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EFFECT OF SUNFLOWER CHLOROTIC MOTTLE VIRUS INFECTION ON SUNFLOWER YIELD PARAMETERS

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SUMMARY

Sunflower chlorotic mottle virus (SuCMoV) was detected in several sunflower (*Helianthus annuus* L.) growing areas, causing a disease characterized by systemic chlorotic mottling. Symptom severity depended on several factors, including the ontogenetic stage at which infections occur. The objective of this study was to determine the effects of artificial infections with SuCMoV at different ontogenetic stages on agronomic yield characters (plant height, stem and capitulum diameters, achene yield, seed width and length, weight of 1000 seeds and oil content). Sunflower seeds of commercial hybrids Dekalb 4030, Contiflor 3N, and ACA 884 were sown in a split plot design with four replications, which were mechanically inoculated with SuCMoV at four growth stages. A negative (non-inoculated) control was included in the experiment. Virus infection was detected by symptoms and by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Virus infections at all stages significantly reduced plant height (16-39%), stem diameter (21-51%), capitulum diameter (27-57%), achene yield (58-87%), seed width (13 -15%), seed length (10-16%) and weight of 1000 seeds (26-28%) compared with healthy controls, independent of hybrid tested. Oil content determined by magnetic nuclear resonance showed no significant differences among treatments.

Key words: **agronomic characters, chlorotic mottling, *Helianthus annuus*, potyvirus, yield losses**

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops in Argentina, planted yearly at more than 3,000,000 ha. Achene production accounts

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for the most important vegetable oil revenue in this country, which is the main sunflower oil exporter in the world.

Several virus-like diseases have been reported in different parts of the country in last 25 years (Delhey *et al.*, 1981, Formento *et al.*, 1994, Muñoz *et al.*, 1980) but the viruses were not thoroughly characterized. During the growing seasons of 1993/94, three poty-like viruses inducing chlorotic mottling, ring spots and mosaic, were isolated and identified from Paraná, in the province of Entre Rios (Lenardon, 1994). The chlorotic mottling disease, characterized by yellow blotches which later coalesce on the leaves of the whole plant, caused most extensive damage because it not only reduced the photosynthetic leaf area but also produced distortions and severe plant stunting. *Sunflower chlorotic mottle virus* (SuCMoV) is the most widespread of the three potyviruses, since, besides Entre Ríos in the East, it has been detected in the central region of the country, in the provinces of Santa Fé, Córdoba and Buenos Aires (Lenardon, unpublished). The virus associated with this disease has been biologically, structurally and serologically characterized as a potyvirus (Dujovny *et al.*, 1998) and based on molecular properties the name of *sunflower chlorotic mottle virus* (SuCMoV) has been proposed (Dujovny *et al.*, 2000).

A potyvirus causing mosaic has been isolated from wild sunflowers in Texas, USA and some properties have been reported (Gulya *et al.*, 1998). Although the American and the Argentinean viruses have been described as potyviruses, they do not share biological and serological properties (Gulya *et al.*, 2000; Lenardon, unpublished).

Symptom severity and yield losses induced by potyviruses usually depend on virus virulence, host tolerance and host growth stages upon infection (Agrios *et al.*, 1985; Shukla *et al.*, 1994). No information about SuCMoV infection on commercial sunflower hybrids is available, thus the objective of this study was to measure the effects of mechanical inoculations with SuCMoV at different plant ontogenetic stages on quantitative and qualitative yield parameters of three commercial sunflower hybrids.

MATERIALS AND METHODS

A trial with three commercial sunflower hybrids was planted at the IFFIVE-INTA experimental field on November 25, 1998. Hybrids Dekalb (DK) 4030, Contiflor (CF) 3N, and ACA 884 comprised the main plots and growth stage at inoculation were the subplots. Each subplot comprised three 5-m rows, spaced at 0.7 m between rows and 25 cm in the row. Plants were mechanically inoculated at V1-2; V3-4; V5-6 and V7-8 true leaf (at least 4 cm long) stages according to Schneiter and Miller (1981). Only the central rows were inoculated with the virus, and a negative non-inoculated control was included for every hybrid. A completely randomized split-plot block design with four replications was used. All plots were treated with acetochlor 2-chloro-N-ethoxymethyl-6´-ethylacet-o-toluidide (900 cc/ha) plus flurochloridrona 3-chloro-4-(chloromethyl)-1-3(trifluromethyl) phenyl-2-pyrrolidone (900 cc/ha) herbicides after sowing to control weeds.

