T1988CROP42

A NEW ALTERNARIA DISEASE OF SUNFLOWER IN GREECE

C.C.Thanassoulopoulos, A.T.Kolokousi
Aristotelian University, Thessaloniki,

SUMMARY

Sunflower was cultivated in approximately 95.000 hectares in Greece during 1987, an area almost hundredfold of those cultivated 10 years ago. A leaf spot disease was observed in all cultivated areas. Main disease symptoms were brown spots on the leaves with concentric rings, which usually appeared before or during flowering. Basic leaves were first attacked and the disease gradually appeared on the top. In serious attack the spots coalesced and the leaf was dry up. The fungus *Alternaria alternata* was permanently isolated and this is the first record of this fungus in this crop in Greece. Pathogenicity tests on sunflower plants were positive in all cases attempted. The fungus had on optimum temperature for growth of 25°C while in 40° C for 7 days was killed. Contrary in temperature even -20° C for 20 days the fungus ceased growing but survive. In 25° C there is 98 % emergence of spores in 24 hours. Begining of emergency happen 3 hours after spores placing in water and 22-24° C. The fungus seemed that penetrated directly the epidermal cells and was growing very fast into the parenchyma of leaves in 25-35°C.

INTRODUCTION

Sunflower crop has been increased almost hundredfold, 1000 hectares in 1980 to 95000 in 1987, in the last decade, mainly in the Northern part of Greece. During 1986 an extensive leaf spot was observed in several areas. Early symptoms as a very tiny, difficulty distinguishable, pinpoint, brown spots with a yellow halo were usually observed on fourth or fifth leaf (V.4 to V.5 stages)(McMullen, 1985) but also later until the V.12 stage. The spots usually had a target spot appearance with brown center, were fast growing and appeared on basic leaves at the begining gradually appearing on the upper leaf pairs. In serious attack the spots coalesced and the major part of leaf lamina or the whole leaf dried up. Spots were not observed on stems and were very rare on petioles and head green parts. The disease appeared to have a higher growth rate in humid enviromment. Disease loss assessment has not been studied but the epidemiology of the disease and the 50 - 80% attack of the leaves in several areas indicates that under favourable temperature and humidity conditions the disease could cause early and severe defoliation.

Pathogen identification, pathogenicity and some other biological properties of the pathogen are given in the present paper.

MATERIALS AND METHODS

Pathogen isolations were effected by transfering diseased leaf tissues previously disinfected with alcohol washing, or hyphae grown in moist chamber, in growth medium. Potato dextrose agar (PDA, Oxoid Ltd, Code CM 139) was used as growth medium throughout the work. During all experiments was used a monospore isolate of the fungus obtained from sunflower plants grown in Nea Mesembria area 25 Km west of Thessaloniki. Growth studies of the fungus were effected in Petri dishes, three for each temperature. Microscopic examinations of spores germinating in water drop were done every half hour until the third hour and then in the 21st and 24th hour. In temperatures below zero the fungus, previously grown or not in Petri dishes or in colony grown in Richard's (Ainsworth et al, 1971) growth medium after filtering and getting the

colony on the filter paper, was placed in refrigarator.

Artificial inoculation were macle on sunflower seedlings grown in test tubes (Neergaard, 1945), in large glass jars,in pots and in field plants in the V.8 to V.10 stage. Seedlings in tubes or glass jars were obtained from seeds previously disinfected with1% mercury chloride for 20min.Plants in pots or in the field were covered with plastic bugs 24 to 48 hours or in other cases were not covered. Inoculations was effected by spraying spores and mycelia solution in water on plant parts slightly injured or not.
Studies of pathogenesis were made on sunflower leaves grown in aseptic comditions. Four to 6 drops of spores and mycelia solution in water were placed on each leaf. The leaves were placed on wet filter paper in petri dishes at 20-25°C in the dark for 48 and 72 hours. At the end of these periods sections were made, of green or decolorized with a mixture of 1:1V/V alcohol 95° and acetic acid (Green et al, 1957), with microtome or by hand and microscopically examined.

RESULTS

Fungus identification. The same fungus was always isolated from all attempts made without exception, and was also observed in straight microscopic examinations of diseased tissues, which easily identified as Alternaria sp. (Ellis, 1971; Joly, 1964; Neergaard, 1945; Simmons, 1967). The fungus spores were dictyosporae in long chains of more than 10 spores in each, light or olive brown, beacked or not, sized 10.0-49.5x5.0-17.5 μm.The length of the main spore was 10.0-39.0μm and that of the beack 2.5-17.5 μm.Comparison of the morphological characters of the colony and the spore and of the sizes of spores with those of other authors (Ellis,1971; Joly, 1964; Neergaard, 1945; Simmons, 1967; Vakalounakis et al, 1982) showed that the pathogen was *A. alternata* (Fr.) Keissler.

