A NEW BACTERIAL ROT OF SUNFLOWER.

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SUMMARY

A new bacterial rot of sunflower has appeared in several countries with different climates. First symptoms are white viscous material between the florets and necrosis of parenchyma at the floret base. Capitula in the course of maturation show brown lesions on the dorsal surface, generally close to the bracts and the lacunate parenchyma is liquified. However, seed maturation is quite normal. The lesions may extend to the stem, forming black spots on petioles.

A single, as yet unidentified bacterial strain has been isolated from early symptoms. Rot symptoms on capitula have been reproduced on isolated plants using a bacterial suspension either sprayed on after wounding or inoculated into the parenchyma or bracts.

If plants are isolated under insect-proof netting, natural attacks do not occur, suggesting that insects may be involved in disease propagation. Honey and bumble bees have no effect but a *Lygus* sp. increases bacterial infection.

INTRODUCTION

A new disease of sunflower capitula appeared in France in 1988. Only certain inbred lines are susceptible.

The symptoms appear during maturation, when the capitulum starts to yellow. A black necrosis appears at the base of the florets, with the presence of white viscous material. Within the capitulum the lacunate parenchyma become liquified, leaving only the fibres, and a characteristic odour is produced. A soft rot spreads to the whole of the capitulum and the dorsal surface turns brown. When exudates from diseased tissues fall on leaves or petioles they cause brown necroses which attract wasps (*Vespa germanica*). These symptoms do not ressemble the bacterial infections described by GUDMESTAD *et al.* (1984) and DURAN (1987). We have observed these symptoms on the same sunflower genotypes in Argentina, Bulgaria and Hungary.

Observations carried out from 1988 to 1991 have shown that this disease is not transmitted by seed and that seed production is reduced around the infection site only by loss of the seed stuck together by the viscous material. Sunflower isolated under insect-proof cages never show the disease, suggesting that insects play an important rôle in its epidemiology.

This paper reports isolation of the pathogen, search for vectors and methods of artificial infection useable as breeding tests.

MATERIALS AND METHODS

Sunflower genotypes : A susceptible inbred line LS and a resistant inbred line LR.

Insects

Honey and bumble bees (*Apis melifera* and *Bombus terrestris*) sunflower pollinators, and a *Lygus* sp. characterised by the presence of a white V on the abdomen and frequent on sunflowers (BADENHAUSER and BOURNOVILLE, 1982) were used in research for insect vectors.

Growth conditions

The sunflowers were grown in the field to observe disease expression in natural conditions and to isolate the pathogen.

For artificial infections and vectors studies, the sunflowers were grown under insect-proof cages covered with nylon mesh with 0,9 mm pores (TOURVIEILLE et *al.*, 1986).

Isolation of bacteria

Capitula whose only disease symptom was viscous liquid between the florets were harvested and 1 to 5 mm diameter

fragments of necrosed parenchyma were extracted and surface sterilised with 95° ethanol. These fragments were then ground in sterile distilled water. The suspension was spread on a buffer medium at 24°C.

Buffer medium

- Buffer solution : 14.5 ml KH_2PO_4 0.5M,
  28.2 ml NA_2HPO_4 0.5M,
  distilled water to make 900 ml,
  15 g agar,
  Autoclaved 20 mn at 110°C.

- Nutritive solution : 10 g glucose,
  1 g yeast extract,
  5 g bactopeptone,
  Distilled water to make 100 ml,
  Autoclaved 15 mn at 110°C.

The two solutions are mixed in sterile conditions.

Inoculum

1. Capitular tissues showing first necrotic symptoms are ground in sterile water. The resulting suspension is then filtered to obtain a sprayable liquid.

2. A bacterial suspension was obtained from 3 day old cultures on the buffer medium in sterile water, with a concentration of 10^8 bacteria/ml.

Sterile water was used as a control.

Infection Methods

1. The floral surface of capitula were sprayed with 5 ml inoculum, following the procedure used, for example by VEAR and TOURVIEILLE (1988) to determine resistance of sunflowers to *Sclerotinia sclerotiorum*.

