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SHORT COMMUNICATION

EFFECTS OF *CUCUMBER MOSAIC VIRUS* ON THE YIELD AND YIELD COMPONENTS OF PEANUT

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SUMMARY

In Argentina, peanut (*Arachis hypogaea*) is naturally infected by *Cucumber mosaic virus* (CMV), subgroup II. The study involved the evaluation of the effects on yield and yield components in response to CMV infection at different growth stages: 4-6 developed nodes on the main axis (V4-6), 12-16 developed nodes on the main axis (V12-16) and onset of bloom (R1). The results showed that CMV infection significantly reduces peanut seed yield, mainly due to a severe decrease in seed average weight which declined approximately by 30%, 20% and 11% when the virus was inoculated at V4-6, V12-16 and R1, respectively. The number of pods, seeds, and seeds per pod were affected only when the virus was inoculated at V4-6. The percentage of confectionery peanut was also significantly affected when CMV was inoculated at vegetative crop stages. In addition, seed size and weight/volume ratio were greatly affected by the virus since the infected plants produced a higher proportion of small seeds. Larger seeds decreased when the plants were infected at earlier growth stages. Finally, infected plants produced a significantly greater number of immature pods than the healthy ones.

Key words: Arachis hypogaea, *Cucumovirus*, yield losses, effect on growth.

Argentina, along with the United States and China, is one of the major peanut (*Arachis hypogaea* L.) exporting countries, leading the market in high quality and edible peanuts. Peanut production has two different uses, the first, of greater economic value, consists of confectionery peanut for the food industry (snacks, peanut butter/paste, etc.). For the second use, production of oils, flours and pellets, peanuts do not have to meet the quality standards required for direct human consumption. The province of Córdoba, located in the center of Argentina, accounts for 96% of the peanut production and has 92% of the planted area (Busso *et al*., 2004).

Cucumber mosaic virus (CMV), the type species of the genus *Cucumovirus*, family *Bromoviridae*, is a ubiquitous virus that infects a wide natural range of plants, inducing losses to many important crops (Agrios, 2005; Gallitelli, 2000). CMV has been detected in peanuts in Argentina and China (de Breuil *et al*., 2005; Xu and Barnett, 1984). The Argentinean CMV isolate belongs to subgroup II and is widespread in peanut crops growing in the southern part of Córdoba province (de Breuil *et al*., 2008). CMV is naturally transmitted by more than 80 aphid species in a non-persistent manner (Gallitelli, 2000) and, in peanut, it is also transmitted through seeds at a rate of *ca.* 2.5%, the highest percentage occurring in the smaller seeds (Lenardon *et al.*, 2002). Infected peanut seeds, weeds and other susceptible crops serve as the primary source of CMV inoculum, while the spread within peanut crops is attributed mainly to migrating non-colonizing aphids.

An evaluation of peanut germplasm showed that most of the commercial varieties planted in the Córdoba area are highly susceptible to CMV (de Breuil *et al*., 2003). Florman INTA, a CMV-susceptible variety, was the main cultivar used from the late '80s until around 2002 and is currently utilized in peanut breeding programmes. Natural CMV infections with incidence levels of 60%, yield losses up to 30% have been reported in peanut fields (de Breuil *et al*., 2006; March *et al*., 2000). Typical symptoms include severe stunting, shortened petioles, malformed and stunted leaflets with chlorotic mottling. However, there is little information regarding distinct peanut yield components losses caused by CMV. The purpose of this work was to evaluate the effects of CMV infection on the yield and yield components of peanut when plants were inoculated at different stages of growth.

The study was carried out at the Experimental field of IPAVE-CIAP-INTA (31° 24´ S, 61° 11´ W), during 2004/2005 and 2005/2006 cropping seasons, with the runner-type cv. Florman INTA.