Growth of the fungus

The fungus does not grow in 0° C and has a slight growth in 1-2°C, while in 5°C the growth is significant. Optimum growth temperature was 25° C while in 40° C the fungus died in 7 days (Fig. 1). In temperatures below 0° C up to -20° C for 20 days colony growth is inhibited but mycelium remain alive and regrowth is certain when the colony is tranferred in 25°C.

Spores germination.

Spores germination begins 2-3 hours after the placing of spores in water and 20 - 22° C. At 5° C germination was not observed but was observed in 8°C. Optimum temperature for germination was 25°C (98% of the spores germinate in less than 24 hours) while in 40°C germination was not observed (Fig.1).

Pathogenicity

Pathogenicity was studies in 40 sunflower plants grown in the field and in more than 100 seedlings, grown under aseptic conditions in test tubes or in glass jars. In all cases, without exception, inoculations were positive and only disease growth rate was diferentiated depenting on humidity of the environment. At 20-25°C and 100%R.H. the tips of young plants succumbed in 48 hours.In natural conditions similar symptoms were not observed. In infected plants were observed spots similar those grown in natural conditions, from which the same fungus was also isolated. Inoculations made with a fungus colony which had remained in -20°C for 20 days were positive without exception and infection caused similar symptoms.

Pathogenesis

Appressorium formation and hypha growth from it (Fig. 2) was

observed in 48 hours on the leaves, while in 72 hours tissue infection was clearly evident by direct penetration of the epidermal cells (Fig. 3) and possibly through stomates. After the penetration process has completed mycelium was growing very fast in the parenchymatous tissues. Slight chlorotic spots appeared around the point of the fungus penetration 3-5 days later or more depending on temperature and humidity conditions.

DISCUSSION

Six Alternaria species have been reported on sunflower, A. helianthi A. iniae, A. alternata, A. helianthicola, A. leucothemi and A. tenuissima (Iliescu et al, 1948; Hedjaroude, 1973; Kolte, 1985; McMullen, 1985; Sackston, 1978) of which A. alternata is usually reported as secondary or weekness pathogen. In Greece was reported A. helianthi (Tjamos, 1983), while A. alternata is first reported in this paper. In these studies was indicated that the fungus has the ability to penetrate directly in the host tissues as a primary pathogen (Smedegaard et al, 1985), under favourable conditio s for spores germination and mycelium development.

In Greece, the optimun temperature conditions for disease development are prevalent in summer when sunflower crop is cultivated. Therefore humidity of the environment seems to be the limiting factor of disease development for areas were humidity due to fog or mist in the morning or rivers and lakes neighbouring, is not to high.

It is so probably that A. alternata could appear under epidemic conditions which would be serious for sunflower industry.

REFERENCES

AINSWORTH G.C., JAMES P.W. and HAWKSWORTH D.L., 1971. Dictionary of the fungi. Commonwealth Mycological Institute, p. 663.

ELLIS M.B., 1971. Dematiaceous hyphomycetes. Commonwealth Mycological Institute, p. 607

GREEN G.J. and DICKSON J.G., 1957. Pathological histology and varietal reactions in Septoria leaf bloth of barley. Phytopathology, 47: 73-79.

ILIESCU H. and POPESCU I., 1984. Possibilities for chemical control of some major diseases of sunflowers. BASF Agricultural News, 2: 8-14.

JOLY P., 1964. Le genre Alternaria. Ed. P.Lechevalier, Paris, p. 250.

HEDJAROUDE GH.A., 1973. La maladie des taches brunes du tournesol (Helianthus annuus L.) en Iran. Phytopath. Z. 78:274-277.

KOLTE S.J., 1985. Diseases of annual edible oilseed crops. Vol. III. C.R.C. Press p. 9-96.

McMULLEN M.P., 1985. Sunflower production and management. N.Dakota Agr.Exp.Sta., Ext. Bull. 25, N.Dakota State University, p. 76.

NEERGAARD P., 1945. Danish species of Alternaria and Stemphylium. Oxford University Press, London, p. 559.

SACKSTON W.E., 1978. Sunflower disease mapping in Europe and adjacent mediterranean countries. Proc. 8th Intern. Sunflower Conf., Minn. U.S.A., p. 7-29.