A SuCMoV isolate maintained on sunflower seedlings in the greenhouses was used as inoculum source. Infected leaves were ground in 0.01 M Na phosphate buffer, pH 7, containing 0.1% $Na_2 SO_3$ (wt/vol) and silicon carbide 600 mesh added as abrassive (0.25 g /10 ml slurry). The inoculum was applied on expanding sunflower leaves, approximately half the final size, using a high-pressure airbrush apparatus. One inoculation with SuCMoV was made per row, at the growth stages mentioned above.

Virus infection was recorded by symptoms. During the growing season, three randomly selected plants from each subplot were tested by DAS-ELISA (Clark and Adams, 1977) to verify that the symptoms were indeed caused by the inoculated virus. Healthy and virus-infected sunflower controls for DAS-ELISA were obtained from greenhouse-maintained plants.

All subplots were evaluated daily to assess 50% of flowers at anthesis (R5), (Schneiter and Miller, 1981). On February 4, all plants at (R7) stage were measured for the following characters: plant height; stem diameter (at first internode) and capitulum diameter. Capitula were individually collected by hand, threshed and achene weight was corrected to 12.5% moisture and converted to kg/ha.

Achene samples from each treatment were evaluated for seed width, length and weight of 1000 seeds. Seed oil content was determined with an Oxford 4000 magnetic nuclear resonance analyzer (Oxford Instruments, Oxon, England).

Statistical analysis

Results were analyzed by the ANOVA test, and means were compared by Tukey´s multiple comparison test at 5% level.

RESULTS

All sunflower hybrids were susceptible to the virus and they differed neither in symptoms nor percentage of infected plants (near 100% of incidence in all cases). Chlorotic mottling symptoms were first observed on plants inoculated at ontogenetic V1-2 and V3-4 leaf stages within 7-8 days post inoculation (Figure 1), whereas plants inoculated at V5-6 and V7-8 leaf stages developed symptoms within 10 and 11 days, respectively.

Infected plants of hybrid ACA 884 opened their ligulate flowers 1 to 4 days earlier than their non-inoculated controls, hybrid DK 4030 flowered 2 to 4 days earlier and hybrid CF3N 3 to 6 days earlier than their respective controls. Furthermore, the ligulate flowers from all infected treatments wilted earlier than those of the non-inoculated controls.

Figure 1: Sunflower plant inoculated at V1-2 growth stage, showing systemic chlorotic mottling symptoms.

After flowering, leaves expressing systemic symptoms changed to a pale green color with dark green blotches, followed by marginal necrosis beginning with the oldest leaves. Infected plants senesced earlier than healthy controls.

DAS-ELISA data confirmed virus infection only in the inoculated rows of the three hybrids (data not showed).

Figure 2: Sunflower plants inoculated at V1-2 growth stage, showing small, reduced capitula and severe dwarfing at flowering stage (R5) in infected plants, and non-inoculated control plants of the same hybrid.

In all hybrids, virus infection at any inoculation stage significantly reduced plant height, stem and capitulum diameter (Figure 2) and achene yield compared with the non-inoculated healthy control. Seeds from all inoculated plants were significantly smaller (seed width, length and weight of 1000 seeds) than their controls, but inoculation stage did not influence seed size.