2. The same spray technique, but after wounding of the floral surface with entomolgical needles.

3. Injection of 10 ml inoculum into the lacunate parenchyma through the dorsal surface of the capitulum.

4. Injection of 10 ml inoculum into the capitular bracts.

RESULTATS

Pathogen Isolation :

Isolations from early symptoms showed the presence only of bacteria (Table 1). In all cases the same characteristics were observed : rounded, shiny, cream, coloured colonies, 1 mm in

diameter. Only for isolation number 3 were any other types bacteria observed (3.2).

Table 1 : Study of bacterial isolates : morphology and pathogenicity.

Isolation nb.	Isolate nb.	Morphology	Pathogenicity
1	11	cream, rounded shiny, ϴ = 1mm	pathogenic/LS non pathogenic/LR
2	21	cream, rounded shiny, ϴ = 1mm	pathogenic/LS non pathogenic/LR
3	31	cream, rounded shiny, ϴ = 1mm	growth problem
"	32	white, rounded shiny, opaque ϴ 1,5mm	non pathogenic/LS non pathogenic/LR
4	41	cream, rounded shiny, ϴ = 1mm	growth problem
6	61	cream, rounded shiny, ϴ = 1mm	pathogenic/LS non pathogenic/LR
7	71	cream, rounded shiny, ϴ = 1mm	pathogenic/LS non pathogenic/LR
8	81	cream, rounded shiny, ϴ = 1mm	

LR = resistant line
LS = susceptible line

The different infection methods discussed below showed that the cream coloured bacteria were pathogenic on the sunflower genotype LS but not on LR. Isolations 6, 7 and 8, from capitula infected with strains 1.1 and 2.1 showed the same morphological and parasitic characteristics as the original isolates.

However, this bacterial strain is difficult to conserve. The buffer medium used did not permit its identification.

When reinfected, strain 3.2 gave no symptoms and studies were not continued.

Infection Methods

The sterile water controls and the spray technique without wounding gave no symptoms on either LS or LR. In contrast the spray technique with wounding and the injections gave symptoms on LS but not on LR (Table 2).

Table 2 : Percentage reproduction of bacterial symptoms by 4 artificial infection techniques.

technique	spraying		spraying after wounding		parenchyma injection		bract injection	
genotype	LS	LR	LS	LR	LS	LR	LS	LR
sterile water	0	0	0	0	0	0	0	0
ground capitulum suspension	0	0	7	0	53	0	87	0
bacterial suspension	0	0	40	0	53	0	47	0

LR = resistant line
LS = susceptible line

Possible insect vectors

The insect colonies were active in the netting cages. The honey and bumble bees collected nectar and pollen, the *Lygus* were observed on all plants and all organs. Results are given in Table 3. The sunflower LR never showed symptoms. In the presence of bees no LS plants showed symptoms, even when they were sprayed with inoculum.

In contrast, in the presence of *Lygus*, 14 % of LS plants showed symptoms after spraying of inoculum. No symptoms were observed on unsprayed plants. *Lygus* do not appear to be vectors of the disease but the wounds they provoke to feed provide entries for bacterial infection.

Table 3 : Percentage bacterial attack in the presence or absence of insects, with and without artificial infections.

Insects	none		*Lygus*		*Apis*		*Bombus*	
Artif. Inf.	+	-	+	-	+	-	+	-
inbred LS	0	0	14	0	0	0	0	0
inbred LR	0	0	0	0	0	0	0	0

LR = resistant line
LS = susceptible line

CONCLUSION

An undentified bacterium, characterised by its shiny, cream coloured colonies causes rotting symptoms on the capitula of certain sunflower genotypes. Reinoculation of bacterial suspension reproduces characteristic symptoms.

Superficial wounding is necessary to obtain infection. This can be obtained artificially using fine needles or naturally by pricks due to feeding of *Lygus* sp. The satisfactory reproduction of symptoms and distinction between sunflower genotypes makes it possible to envisage tests in resistance breeding programes.

Insects do not appear to transmit the disease, but presence of *Lygus* spp. may facilitate its development. Direct chemical control in the field is not possible since large scale use of antibiotics is not authorised, and insecticides to reduce *Lygus* populations during flowering would destroy pollinators. Genetical control therefore appears the most satifactory method to limit the incidence of this bacterial disease.

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