DRECHSLERA HELIANTHI nov. sp. ISOLATED FROM A SUNFLOWER STALK

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In July 1973 the mass appearance of disease symptoms generally manifested by different levels of leaf drying, brown lesions with various shapes and sizes on stalks, petioles and heads, and partial or total empty seeds was reported in a sunflower field of Constantza district.

Several genera and species of fungi were isolated on nutrient media from different diseased tissues and organs, some of them such as *Rhizopus stolonifer* (Ehrenb. ex. Fr.) Lind., *Septoria helianthi* Ell et Kell., *Phoma oleracea* var. *helianthi tuberosi* Sacc., *Alternaria tenuis* Auct., *Botrytis cinerea* Pers., being well known as sunflower pathogens in Romania. Different new pathogens had been reported in Romania on other cultivated species except two fungi. One of them was identified and described as *Alternaria helianthi* (Hansf.) Tubaki and Nishihara (Hulea, Iliescu, Bunescu, 1973). The second fungus, described here, in many respects similar to *Helminthosporium* was provisionally designated *Drechslera helianthi* n. sp. in order to avoid any confusion with the two *Helminthosporium* species described in 1943 by Hansfeld in Africa and in 1964 by Pavgi and Upachyay in India (both cited by Tubaki and Nishihara, 1969) these latter being considered in 1969 by Tubaki and Nishihara as identical to *Alternaria helianthi* studied by them in Japan.

The fungus was isolated from a sunflower stalk on potato-dextrose-agar and 3 to 4 days later developed dark colonies, occasionally transparent due to the absence of aerial mycelium, or opaque and scarcely fluffy. In some colonies the outline was smooth and complete, but in others very irregular or festooned, and with the opposite side dark or dark-brown.

The aspect of the colonies varied with the environmental factors. The colonies were either uniform on the whole surface, or developed radially and at the same time concentrically or as waves with different shapes.

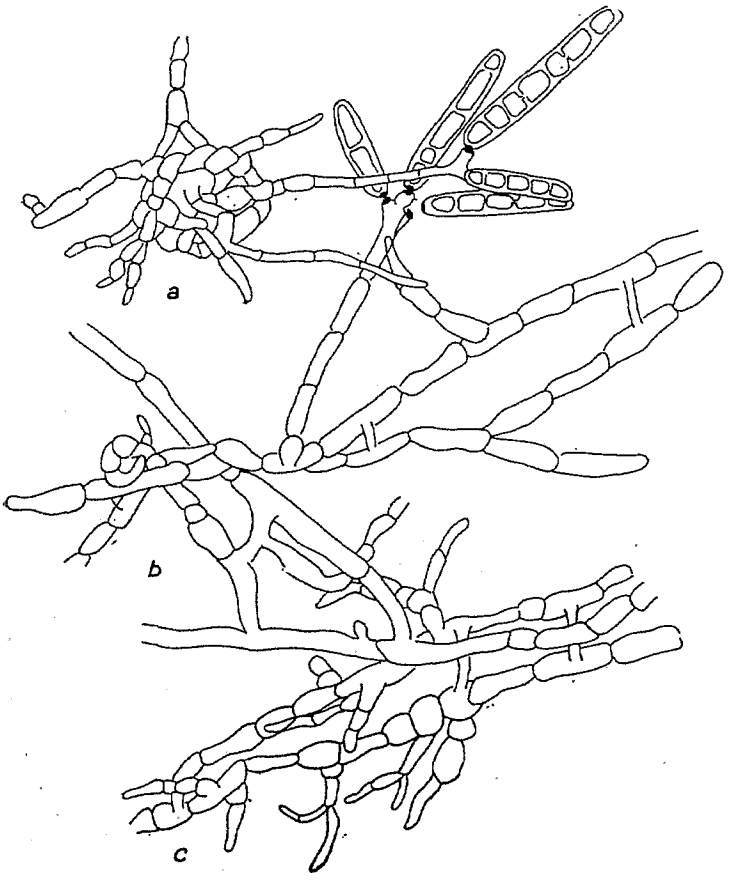

Fig. 1 — Different types of mycelial filaments in *Drechslera helianthi* *a*—tightly weawed filaments forming a sterile structure; *b*—anastomosed filaments with a trend to coilling up; *c*—strongly branched and anastomosed articulated filaments.

The fungal mycelium consisted of filaments that were submerged or tightly adhering to the substrate, or in a few cases even grew upwards. The mycelial filaments were dark, varying in thickness, abundantly branched and septate, particularly the thicker ones which appeared articulated (fig. 1).