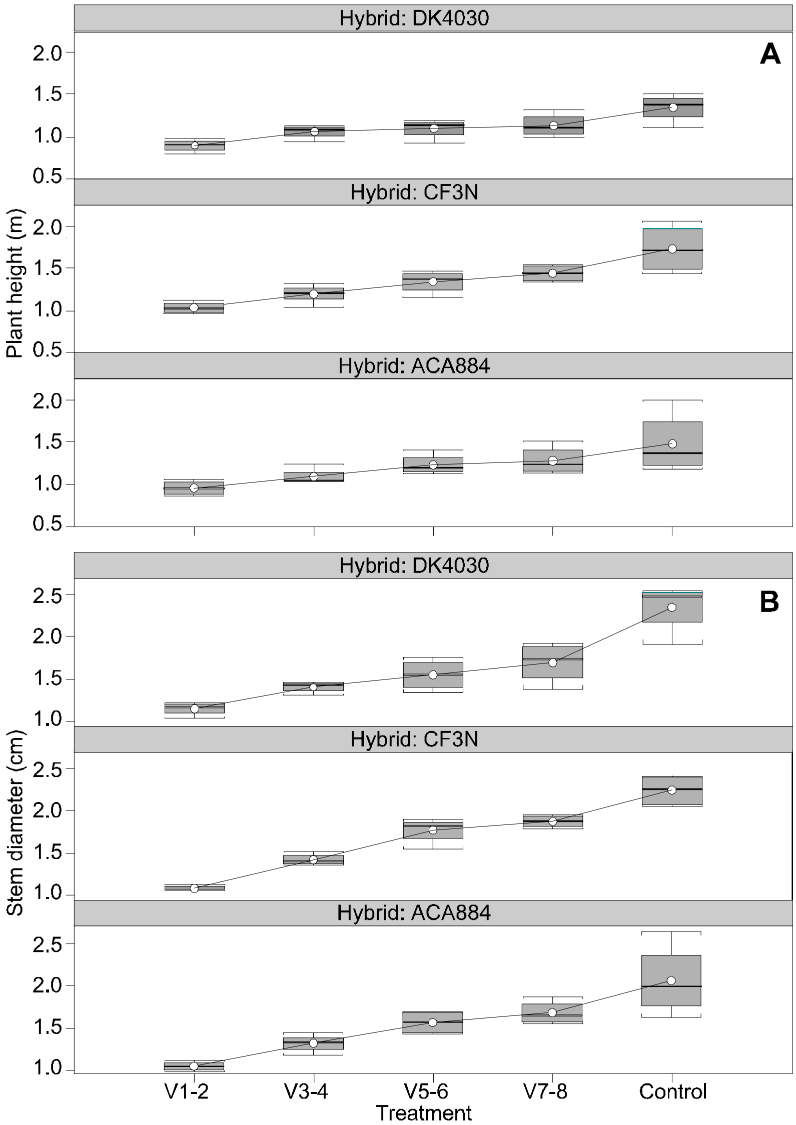

Figure 3: Effect of SuCMoV on three sunflower hybrids inoculated at four growth stages; (A) plant height (m) and (B) stem diameter (cm). Controls were not inoculated.

Virus infections at any stage of growth resulted in smaller plants, with height reductions ranging from 17% in V1-2 to 39% in V7-8 (Figure 3A), and thinner stems with diameters ranging from 21% to 51% of controls (in V1-2 and in V 7-8, respectively, Figure 3B). Plants infected earliest were most drastically dwarfed and thinned, and later inoculations produced proportionally smaller reductions.