The trials were established on a salty loam Typical Haplustoll soil and under an insect-proof net to reduce

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insect colonization. The experimental design was completely randomized with 18 replications planted during two consecutive years in order to increase the number of replications. The experimental units were 1.43 m long rows (equivalent to 1 m^2) placed in the center of 2.5 m long plots. Treatments consisted of mechanical inoculation of peanut plants with CMV at different phenological stages (Boote, 1982): 4-6 developed nodes on the main axis (V4-6), 12-16 developed nodes on the main axis (V12-16), beginning bloom (R1), and healthy control (HC, inoculated with buffer solution). The planting dates were November 29th, 2004 and November 28th, 2005; before sowing, peanut seeds were treated with carboxin plus thiram (Vitavax-Flo, Aventis) at 2.5 ml kg^{-1} of seed, and hand-planted in 2.5 m rows, 0.70 m apart. Plant density was as recommended for commercial peanut production, with additional seeds sown to secure a stand of 14 plants per m^2. Plots were maintained by manual removal of weeds. Tebuconazole (Folicur 25 EW, Bayer) 500 ml ha^{-1} was used for preventing foliar fungal diseases; deltamethrin (Decis 12 EC, Aventis) 12 g ha^{-1} was used as insecticide, and abamectina (Vermitec 018 EC Syngenta) 500 ml ha^{-1} was used against mites. Irrigation was applied when necessary.

Viral inocula were prepared from 7- to 8-week-old *Nicotiana glutinosa* plants systemically infected with CMV subgroup II. Young leaves were homogenized at a ratio of 1:10 (w/v) with sterilized pestles and mortars in 0.01 M sodium phosphate buffer, pH 7.5, containing 0.1% sodium sulphite (Na_2SO_3), and carborundum (0.03 g ml^{-1} buffer), filtered in cheesecloth and kept at 4°C. In each treatment, the inoculum was applied to the two youngest fully expanded leaves on the main axis, using an air-brush apparatus at 8 bars pressure. Plants were monitored daily for leaf symptom expression. To assess CMV infection, young symptomatic leaves from mechanically inoculated plants were randomly collected from each plot. Collected leaves were younger than inoculated leaves. Samples were analyzed by transmission electron microscopy (TEM) using leaf-dip preparations and by DAS-ELISA (Clark and Adams, 1977) with a commercial kit (Agdia, USA) following the manufacturer's protocol.

In both crop seasons, plants were hand harvested 153 days after sowing. The following parameters were evaluated: numbers of pods, seeds and seeds per pod, seed average weight (SAW), percentage of confectionery peanut, percentage of mature pods and seed yield. Pods were counted manually, then were hand-shelled. Seeds were counted and weighed for seed yield determination. Yield was determined adjusting the weight to 0% moisture using: "final weight = [initial weight_(100-moisture content of seeds)]/100". The number of seeds per pod was calculated as the quotient between number of seeds and number of pods. SAW was determined as the quotient between seed yield and seed number. The percentage of confectionery peanut was calculated as the relationship between the weight of seeds that were retained on a 7 mm sieve and the total seed weight. The proportion of mature pods was determined only in 2004/2005 crop season according to Gilman and Smith (1977).

For each treatment, seeds were sorted with a sieve into size classes of 11, 10, 9, 8, 7 mm diameter, and smaller than 7 mm. The total number of seeds in each seed size class and their weight were determined, and they were classified into different categories of weight/volume ratio based on the number of seeds in 28.35 g (1 ounce): 60 seeds or less, 60-70 seeds, 70-80 seeds, 80-100 seeds, 100-120 seeds, 120-140 seeds, and 140 or

Table 1. Yield parameters for plants inoculated at different phenological growth stages (V4-6, V12-16 and R1) with CMV subgroup II, and for healthy control.

