ALTERNARIA RESEARCH DEVELOPMENT IN SÃO PAULO STATE, BRAZIL

Maria Regina Gonçalves Ungaro,. Scientific Researcher, Dr., CEGRAN, IAC.
Caixa Postal. 28. 13001-970 Campinas, SP, Brazil.
Fax: 55 11 4343483; e-mail:ungaro@cec.iac.br
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Summary:

In Brazil, *Alternaria helianthi* was observed for the first time in 1969, in the State of Pernambuco, and, in 1974, was observed in the State of São Paulo. Field evaluations of different sunflower (*Helianthus annuus* L.) genotypes which took place between 1976 and 1978, in the State of São Paulo, indicated the great destructive power of the fungus to which there are no resistant genotypes. The first research with the species consisted of determining the laboratory conditions for its development, followed by green-house inoculations. Field mass selection, chemical control under field conditions, recurrent selection based on half-sib families, studies of disease development in different sunflower genotypes and sowing dates are some of the research topics developed by São Paulo State Government. The results obtained for this research showed that: *A. helianthi* is the most pathogenic species of *Alternaria* for sunflower; chemical control is expensive and does not shows good efficiency; the use of gamma-rays might be a tool for the development of less susceptible genotypes; recurrent selection based on half-sib families is efficient for the development of less susceptible genotypes; disease development is highly dependent on rain intensity.

INTRODUCTION

In 1969, Aquino *et al*. (1971) observed the occurrence of *Alternaria* disease, caused by *A. helianthi* on sunflower in the State of Pernambuco, and Ribeiro *et al*. (1974) reported it in São Paulo State, Brazil. Field trials carried out between 1976 and 1978, in Mococa, SP, showed the destructive potential of *Alternaria* species and the susceptibility of the sunflower genotypes studied. Field and green-house evaluations indicated a greater damage level due to *A. helianthi*, when compared to other *Alternaria* species occurring together. The objective of this paper is to report the development of *Alternaria* research that has been done by São Paulo public research, specially in the Agronomic Institute – IAC.

MATERIAL AND METHODS

Experiment 1- Growth and sporulation under different light regimes

Growth and sporulation of some isolates obtained in São Paulo State were evaluated under 20 and 25°C and three light regimes: a) continuous dark; b) continuous day light; c) fluorescent light. Potato dextrose agar (PDA) was used as growth medium. After 7 and 14 days of incubation the radial growth of the colonies was recorded. Sporulation was evaluated through the spore concentration in a liquid suspension.

Experiment 2- Pathogenicity

Pathogenicity of the isolates obtained from sunflower leaves, petioles, and stems in different regions of São Paulo State, was evaluated through green-house inoculations of 3 sunflower genotypes. The inoculations were made on 45 sunflower plants grown in vases maintained in a humid chamber for 48 h after the artificial inoculation.

Experiment 3- Chemical control

A trial was carried out under field conditions in IAC Experimental Station of Mococa, SP., in a randomised block design with four replications. Each plot was formed by four rows of 5 m long, with five treatments. Spraying was done at 7-day intervals. The treatments were: a) eight applications starting at stage 3.1 (Siddiqui *et al*., 1975); b) four applications from 3.3; c) four applications from 4.1; d) four applications from 4.2; e) control, without fungicides. The evaluations included total number of leaves, percent of senescent leaves, percentage of infection according to Allen *et al*. (1983). The fungicide Difolatan 480 was used at the rate of 2.0 l a.i./ha.

Experiment 4- Environmental influence on disease development.

Alternaria leaf spot (*Alternaria helianthi)* progress curves were evaluated for three sunflower cultivars, IAC-Anhandy, VNIIMK and Contisol 621, at twelve sowing dates. The sowings were made monthly in a split-plot experimental design. Disease severity was recorded during the plant cycle, at 10-day intervals, up to physiological maturity. In the evaluations made on 10 plants per plot, were studied the percentage of dried leaves and the foliar area affected by the fungus using a diagrammatic scale proposed by Allen *et al*. (1969). With these data, a disease index was calculated for each evaluation and the disease amount in each cycle was obtained. The disease index was calculated using the following expression:

$DI = [(A_1 + A_2)/2 + DL] /2$ where,

A_1 = % of disease level on the first live bottom leaf

A_2 = % of disease level on the second live bottom leaf

DL = number of dried leaves multiplied by 100%

With this index, determined for each evaluation, an *Alternaria* leaf spot progress curve was constructed, and the disease amount was calculated using the Area Under Disease Progress Curve (AUDPC), according to Moraes *et al*. (1988):

$AUCDP = \sum\{[(Y_{i+1} + Y_i)/2\} \{(X_{i+1} - X_i)]\}$ where,

Y_{i+1} is the % of disease infected area in the i_{th}+1 observation

Y_i is the % of disease infected area in the i_{th} observation

X_{i+1} is the number of days from sowing in the i_{th}+1 observation

X_i is the number of days from sowing in the i_{th} observation

All data acquired were transformed to DI and AUDPC and submitted to analysis of variance. The Duncan test at 5% was used for mean comparisons.