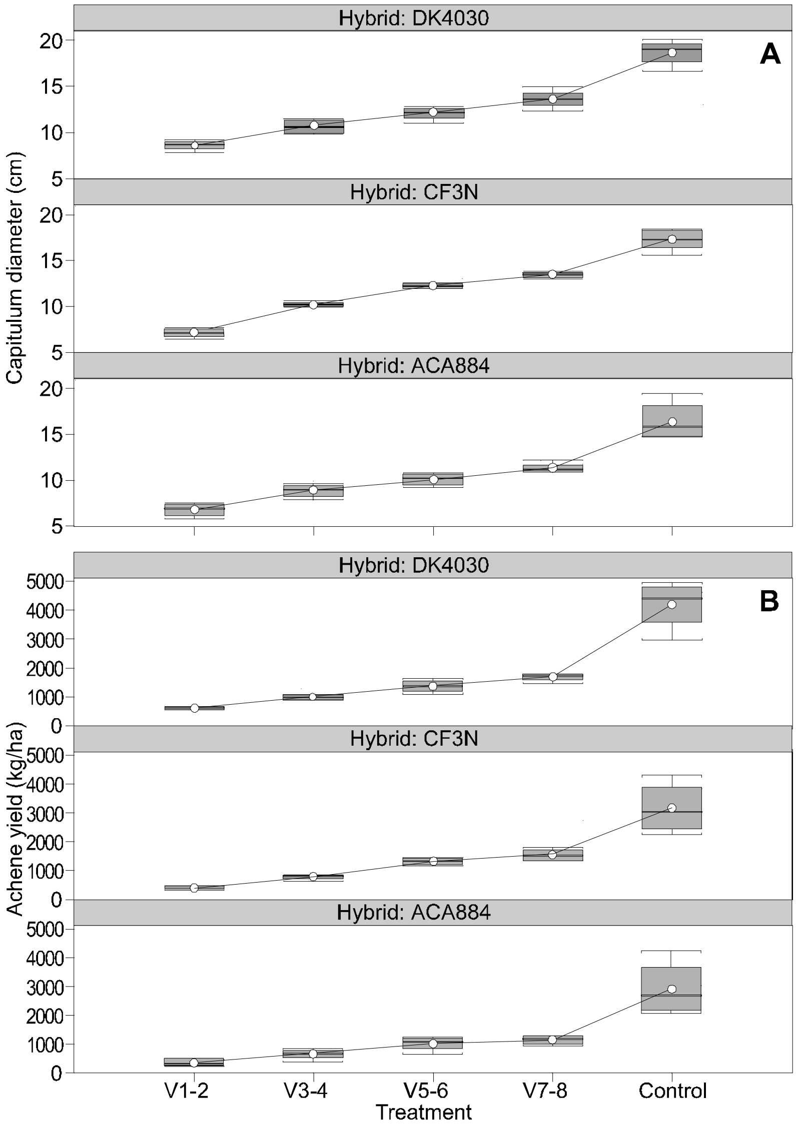

Figure 4: Effect of SuCMoV on three sunflower hybrids inoculated at four growth stages; (A) capitulum diameter (cm) and (B) achene yield (kg/ha). Controls were not inoculated.

Reductions in capitulum diameter and achene yield were directly related to the earliness of plant infection. Early inoculations (V1-2 leaf stage) reduced capitulum diameter by one half and later inoculations (V7-8 leaf stage) reduced it by 25% (Figure 4A). Achene yield (kg/ha) decreased in proportion to capitulum reductions and