SIMMONS E.G., 1967. Typification of Alternaria, Stemphylium and Ulocladium. Mycologia, 59: 67-92.

SMEDEGAARD-PETERSEN V. and TOLSTRUP K., 1985. The limiting effect of disease resistance on yield. Ann.Rev.Phytopathol. 23:475-490

TJAMOS E.C., 1983. Occurence of Alternaria helianthi of sunflower in Greece. 9th Interbalcanic Plant Prot. Conf., Athens.

VACALOUNAKIS D.I. and MALATHRAKIS N.E., 1982. An Alternariosis of cucumber due to Alternaria alternata. 2nd Scientific Meeting on Covered Vegetables and Flowers. p. 54.

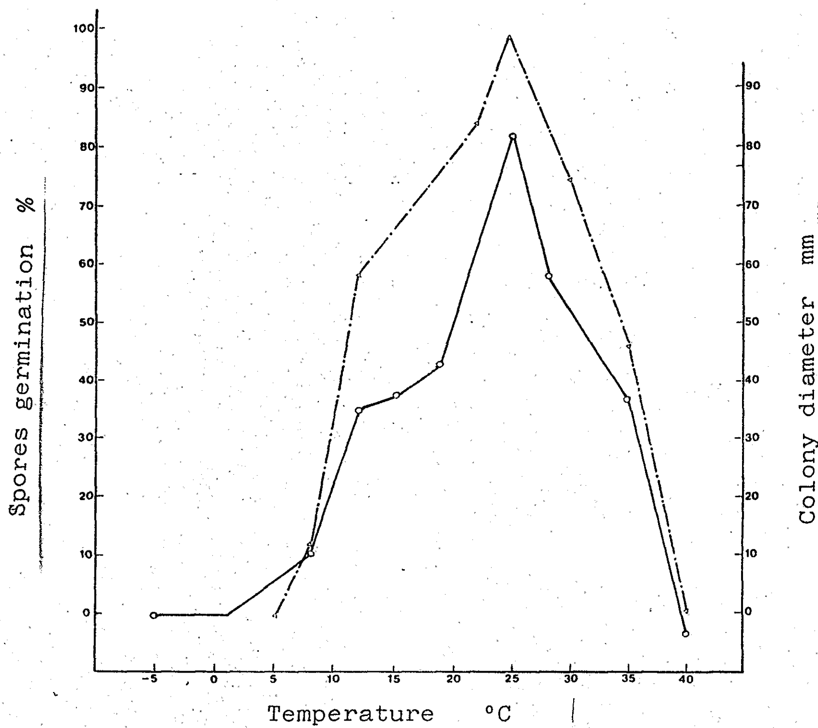

Fig. 1. Growth (————) and spores germination (— . — . —) of the fungus Alternaria alternata

Fig. 2. Appressorium formation on Sunflower leaf by Alternaria alternata 48 hours after inoculation

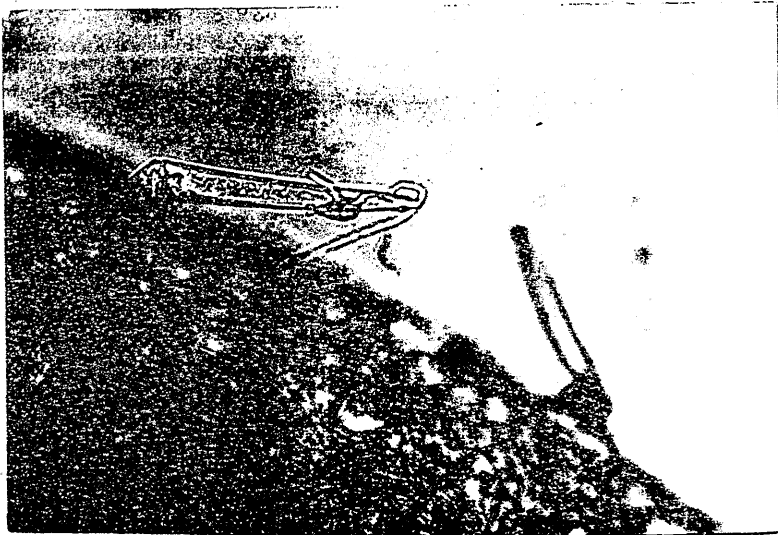

Fig. 3. Direct penetration of epidermal cells by Alternaria alternata of Sunflower leaf, 72 hours after inoculation