Brown, septate, simple conidiophores with different lengths and nodoses at the freed end grew on the mycelial filaments (fig. 2). One to five large, cylindrical, light brown to dark brown, strainght or bent, 1—8 septate acropleurogenous conidia with thick walls developed. A ring-like hilum was apparent at one end. The septa become gradually visible at a single end in some conidia, in others at both ends simultaneously, and in others in the center only and, thus, in the microscopical field both long 1—2 septate conidia and 6—8 septate conidia with a

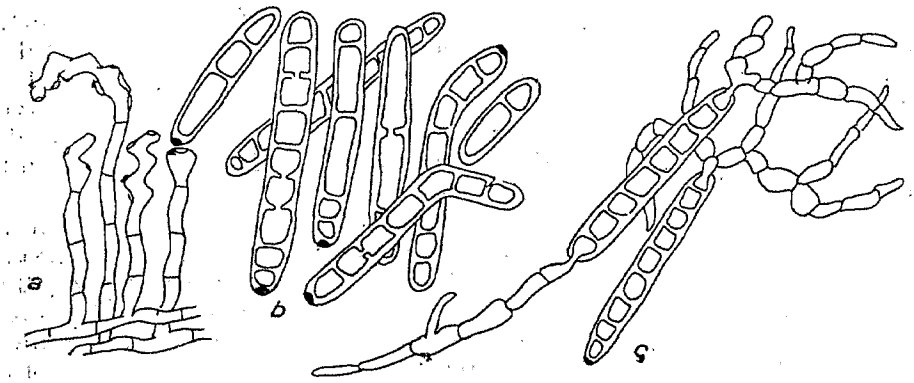

Fig. 2—Conidiophores (a) and dormant (b) and germinated (c) conidia of *Drechslera helianthi*.

similar length were visible. The rings between septa appeared as pierced by a small pore of communication between two cells, in others this pore was absent. Conidial sizes ranged between 53 and 180 μ in length and 12—15 μ in width.

According to the characteristics described above, this fungus belongs to the genus *Helminthosporium*, the subgenus *Cylindrohelminthosporium*, distinguished by Nishikado in 1962 on the basis of its cylindrical conidia and later (1930) included by Ito (both cited by Luttrell, 1954) in the genus Drechslera (Deschpande and Deschpande, 1968; Luttrell, 1954; Schoemaker, 1959).

Optimum conidial germination occurs at 22—24°C. Usually each spore develops a filament at one or both ends (fig. 2), and seldom from the intermediary cells. The germination threads grow rypidly, they are abundantly branched and, frequently, septate or they seem articulated. Anastomoses occur between two adjacent filaments originating from two different conidia.

Here and there the ramifications of the germination threads weave themselves forming ball-like structures, first brown coloured and later, as they grow older, their outline becomes marked; brown, septate, fulcrum-like thread ends with different length persist. Some of these filaments grow into conidiophores bearing 1 to 3 conidia at their end (fig. 1).

Such black structures, formed by filament interweaving, generally were round or ovally shaped (fig. 3); others developed a tiny conical

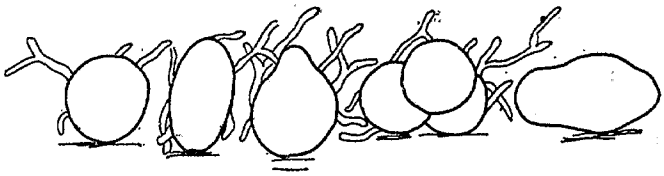

Fig. 3—Different forms of sterile structures in *Drechslera helianthi*.

neck like in perithecia and still others merged by twos or threes forming small, unevenly-shaped groups. On some substrates such structures developed abundantly and even formed a black, dense and hard crust, but on other substrates fewer structures developed ; on media such as Czapek-Dox they were very rare and slightly submerged in the substrate or absent. If pressed on the slides, only a mass of oily droplets with different sizes was obtained.

The relationships between *Drechslera*-type conidia and these microsclerote-or perithecium-like structures, were established by means of single-conidium colonies obtained from a single sterile structure, the same type of colony being always formed. In both cases when the mycelium developed, *Drechslera*-like conidia were formed in the centre and subsequently, the dark structures became differentiated. The proportion between conidia and these structures fluctuated in terms of the nutrient elements, culture media, temperatures, pH, etc.