Growth stages	Yield parameters						
	Seed yield[Y] (kg ha^{-1})	Pods (No. m^{-2})	Seeds (No. m^{-2})	Seeds per pod	SAW[YZ] (g)	Confectionery peanut (%)	Pod maturity (%)
V4-6	1136.72 a	212 a	359 a	1.59 a	0.32 a	88.5 a	40 a
V12-16	1738.94 b	258 b	455 b	1.65 b	0.37 b	92.3 b	36 a
R1	1938.14 b	274 b	475 b	1.69 b	0.41 c	95.0 c	47 b
Healthy control	2205.06 c	283 b	493 b	1.71 b	0.46 d	95.7 c	58 c

[Y] Based on seeds with 0% moisture.
[Z] SAW: Seed Average Weight.
Different letters are significantly different (_ = 0.05) based on DGC test.

Fig. 1. CMV-inoculated peanut plant showing severe chlorotic mottling and line patterns in leaves.

more seeds, according to procedures established by the Secretaría de Agricultura, Ganadería, Pesca y Alimentación (SAGPyA), for peanut marketing (Fernandez and Giayetto, 2006). For each seed size class the 1000-seed weight (g) was calculated.

The statistical analyses were carried out with InfoStat statistical software (Version 2004, Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina). The data were analyzed using generalized linear mixed model procedure (GLMM) with fixed effect of "treatment" and random effects of "crop season". Treatment means comparisons were done a posteriori using Di Rienzo, Guzmán and Casanoves (DGC) test (Di Rienzo *et al.*, 2002) at _ = 0.05.

All CMV-inoculated plants at different phenological stages developed symptoms which occurred approximately 15 days after inoculation. Infected plants showed severe chlorotic mottling and line patterns in the leaves (Fig. 1). Plants inoculated at V4-6 also showed severe stunting and all leaves presented symptoms. In contrast, when inoculation was delayed (V12-16, R1), symptoms were observed only in some branches of the plant.

All random samples evaluated from the different treatments reacted strongly in ELISA and TEM revealed only particles with a typical cucumovirus morphology. Healthy controls were ELISA- and TEM-negative (not shown).

CMV infection affected both peanut yield and yield components. Results showed significant seed yield loss (between 12 and 48%) following CMV inoculation (Table 1). There were no significant differences among the treatments regarding the number of pods and seeds per m^2, and the number of seeds per pod, except when the virus was inoculated at the V4-6 stage. In contrast, CMV infection had a negative impact on seed weight, SAW being the main parameter that explains seed yield differences among treatments. At V4-6, V12-16 and R1, SAW was 30%, 20% and 11% less than that of the healthy control, respectively (Table 1).

The percentage of confectionery peanut was also significantly affected by CMV inoculated at stages V4-6 and V12-16. In terms of fruit maturity, at the time of harvest the healthy control had significantly more mature pods than infected plants (Table 1). Moreover, peanut seed weight/volume ratio was affected by CMV infection, showing, in the healthy control, a higher proportion of grains belonging to the 60 seeds or less per 28.35 g category (Fig. 2). When the virus was inoculated at the beginning bloom there was a significant decrease