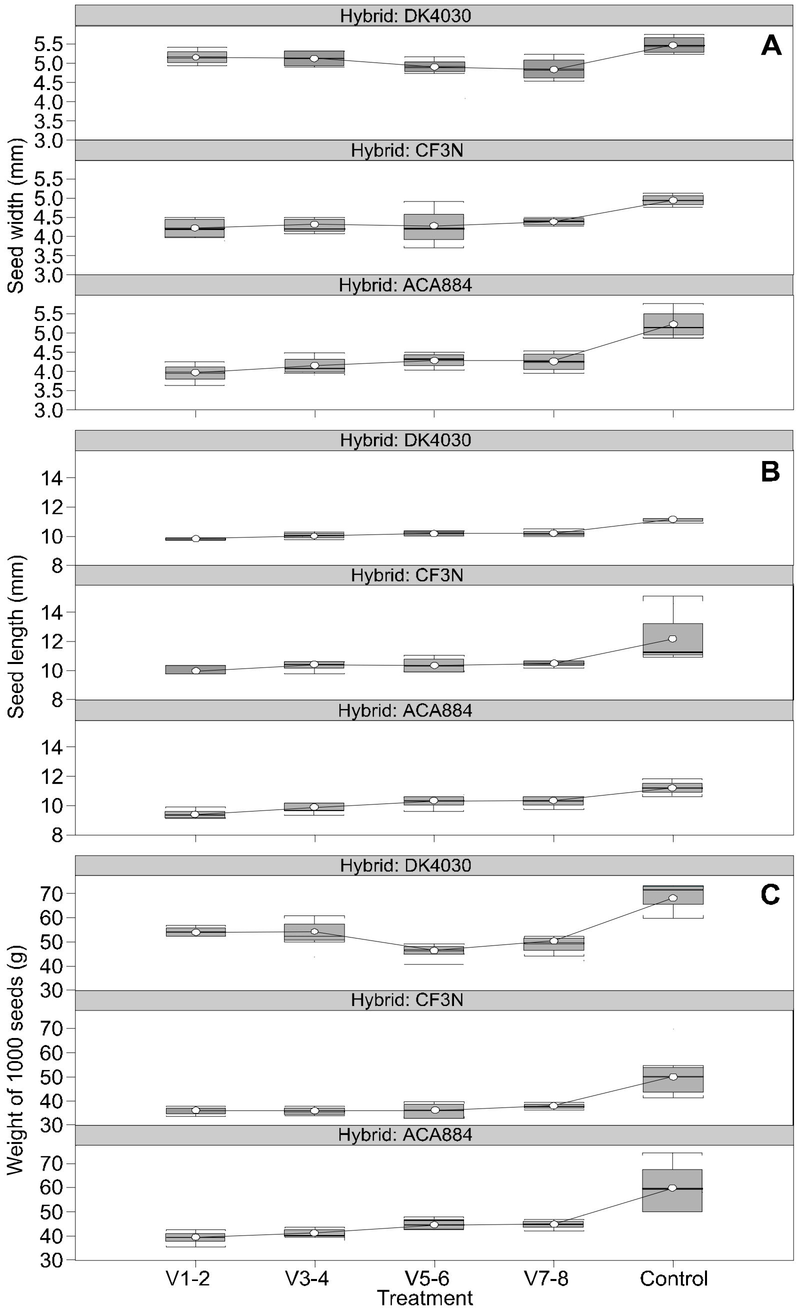

Figure 5: Effect of SuCMoV on three sunflower hybrids inoculated at four growth stages; (A) seed width (mm), (B) seed length (mm) and (C) weight of 1000 seeds (g). Controls were not inoculated.

the youngest inoculated sunflower plants suffered the highest loss in yield (87%) while the latest inoculated plants yielded 58% less than the controls (Figure 4B).

Seed width and length, as well as weight of 1000 seeds were lower in the inoculated plants than in the controls (Figure 5C), however, no significant differences were observed among inoculation treatments for any of these parameters.

Total achene oil content was not affected by virus inoculation, irrespectively of the hybrid used.

A few virus-infected plants were seen outside the inoculated rows, which could indicate that the virus was naturally spread by aphids.

DISCUSSION

The extent of losses due to viruses in any particular crop depends on several factors like vector population infectivity, crop susceptibility, virus strain, time of virus infection, and crop growth conditions (stresses) (Matthews, 1991). Our field experiments demonstrate that several commercial sunflower hybrids with different genetic backgrounds, mechanically inoculated with SuCMoV, show a similar rate of infection and that symptoms varied according to the stage at which virus infection occurred. Quantitative and qualitative modifications which caused significant yield losses occurred in all infection treatments, however, yield reductions were higher when plants were inoculated at an early growth stage rather than later in the season. In fact, decreases in plant height, stem and capitulum diameter and achene yield were generally proportional to the earliness of plant inoculation, suggesting that the plant growth stage at infection determines the extent of yield reduction. This supports previous reports showing that the earlier virus infection occurs the more severe reductions in yield parameters (Agrios *et al.*, 1985; Wakman *et al.*, 1989).

No significant differences were observed in the achene oil content from the distinct SuCMoV inoculation treatments against their respective non-inoculated controls, however, total seed oil decreased in soybeans infected with *Tobacco ring spot virus* (Matthews, 1991).