Values of the disease apparent infection rates, determined by logistic transformation, were correlated with the following climatic variables: mean air temperature (TMED); maximum air temperature (TMAX); minimum air temperature (TMIN); total rainfall (RAIN); wet period duration (WPD); number of rainy days (NRD); number of days with WPD greater than 10 hours (NDWPD10). For this analysis only eleven sowing dates were considered.

Experiment 5- Use of mass selection in the development of less susceptible genotypes

The initial germplasm originated from a bulk between eight different genotypes. During four years, about 1% of the best plants were selected and formed the next generation. The selection procedure occurred in two phases: 1)elimination of the more susceptible plants before flowering; 2)elimination of all unselected plants during flowering. After that, only plants with the least disease symptoms remained to be harvested after physiological maturity. The field disease evaluation was based on disease progress. Plants with disease spots on the upper leaves were cut off before flowering. The second selection was based on disease level and leaf-spot size. After four cycles of selection, an evaluation trial was done, comparing the original disease level with the disease level in cycles 1, 2 and 3, in a randomised block design, with 4 replications.

Experiment 6- Intrapopulational recurrent selection based on half-sib families

Seeds of the French germplasm PIGB were irradiated with 18 Krad of gamma rays. After two recombination cycles, selected heads were individualised as half-sib families. After evaluation under lattice design, the best families were recombined for the next selection cycle. The disease evaluation was made at stages 4.1 and 4.3 (Siddiqui et al., 1975), according to the following degrees: 1- no visible symptoms; 2- some small necrotic leaf-spots, on the basal leaves, not exceeding 5% of the leaf tissue; 3- between 5 and 15% of the leaf tissue with necrotic spots, but no spots in the upper part of the plant; 4- necrotic spots in the upper leaves; lower leaves with no more than 50% of infected leaf tissue; 5- necrotic spots spreading all over the plant, including the head, with necrosis and death of the lower leaves. The hybrid DK-180 was used as check. A comparison was made between irradiated and non-irradiated families.

RESULTS AND DISCUSSION

Experiment 1- Growth and sporulation under different light regimes

The radial growth of the fungus colonies was greater at 25°C at all light regimes. Under 20°C, continuous dark presented better colony growth; at 25°C continuous dark showed the lower colony growth. The isolates sporulated more at 20°C, under both day and fluorescent light, after 7 days of incubation. But after 14 days, continuous dark at 20°C presented a higher spore concentration (Moraes *et al*., 1983).

Experiment 2- Pathogenicity

The first symptoms appeared 48 h after inoculation as small black points spread all over the leaves. After 6-7 days, the leaves presented normal disease symptoms, but with some peculiarities, according to the leaf age. The apical part of the plant (younger leaves) showed isolated necrotic lesions with a characteristic chlorotic halo; the older leaves from the bottom part showed coalescent lesions, with drying and leaf drop 7 days after inoculation. Five days after inoculation, all the leaves of cv. VNIIMK dried and dropped off (Moraes *et al*., 1983). This is an indicative of the greater susceptibility of this genotype and also the high pathogenicity of the fungus.

Experiment 3- Chemical control

Some disease control was obtained with 4 spray treatments starting at stage 4.1 but, as a rule, chemical control does not work (Silveira *et al*., 1986), especially when the disease spreads at the end of vegetative period, and the plants are too tall to be easily sprayed.

Experiment 4- Environmental influence on disease development.

The level of disease incidence seems not to be strongly related to the primary inoculum as the disease evaluations were carried out during the second year of trial, in an area with a great amount of initial inoculum; the disease appeared in the previous sowings and the debris stayed in the field. The variation in the disease level (Figure 1) is clearly related with climatic factors, already discussed by Sentelhas *et al*. (1996). Figure 1 shows the response of disease development in different sowing dates and cultivars.

Figure 1- Seasonal patterns of DI in three sunflower genotypes sown in August and October.