Drechslera helianthi colonies grew well at temperatures ranging between 16° and 35°C, and optimum growth occurred at temperatures ranging between 18° and 30°C. Colony growth and aspect however, varied in terms of temperature. Thus, at 16°C colony growth was concentric and its outline very irregular. At 18°C the colonies developed in the form of more dense and transparent waves depending on the density of conidia and sterile structures. The margins of these colonies were unevenly wrinkled. At 20° and 30°C, the aspect of the colonies was more uniform, and its outline more or less even.

Fungal development also varied with the nutrient substrate. As a rule the fungus grew well on all the media tested except on sunflower leaf extract, on which the growth was poor, on plum leaf extract as well as on the synthetic Czapek-Dox medium ; on these latter no conidia were formed, but only sterile structures, and even these were rare. The colonies sporulated best on potato, malt, and carrot-leaf extracts, on potato slices, rice kernels, etc. On carrot leaf extract many conidia and rare sterile structures developed ; on this latter substrate, the colonies showed a characteristic aspect, as they were polychrome and with well defined concentric rings.

Fungal growth and sporulation was little affected by the pH value of the substrate. Generally, growth took place at pH values ranging between 2 and 12 (table 1) with a peak between pH 5 and 6 ; at these values, the area of the colonies was 51 cm^2 and 38 cm^2, respectively.

The energetic requirements were determined on Czapek-Dox-agar medium and solution, in which glucose was substituted with other 10 sugars, their amounts being estimated in C equivalent. The observations were made during 9 days on agar substrate and 30 days on Czapek solution. In the first case colony development was evaluated by surface measurement, and in the second one by weighing several times the vegetative mass.

The results (table 2) showed that starch, arabinose, galactose and dextrin are more readily and rapidly metabolized by *Drechslera he-*

Table 1

The surface areas of *Drechslera helianthi* colonies grown on PDA at different pH values (averages of 10 colonies)

pH value	Colony area after 9 days (cm^2)	Observations
2	11.78	Abundant mycelial growth, cottony aspect. Few conidia and sterile structures.
3	14.76	Abundant mycelial growth, cottony aspect. No conidia and sterile structures formed.
4	22.05	Abundant growth, irregular outline. Many sterile structures.
5	50.65	Very abundant growth. Numerous conidia and sterile structures.
6	37.95	Very abundant growth. Numerous conidia and sterile structures.
7	28.80	Abundant growth. Rare conidia, numerous sterile structures.
8	21.42	Abundant growth. Rare conidia, numerous sterile structures.
9	32.64	Abundant growth. Irregular outline, numerous conidia, fewer sterile structures.
10	23.92	Abundant growth, colonies almost circular. Numerous conidia and sterile structures.
11	24.00	Abundant concentric growth. Numerous conidia and rare sterile structures.
12	6.83	Poor growth. Conidia and sterile structures absent.

lianthi, colony surfaces ranging between 6 to 1 cm^2 and vegetative mass weight between 0.9238 and 1.0962 g.

Conidium-sterile structure ratios varied also as regards the sugars, i.e. the sterile structures were more abundant on media containing fructose, rhamnose, mannite, galactose and starch, and conidia were predominant on cellulose media.

Plastic substance requirements were assessed on Czapek-Dox-agar substrate by linear measurement of colonies, and on Czapek solution by weighing the vegetative mass. Mineral salts, organic substances (urea, asparagin and peptone) and amino acids (alanine, valine, serine, guanidine and leucine) were used as nitrogen sources. The results listed in table 3 show that, generally, fungal growth was best promoted by amino acids, serine being the most readily metabolized.

The most favorable organic and mineral nitrogen substances were peptone and ammonium nitrate, respectively. Fungal growth was hardly stimulated by ammonium sulfate, the surface of the colonies that contained this substances being almost equal to that of nitrogen-free colonies.

On amino acid media, the proportion between conidia and sterile structures was in favour of conidia, whereas on mineral nitrogen media,

Table 2

The growth of *Drechslera helianthi* on Czapek media containing different carbon sources