Also, we present evidence demonstrating that virus infection influences plant maturity. In fact, plants infected at any stage flowered earlier and induced earlier the wilting of ligulate flowers than their healthy controls. Further to this matter, virus infections may induce premature or retarded flowering depending on the virus-host association involved. Also, it has been shown that *Tobacco streak virus* infection on sunflower induces pronounced epinasty of floral bracts (Gulya, *et al.*, 1997). Most drastic effect on virus infection were observed when flowering and seed production were reduced or stopped, as in broad bean infected with *Bean leaf roll virus* (Gibbs and Harrison, 1976). These flower modifications may be due to some metabolic changes induced by the virus infection, which are associated with plant senescence (Gibbs and Harrison, 1976).

SuCMo is a plant virus disease detected in several sunflower-growing regions, appearing with increasing incidence levels in Miramar and Necochea, southeast Province of Buenos Aires. The wide crop distribution in the country and the dramatic virus effects on achene yield, suggest that this virus could potentially threaten the extended Argentinean sunflower production system if an epidemic breaks out.

The information reported in this work show that any sunflower management strategy aimed at preventing an early SuCMoV infection, or delaying it, may potentially increase yield by avoiding negative effects on quantitative and qualitative agronomic parameters.

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REFERENCES

Agrios, G., Walker, M.E. and Ferro, D.N., 1985. Effects of cucumber mosaic virus inoculation at successive weekly intervals on growth and yield of pepper (*Capsicum annuum*) plants. Plant Dis., 69: 52-55.

Delhey, R., Kiehr-Delhey, M., Lesemann, D. and Weidemann, H., 1981. Estudios sobre el mosaico necochense del girasol. In: Actas IV Jornadas Fitosanitarias Argentinas. Universidad Nacional de Córdoba. Córdoba, Argentina, pp. 20.

Clark, M.F. and Adams, A.N., 1977. Characteristics of the microplate method of enzyme linked immunosorbent assay (ELISA) for the detection of plant viruses. J. Gen. Virol., 34: 475-482.

Dujovny, G., Usugi, T., Shohara, K. and Lenardon, S.L., 1998. Characterization of a potyvirus infecting sunflower in Argentina. Plant. Dis., 82: 470-474.

Dujovny, G., Sasaya, T., Koganesawa, H., Usugi, T., Shohara, K. and Lenardon, S.L., 2000. Molecular characterization of a new potyvirus infecting sunflower. Archives of Virology, 145: 2249-2258.

Formento, N., Rodriguez, P., Alegre, A., Truol, G. and Laguna, I.G., 1994. Potyvirus, causal organism of sunflower mosaic in Argentina. In: Actas VII Congreso Latinoamericano de Fitopatología. Pontificia Universidad Católica de Chile. Santiago, Chile, pp. 58.

Gibbs, A. and Harrison, B., 1976. Plant Virology. John Wiley & Sons, New York, pp. 1-292.

Gulya, T.J., Rashid, K.Y. and Maširević, S., 1997. Sunflower Diseases. In: Schneiter, A. A. (ed.), Sunflower Technology and Production, Agronomy Series, Vol. 35, Madison, Wisconsin, pp. 263-379.

Gulya, T.J. , Berger, P.H., Shiel, P.H., Freeman, T.P. and Isakeit, T.S., 1998. Mosaic-causing potyvirus on wild sunflower from Texas. Phytopathology, 88: S34.

Gulya, T.J. , Berger, P.H., Shiel, P.H., Freeman, T.P. and Isakeit, T.S., 2000. Characterization and host range of sunflower mosaic potyvirus. In: Actas 15th International Sunflower Conference, Toulouse, France. Vol. II, pp. 185–189.

Lenardon, S.L., 1994. Síntomas de etiología viral en cultivos de girasol. In: Enfermedades del girasol en la Argentina. V.R. Pereyra and A.R. Escande, eds. INTA, Balcarce, Argentina, pp. 99–103.

Matthews, R.E.F., 1991. Plant Virology. 3rd ed. Academic Press, California, pp. 1–835.

Muñoz, J., Giorda, L., Teyssandier, E. and Lenardon, S.L., 1980. Una virosis del girasol (*Helianthus annuus* L.) en la República Argentina. In: Actas IX International Conference of Sunflower. Torremolinos. Málaga, España, pp. 61.