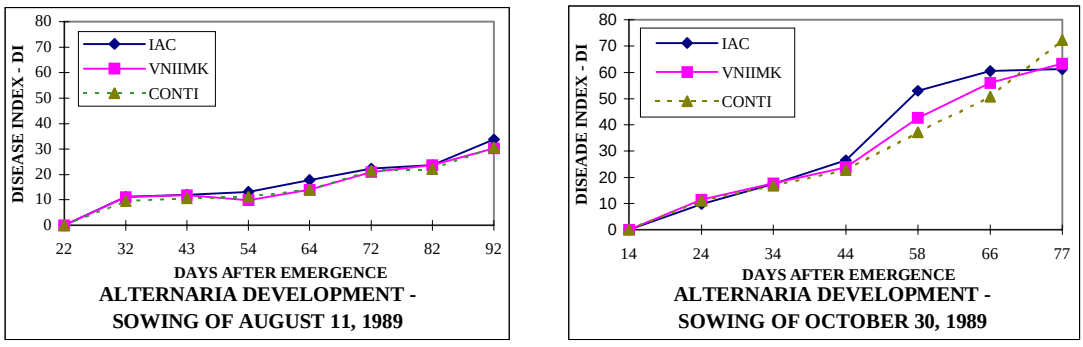

Table 2 shows the AUDPC for all cultivars and sowing times. Mean AUDPC of July and January sowings was 15% and 33% higher, respectively, than August sowing; however, the AUDPC for Conti 621 was not statistically different in those three sowing dates. May and June presented intermediate data for AUDPC. The AUDPC for May was statistically equal to January sowing date, except for Conti 621. November showed the

highest AUDPC, followed by December (Table 2). Data of AUDPC for the sowing of March, April and October were intermediate (Dudienas *et al*., 1998).

Table 1. Area under the curve of disease progress (AUCDP) in 3 sunflower genotypes evaluated in 12 sowing dates under natural field disease infection. Means of 4 replications.

	Genotypes							
Sowing date	IAC-Anhandy		VNIIMK 8931		Conti 621		Mean	
30/10/89	2024.47 a	A	1939.22 a	AB	1854.48 a	B	1939.39 a	
15/12/89	1875.15 abc	A	1836.95 abc	A	1819.47 a	A	1843.86 b	
03/10/89	1856.06 bc	A	1842.93 abc	A	1765.00 a	A	1821.33 b	
02/02/89	1522.42 ef	B	1943.17 a	A	1579.42 b	B	1691.67 c	
05/04/89	1758.27 cd	A	1762.43 bcd	A	1542.54 b	B	1687.75 c	
07/03/89	1978.36 ab	A	1693.60 cde	B	1333.77 cd	C	1668.58 c	
01/09/89	1647.65 de	A	1608.90 de	A	1465.29 bc	B	1573.95 d	
02/06/89	1489.62 ef	B	1896.77 ab	A	1213.30 d	C	1533.23 d	
05/05/89	1560.83 ef	A	1589.25 e	A	1355.26 cd	B	1501.78 de	
19/01/90	1573.26 e	A	1642.22 de	A	1068.18 e	B	1427.88 e	
05/07/89	1410.30 f	A	1304.38 f	A	1008.20 e	B	1240.96 f	
11/08/89	1154.34 g	A	1050.61 g	A	1027.11 e	A	1077.35 g	
Mean	1654.23	A	1678.37	A	1419.34	B		

CV(%) = 6.45. Means followed by the same letter in the column and the same capital letter in the line are not statistically different by Duncan at 5%.

Experiment 5- Use of mass selection in the development of less sensible genotype

The results show that mass selection is not effective to develop genotypes less susceptible to *Alternaria* leaf spot.

Experiment 6- Intrapopulational recurrent selection based on half-sib families

According to Table 2, the mutagenic treatment decreased the genetic variability for *Alternaria* resistance. On the other hand, the recurrent selection decreased disease sensibility level in both populations (Ungaro & Miranda Filho, 1996).

CONCLUSIONS

- For artificial inoculation, spore production is easily obtained in PDA medium, at 20 or 25°C, after 15 days under continuous dark;
- Different genotypes show different levels of disease susceptibility;
- Chemical control does not show good efficiency;
- The level of disease incidence is not strongly related to primary inoculum;
- There is a clear relation between disease level and climatic factors;
- Total rain and air minimum temperature are the most important climatic factors for *Alternaria* development;
- Mass selection does not seems to be efficient for the development of less susceptible genotypes;
- Recurrent selection based on half-sib progenies is efficient in developing genotypes less susceptible to *Alternaria* leaf spot.

- The disease development is directly dependent of the interaction genotype x behaviour;

Table 2- Mean results obtained for subpopulations A (untreated) and B (mutagenic treated) and for check, in the 2nd and 3rd cycles of recurrent selection. Means estimates for environmental coefficient of variation (CVe%), genetic coefficient of variation (CVg%), ratio b (CVg%/CVe%), heritability(h^2m), progeny, additive and phenotypic variance (σ^2_P, σ^2_A, σ^2_F) for *Alternaria* in two selection cycles.

Popul.	Year	*Alternaria* notes #	CVe%	CVg%	b	h^2m	σ^2_P	σ^2_A	σ^2_F
A	1991	2.69	9.46	3.35	0.35	27.37	4.2	16.8	15.2
B		2.88	8.11	2.73	0.34	25.22	2.9	11.6	11.5
T		2.34							
A	1994	1.57	10.10	7.28	0.72	61.54	13.0	52.0	22.0
B		1.75	10.04	5.32	0.53	45.73	8.7	35.0	19.0
T		2.00							

Notes: 0 (resistant) to 5 (susceptible)

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