engelm. plantlets to nitrates, phosphates, and potassium, found greater increases of dry matter positively correlated with increases in nitrogen. However, nutrient deficiencies can increase pest and disease incidence (Schroth et al. 2000). The objective of this work was to determine the relationship between the degree of damage caused by *S. acupunctatus* and the amounts of reducing sugars and nitrogen, phosphorus, and potassium in *A. angustifolia*.

Materials and Methods

This study was done at Tlacolula de Matamoros in the Central Valleys of Oaxaca [16º 57´ north latitude, 96º 28´ west longitude, 1,614 m above sea level, 600-800 mm annual precipitation, mean annual temperature 18 to 22°C, subhumid warm climate] and in the Control Laboratory of the CIIDIR Oaxaca in Santa Cruz Xoxocotlán, Oaxaca. The amount of damage was determined according to the scale proposed by the Tequila Regulating Council (Consejo Regulador de Tequila 1999, Table 1).

During 1.5 years, 25 adult *A. angustifolia* plants 8 to 10 years old were checked monthly. After the plants were chosen randomly for diagnosis, they were uprooted and divided by hand into four sections. Recorded for each plant were the weight, number of *S. acupunctatus* larvae and adults, and number of lesions. With the information, the relationship between the degree of damage by the insect pest and stem rot of the agave was determined as mentioned by Solís-Aguilar et al. (2001).

A completely randomized design was used. The independent variable was the degree of damage (1 to 5), and the dependent variable was the number of weevil adults and larvae during each sampling (each sampling represented one replication). Five *A. angustifolia* plants were used for each degree of damage to determine total reducing sugars. Extracts from *A. angustifolia* were obtained by mashing the fresh samples diluted in water and shaking for 15 minutes at 80ºC. They were later cooled and diluted to 100 ml in distilled water. Total reducing sugars were determined by using the method described by the AOAC (1984).

The samples of *A. angustifolia* were taken from the top, middle, and bottom sections of the core of the piña (mezontle) and leaves as indicated by Terán (2004). Chemical analyses were done according to the method of Alcántar and Sandoval (1999). Total nitrogen was determined by the microkjeldahl method. For phosphorus and potassium, the sample was analyzed through the humid digestion method, using the tri-acid mix (sulfuric and perchloric acid).

Phosphorus was determined by the chlorimetric method: vanadate-molybdate-yellow, doing measurements in a Gilford spectrophotometer (model 260, Instrument Laboratories Inc. Oberlin, OH) at a wavelength of 470 nm. Potassium was determined with a Coleman flame spectrophotometer (Instrument Laboratories Inc. Oberlin, OH).

Analysis of variance (ANOVA) was used to determine the relationship between the degree of damage and number of *S. acupunctatus* adults and larvae, besides the different degrees of infestation and damage categories. The Tukey test ($P \leq 0.05$) was used to separate means. Correlation analyses were done ($P \leq 0.05$) to determine the degree of damage of *A. angustifolia* by the number of lesions. Statistical analyses were done with SAS version 9.0 statistical software (SAS Institute 2002).

Table 1. Amount of Damage Caused by *S. acupunctatus* in *A. angustifolia*

Degree of damage	Symptoms
1	Healthy plant (without aqueous lesions, Fig. 1a,b)
2	1 to 5 aqueous lesions on the leaf, 1 to 30 cm long (Fig. 1a,c)
3	More than six lesions on the leaf, 5 to 30 cm long.
4	Necrotic lesion on the base and healthy piña (Fig. 2a,c)
5	Completely damaged stem and piña (dead plant, Fig. 2b,d)

Fig. 1. *A. angustifolia* a) healthy plant (left) and plant with degree of damage of 3 by agave weevil (right), b) healthy piña, and c) piña damaged by agave weevil.

Fig. 2. *A. angustifolia* a) degree of damage 4, b) useless plant with degree of damage greater than 5. The stem breaks off easily in the damaged plant. c) damaged section of the piña (mezontle) with agave weevil larvae. d) rotted piña (mezontle) and adult agave weevils.

Results and Discussion

For all the variables analyzed, the presence of *S. acupunctatus* in *A. angustifolia* plants to a degree of damage of 5 had a significantly ($P \leq 0.01$) greater effect than on plants with a degree of 1 (Table 2). Likewise, there was a direct relationship between the degree of damage of the plant and its mean weight, number of lesions, and presence of *S. acupunctatus* larvae and adults. *A. angustifolia* plants with a degree of damage of 5 weighed less (33.24 kg plant^{-1}) and had greater numbers of lesions (33.2), weevil adults (30.5), and larvae (40) per plant.