Schneiter, A.A. and Miller, J.F., 1981. Description of sunflower growth stages. Crop Science, 21: 901-903.

Shukla, D., Ward, C.W. and Brunt, A.A., 1994. The Potyviridae. CAB International, Cambridge, UK, pp. 1-516.

Wakman , W., Pakki, S. and Hasanuddin, A., 1989. Yield losses of groundnut due to peanut stripe virus. In: Summary Proceedings of the Second Coordinator´s Meeting on Peanut Stripe Virus, 1-4 August 1988. ICRISAT, Patancheru, India, pp. 11.

EFECTOS DE LA INFECCIÓN CON EL VIRUS DEL MOTEADO CLORÓTICO DEL GIRASOL SOBRE PARÁMETROS DE LA PRODUCCIÓN DEL GIRASOL

RESUMEN

El virus del moteado clorótico del girasol (SuCMoV) ha sido detectado en varias regiones girasoleras de Argentina ocasionando un característico moteado clorótico sistémico. La severidad de los síntomas dependen de varios factores entre ellos el estado de desarrollo del cultivo al momento de la infección. El objetivo de este estudio fue determinar los efectos de infecciones artificiales con el SuCMoV en distintos estados de crecimiento del girasol sobre caracteres agronómicos de la producción (altura de la planta, diámetro del tallo y del capítulo, producción de aquenios, ancho y largo de las semillas y contenido de aceites). Semillas de los híbridos comerciales Dekalb 4030, Contiflor 3N y ACA 884 fueron sembradas en un diseño de parcelas divididas con cuatro repeticiones las que fueron mecanicamente inoculadas con el virus en cuatro estados de crecimiento. Un control negativo (no inoculado) fue incluído en el experimento. La infección viral fue determinada por sintomas y por DAS-ELISA y en cualquiera de los estados de crecimiento reducen la altura de las plantas (16-39%), diámetro de tallos (21-51%), diámetro de capítulos (27-57%), producción de aquenios (58-87%), ancho de las semillas (13-15%), largo de las semillas (10-16%) y peso de 1000 semillas (26-28%) comparadas con los testigos sanos, independientemente del híbrido utilizado. El contenido de aceites determinado por resonancia nuclear magnetica no mostró diferencias significativas entre los tratamientos.

EFFET DE L´INFECTION AVEC LE VIRUS "SUNFLOWER CHLOROTIC MOTTLE VIRUS" (SuCMoV) SUR LES PARAMETRES DE LA PRODUCTION EN TOURNESOL

RÉSUMÉ

Le virus "Sunflower chlorotic mottle virus" (SuCMoV) a eté detecté en plusieurs regions cultiveés avec tournesol provocant chez les plantes une chlorosis systemique. La severite des symptomes depends de plusieurs facteurs y compris l´etat du developpement ontogenique au moment de l´infection. Le but de cet étude a eté determiner les effets des infections provoquées artificiellement á differents etapes de l´ontogénese sur les characteres agronomiques (taille des plantes, diametre de la tige principale et du capitule, production d´akénes, largeur et longueur des graines, poids de 1000 graines et contenu des huiles).

Les graines des hybrides commerciaux Dekalb 4030, Contiflor 3N et ACA 884 out eté semes en parcelles divisés avec quatre repetitions lesquelles etaient inoculeés avec le virus en quatre stades du developpement. Un temoin negatif (non inoculé) a ete utilisé comme control. L´infection virale a eté determinée par la symptomatogie visuel et par DAS-ELISA. Dans tous les etats du developpement on constate une diminution de la taille des plantes (16-39%), du diametre des tiges (21-51%), diametre des capitules (27-57%), production d´akénes (58-87%), largeur des graines (13-15%), longueur des graines (10-16%) poids de 1000 graines (26-28%) par rapport aux temoins non inocules. Ces resultats ont eté constatés en tous les hybrides utilisés. Le contenu en huile, analysé par reaonance magnetique nuclear, n´a pas des differences significatives entre les traitements.