Carbon sources	Colony area after 9 days (cm^2)	Weight of vegetative mass (g)	Observations
Glucose	19.89	0.9003	Irregular dark-brown colonies. Numerous conidia, sterile structures absent.
Arabinose	30.50	0.9933	Abundant growth, irregular outline. Numerous conidia and sterile structures.
Rhamnose	15.04	0.8013	Good growth, colonies with irregular outline. Rare conidia and sterile structure.
Fructose	23.65	0.9117	Round cottony colonies. Rare conidia, sterile structures agglomerated in the centre.
Raffinose	8.32	0.7682	Downy colonies. Many conidia peripherically, sterile structures in the centre.
Mannite	19.11	0.8993	Abundant growth. Conidia absent, numerous sterile structures.
Lactose	12.40	0.7873	Colonies with irregular outline. Very rare conidia, small and malformed sterile structures.
Galactose	25.76	0.9136	Abundant growth. Rare conidia, numerous sterile structures.
Cellulose	18.40	0.7935	Poor growth. Many conidia, fewer sterile structures.
Starch	30.87	1.0962	Abundant growth, more sterile structures in the centre.
Dextrin	28.42	0.9238	Abundant growth, colonies almost circular, numerous conidia and sterile structures.
Sugar-free (check)	4.18	0.0111	Poor growth. Few conidia and sterile structures.

more sterile structures than conidia appeared. Media containing organic nitrogen stimulated in equal proportions the development of sterile structures and conidia.

The results appeared in the same order on liquid media. The greatest weight was that of the colonies growing on amino acid solutions, and among these serine ranked first, followed by the colonies developing on organic (peptone) and mineral nitrogen sources. The growth of the vegetative mass was best promoted by ammonium nitrate and least by ammonium sulfate.

Fungicides such as Panogen, Cryptodin, Orthocide 50, Tiradin 75 and Maneb 80, added to the nutrient substrate, completely inhibited the development of the fungus, their highly toxic effects on this fungus being thus proved.

Table 3

Growth of *Drechslera helianthi* colonies on solid and liquid Czapek media containing different nitrogen sources — (average values of 10 colonies)

Nitrogen source	Colony surface on agar-Czapek media (cm^2)	Weight of vegetative mass in Czapek solution (after 28 days) (g)	
Na nitrate	15.99	0.9013	Many sterile structures, few conidia.
Na nitrate	14.40	0.8971	
K nitrate	14.82	0.8961	
K nitrate	11.16	0.7136	
Ammonium nitrate	12.48	0.8135	
Ammonium nitrate	12.48	0.8135	
Ammonium sulfate	6.30	0.5367	
Urea	12.60	0.7938	Numerous sterile structures and conidia.
Asparagine	17.63	0.9163	
Peptone	18.48	0.9935	
Alanine	13.12	1.2166	Numerous conidia, fewer sterile structures
Valine	28.20	1.2165	
Serine	50.10	1.4331	
Guanidine	24.38	1.1613	
Leucine	23.50	1.0170	
Free of nitrogen (check)	5.06	0.5467	

As to the light, it was found that the development of *Drechslera helianthi* colonies was favoured by continous day light, under such conditions the surface averaging 38.3 cm^2; alternative lighting (12 hours in the light + 12 hours in the dark) only allowed a growth of 27 cm^2. Sporulation was more abundant under continous light.

Conidial development was most influenced by UV light (dark bulbs) to the detriment of the sterile structures when exposed 3 times for 12 hours daily; but the conidium-sterile structure ratio was equal when exposed for only 12 hours.

Drechslera helianthi n.sp. was not pathogenic to sunflower seedlings, neither to cotyledons nor to seedlings in the 2 to 4 true leaf stage. None of the infection methods tested (conidial suspensions sprayed on intact and wounded leaves, suspension droplets laid on detached sunflower leaves at different humidity (70—100%) and temperature (10° to 30°C) was able to induce symptoms. Under such conditions, the development and sporulation of the fungus on the leaf occurred in association with a common *Alternaria* species, and in the form of spots on the filter paper surrounding the leaves sprayed with fungal suspensions.

The wheat, barley and oat seedlings infested with the same suspension were free of symptoms, a fact favouring the assumption that this fungus is not a graminicolous species that arrived accidentally on the sunflower stalk from where it was isolated.

These repeated failures allowed us to draw the conclusion that *Drechslera helianthi* is a saprophytic fungus infesting the sunflower stalks that are either drying or growing weak due to other causes.

CONCLUSIONS

Preliminary studies showed that the fungus isolated by us out of the associated fungi occurring on sunflower plants, — can be included in the genus *Drechslera* on the basis of the morphological characters of conidia and conidiophores. The abundant black structures formed on some media may be considered as sterile peritechia or microsclerotes. The presence of the microsclerotes in several *Helminthosporium* and *Drechslera* genera was already mentioned in the literature (Hochreakov, 1953; Ramachandra-Reddy, 1963). The fungus isolated by us from a drying sunflower stalk shows a marked saprophytic character and, therefore, is practically unimportant.

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