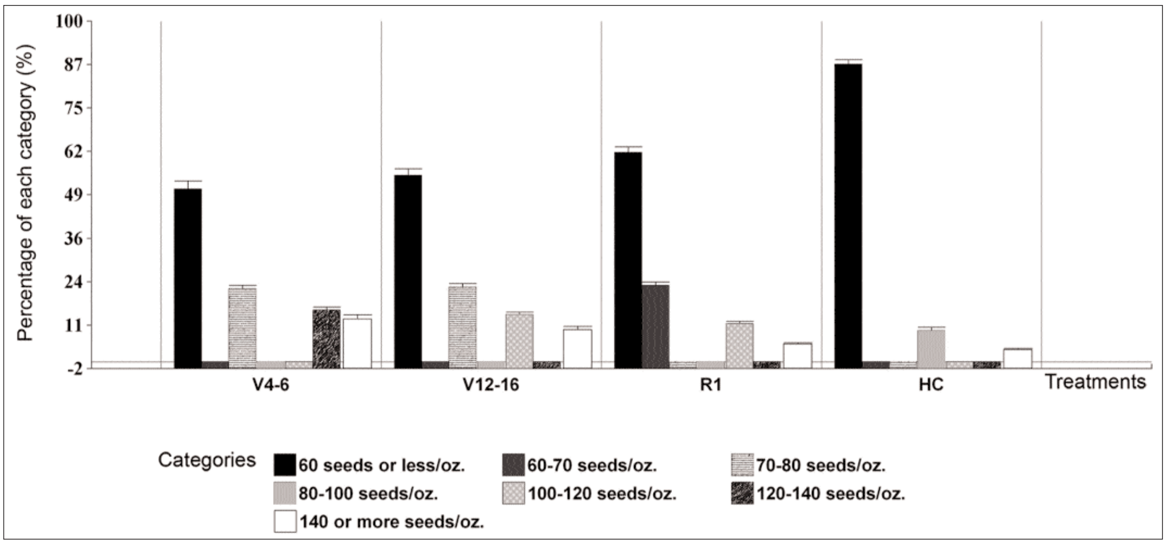

Fig. 2. Percentage of weight/volume ratio categories, based on the number of seeds contained in 28.35 g (1 oz.), for peanuts inoculated at different phenological growth stages (V4-6, V12-16 and R1) with CMV subgroup II, and healthy control (HC).

in the percentage of grains corresponding to the 60 seeds or less per 28.35 g category, while grains of the 60-70 seeds per 28.35 g category increased. When the virus was inoculated at vegetative stages, the fraction corresponding to 60 seeds or less declined further, and the plants produced a higher percentage of grains in the 70-80 seeds per 28.35 g category, and the other categories with smaller seeds, compared to healthy controls (Fig. 2). Moreover, when the weight of 1000 seeds was analyzed separately for each seed size fraction, differences between treatments were noticed for the fraction of seeds in 7, 8 and 9 mm diameter sieves. The results indicated that in such size classes, seeds from infected plants are lighter than those of equal diameter from healthy controls.

The results obtained in this study showed that CMV significantly reduces peanut yield. In addition, an early viral infection during the vegetative stage had the greatest impact on yield, showing the importance of the crop growth stage at the time of infection. This has agronomical implications since a crop planted early in the season will have less sensitive plants when insect vectors arrive to the crop. In fact, we observed that plants infected in more advanced growth stages develop a smaller proportion of symptomatic tissue, probably due to restrictions in systemic virus movement (Dufour *et al*., 1989; Guerini and Murphy, 1999). Peanut yield losses caused by CMV were due to seed weight decrease. As shown with other CMV host species, weight seed reduction in CMV-infected plants could be related by an interference with the regular leaf cell carbohydrate metabolism, impairing assimilates source-sink relationship (leaves-seeds) (Shalitin *et al*., 2002; Shalitin and Wolf, 2000; Técsi *et al*., 1994a; Técsi *et al*., 1994b, 1996). Carbohydrate decrease in the reproductive organs would directly affect the seed filling stage, resulting in lower weight seeds. This fact could be also supported by the higher weight of 9 mm or smaller seeds from the healthy treatment compared to the infected ones. Other important effects of CMV infection were the reduction in the numbers of pods, seeds and seeds per pod when the virus was inoculated at the V4-6 early crop stage and the decrease in the percentage of confectionery peanut when CMV was inoculated at vegetative growth stages.

The value of a peanut crop is determined by yield and grade, which largely depends on seed size distribution. This is an important issue, since confectionery peanuts for export are priced according to their size. CMV is of special concern to peanut production, since seed size and weight/volume ratio are parameters greatly affected by virus infection. Our results showed that the infected plants produce higher proportion of small seeds while the percentage of larger seeds decreases when the plants are infected at earlier stages of growth. Moreover, the uniformity in size is a desirable feature in peanut cultivars as it improves the efficiency of seeding, blanching and roasting. In addition, a crop with smaller seeds leads to increased mechanical losses during harvesting since the machine cannot lift the small seeds that are left in the field. It is also known that smaller grade sizes have more immature peanut seeds (Sanders, 1995).