Table 2. Degree of Damage by *S. acupunctatus* on *A. angustifolia*

Degree of damage	Weight (kg plant^{-1})	Number of adults	Number of larvae	Number of lesions plant^{-1}
1	63.21 a*	0.0 a	0.0 a	0.0 a
2	57.67 ab	2.1 a	13 b	4.1 ab
3	53.50 ab	4.2 a	21 b	15.1 bc
4	43.26 ab	12.8 a	28 c	29.5 bc
5	33.24 b	30.5 b	40 d	33.2 c

*Different letters in a column indicate significant difference (Tukey, 0.05)

When the *A. angustifolia* plant showed a degree of damage of 3 to 5, losses were 2,473 and 3,086 kg ha^{-1}, respectively. This represented an average yield loss of 52.6% per plant. These results are greater than those by Halffter (1957) and Ramírez (1993) who found yield losses of 40% from *S. acupunctatus* in *A. fourcroydes* Lemaire (henequen) in Yucatan, Mexico.

On the other hand, a significant ($P \leq 0.05$) correlation was found between the degree of damage on *A. angustifolia* plants and numbers of lesions (r = 0.981), weevil adults (r = 0.908), and larvae (r = 0.994). This proves the relationship between damage and *S. acupunctatus*. Losses caused by the agave weevil were estimated at 10,803 kg ha^{-1}.

A. angustifolia plants with a degree of damage of 5 had the greatest number of lesions, weevil adults, and larvae. Solís-Aguilar et al. (2001) proposed eliminating damaged plants as a phytosanitary measure because they host large numbers of agave weevils. Controlling the pest is difficult if the phytosanitary measure is not used. Adult and larval weevils continue feeding on healthy agave plants near damaged ones, and thus continue their life cycle (Fucikovsky 2001, Knox 2010).

A. angustifolia plants with a degree of damage of 1 attained maximum sugar content (16.42 mg g^{-1}) (Table 3). But, when the degree of damage was 5, sugar concentration was significantly less (11.22 mg g^{-1}, $p \leq 0.05$). Sugar content in agave is important because alcohol obtained from fermentation depends on the amount of reducing sugars (Téllez, 1998).

Table 3. Nutrient and Total Reducing Sugar Content of *A. angustifolia* with Different Degrees of Damage by *S. acupunctatus*

Degree of damage	Nitrogen %	Phosphorus %	Potassium %	Total reducing sugar (mg g^{-1})
1	2.8 a*	0.60 a	3.48 a	16.42 a
2	3.5 a	0.48 b	3.16 a	14.02 ab
3	0.7 b	0.40 b	2.86 b	13.74 ab
4	0.4 b	0.31 b	2.90 b	13.36 b
5	0.7 b	0.36 b	2.37 b	11.22 c

*Different letters in a column indicate significant difference (Tukey test, $p \leq 0.05$)

A. angustifolia plants with a degree of damage of 1 had greater nitrogen-phosphorus-potassium content, while those with degrees of damage of 3, 4, and 5 had considerably less of each macronutrient. This is also related to less total reducing sugars (Table 3), mainly because of the effect of phosphorus in the plant. Mengel et al. (2001) mentioned phosphorus was an element used in small amounts, which related with quality, intervened in sugar and starch utilization. A deficiency impedes exportation of triose phosphates of the chloroplast, and therefore sugar synthesis.

It is probable that when nutrients are reduced in the plants because of agave weevil, the metabolic pathway of *A. angustifolia* might have been altered as was noted in other agave species (Nobel 1983, 1988; Nobel et al. 1989). This leads to visible abnormalities of chlorosis and wilting (Epstein and Bloom 2005) like those observed in *A. angustifolia* with a degree of damage of 5. Moreover, potassium is an activator of enzymes essential in photosynthesis and respiration; it activates enzymes necessary for production of starch and proteins and is involved in the transportation of photoassimilates (Swietlik 2003). It also gives firmness to plant tissues and thickness to cell walls. A potassium deficiency results in decomposition of the parenchymatous tissue (Black 1975). Also, potassium participates in osmotic regulation of the cell, and deficiency causes loss of firmness and wilting (Bonilla 2000), probably because of reduction in the amount of potassium in *A. angustifolia*, caused by many agave weevils (degree 5) that caused the plant to wilt and finally die.

Conclusions

Weight losses in *A. angustifolia* from degrees of damage of 2 and 5 by agave weevils were 5.54 and 29.97 kg per plant, respectively. More *S. acupunctatus* significantly decreased the amount of reducing sugars and nitrogen-phosphorus-potassium in the plant.

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