Pod maturity is another important quality parameter since it directly affects flavor, conservation capacity and resistance to the organoleptic deterioration of fruits (Sanders, 1995; Bragachini *et al*., 1994; Worthington *et al*., 1972; Young and Waller, 1972). In the Córdoba peanut-growing area, runner cultivars reach maturity percentages of approximately 40-60%, which is considered optimal for digging (Pérez *et al*., 2004). The results obtained in this study showed that plants infected with CMV at vegetative stages did not reach the optimal percentage of fruit maturity for an effective harvest because harvest timing is determined by environmental conditions (reduced irradiance and low temperature) (Bell *et al*., 1994a, 1994b; Haro *et al*., 2007). It is probable that a delay in the harvest date may not result in a significant increase of mature pod percentage. However, since maturity was evaluated only in the 2004/2005 crop season, further investigations are needed to elucidate this point.

In conclusion, this study has revealed the economic importance of CMV for peanut crop, thus our ongoing efforts are being focused on the identification of CMV-resistant peanut genotypes for future breeding.

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REFERENCES

Agrios G.N., 2005. Plant disease caused by viruses. In: Agrios G.N. (ed.) Plant Pathology, pp. 724-822. Elsevier-Academic Press, San Diego, CA, USA.

Bell M.J., Gillespie T.J., Roy R.C., Michaels T.E., Tollenaar M., 1994a. Peanut leaf photosynthetic activity in cool field environments. *Crop Science* **34**: 1023-1029.

Bell M.J., Michaels T.E., McCullough D.E., Tollenaar M., 1994b. Photosynthetic response to chilling in peanut. *Crop Science* **34**: 1014-1023.

Boote K.J., 1982. Growth stages of peanut (*Arachis hypogaea* L.). *Peanut Science* **9**: 35-40.

Bragachini M.A., Bonetto L.A., Bongiovanni R.G., 1994. Inicio de la cosecha. In: Bragachini M.A., Bonetto L.A., Bongiovanni R.G. (eds). Maní, implantación, cuidados culturales, cosecha, secado y almacenaje, pp. 58-68. INTA-EEA Manfredi, Córdoba, Argentina.

Busso G., Civitaresi M., Geymonat A., Roig R., 2004. Situación socioeconómica de la producción de maní y derivados en la región centro-sur de Córdoba. 1st Ed. Universidad Nacional de Río Cuarto, Córdoba, Argentina.

Clark M.E., Adams A.N., 1977. Characteristics of microplate methods of enzyme linked immunosorbent assay for detection of plant viruses. *Journal of General Virology* **34**: 475-483.

de Breuil S., Giolitti F., Lenardon S.L., 2003. Evaluación de germoplasmas comerciales de maní a *Cucumber mosaic virus*. *XVIII Jornada Nacional de Maní*, *Gral Cabrera, Córdoba, Argentina*: 36-37.

de Breuil S., Giolitti F., Lenardon S., 2005. Detection of *Cucumber mosaic virus* on peanut (*Arachis hypogaea* L.) in Argentina. *Journal of Phytopathology* **153**: 722-725.

de Breuil S., Moresi A., Lenardon S., 2006. Nueva epidemia de *Cucumber mosaic virus*. *XXI Jornada Nacional de Maní, Gral Cabrera, Córdoba, Argentina*: 25-26.

de Breuil S., Nievas M.S., Giolitti F.J., Giorda L.M., Lenardon S.L., 2008. Occurrence, prevalence, and distribution of viruses infecting peanut in Argentina. *Plant Disease* **92**: 1237-1240.

Di Rienzo J.A., Guzmán A.W., Casanoves F., 2002. A multiple-comparisions method based on the distribution of the root node distance of a binary tree. *Journal of Agricultutal, Biological, and Environmental Statistics* **7**: 129-142.

Dufour O., Palloix A., Gebre-Selassie K., Pochard E., Marchoux G., 1989. The distribution of cucumber mosaic virus in resistant and susceptible plants of pepper. *Canadian Journal of Botany* **67**: 655-660.

Fernandez E.M., Giayetto O., 2006. Calidad comercial y alimenticia de los granos. In: Fernandez E.M., Giayetto O. (eds). El cultivo de Maní en Córdoba, pp.49-69. Universidad Nacional de Río Cuarto, Córdoba, Argentina.

Gallitelli D., 2000. The ecology of *Cucumber mosaic virus* and sustainable agriculture. *Virus Research* **71**: 9-21.

Gilman D.F., Smith O.D., 1977. Internal pericarp color as a subjective maturity index for peanut breeding. *Peanut Science* **4**: 67-70.

Guerini M.N., Murphy J.F., 1999. Resistance of *Capsicum annuum* 'Avelar' to pepper mottle potyvirus and alleviation of this resistance by coinfection with cucumber mosaic cucumovirus are associated with virus movement. *Journal of General Virology* **80**: 2785-2792.

Haro R.J., Otegui M.E., Collino D.J., Dardanelli J.L., 2007. Environmental effects on seed yield determination of irrigated peanut crops: links with radiation use efficiency and crop growth rate. *Field Crops Research* **103**: 217-228.

Lenardon S., de Breuil S., Oddino C., Kearney M., Marinelli A., 2002. Transmisión del *Cucumber mosaic virus* (CMV) en semillas de maní. *XI Jornadas Fitosanitarias Argentinas, Río Cuarto, Córdoba, Argentina* : 74.

March G.J., Marinelli A., Lenardon S.L., Pedrozo F., Kearney M., Oddino C., Remedi D., 2000. Incidencia, distribución espacial y pérdidas por *Cucumovirus* en maní. *XV Jornada Nacional de Maní, Gral Cabrera, Córdoba, Argentina: 34.*

Pérez M.A., Cavallo A.R., Pedelini R., 2004. Indicadores de madurez en frutos de maní (*Arachis hypogaea* L.) cv. Florman para la producción de semillas en Córdoba, Argentina. *Agriscientia* **21**(**2**): 77-83.

Sanders T.H., 1995. Harvesting, storage, and quality of peanuts. In: Melouk H.A., Shokes, F.M. (eds). Peanut Health Management, pp. 23-31. APS Press, St. Paul, MN, USA.

Shalitin D., Wang Y., Omid A., Gal-On A., Wolf S., 2002. *Cucumber mosaic virus* movement protein affects sugar metabolism and transport in tobacco and melon plants. *Plant, Cell and Environment* **25**: 989-997.

Shalitin D., Wolf S., 2000. *Cucumber mosaic virus* infection affects sugar transport in melon plants. *Plant Physiology* **123**: 597-604.

Técsi L.I., Maule A.J., Smith A.M., Leegood R.C., 1994a. Metabolic alterations in cotyledons of *Cucurbita pepo* infected by *Cucumber mosaic virus*. *Journal of Experimental Botany* **45**: 1541-1551.

Técsi L.I., Maule A.J., Smith A.M., Leegood R.C., 1994b. Complex localised changes in CO_2 assimilation and starch content associated with the susceptible interaction between *Cucumber mosaic virus* and cucurbit host. *The Plant Journal* **5**: 837-847.

Técsi L.I., Smith A.M., Maule A.J., Leegood R.C., 1996. A spatial analysis of physiological changes associated with infection of cotyledons of marrow plants with *Cucumber mosaic virus*. *Plant Physiology* **111**: 975-985.

Worthington R.E., Hammond R.O., Allison J.R., 1972. Varietal differences and seasonal effects on fatty acid composition, stability of oil from 82 peanut genotypes. *Journal of Agricultural and Food Chemistry* **20**: 727-730.

Xu Z., Barnett O.W., 1984. Identification of a *Cucumber mosaic virus* strain from naturally infected peanuts in China. *Plant Disease* **68**: 386-389.

Young C.T., Waller G.R., 1972. Rapid oleic/linoleic microanalytical procedure for peanut. *Journal of Agricultural and Food Chemistry* **20**: 1116-1118.

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