problem becomes a statistical one. It will be necessary to determine the sample size required to assure the identification of individual infected or infested seeds at various tolerance levels.

We have applied the ELISA test only to spore suspensions, young seedling tissues, and seeds. If in our pending studies we consistently obtain positive results with tissues of inoculated plants which survive infection and produce seeds, ELISA can have an immediate application under Australian conditions. Sunflower seed imported for research or other purposes must be grown for two generations under quarantine. If ELISA testing of plant tissues when the seed of the first crop is mature gives negative results, it might be accepted as evidence of freedom from *P. halstedii* infections, and might permit release of the seed lot for increase after only one generation in quarantine.

ACKNOWLEDGEMENTS

We are grateful for the financial assistance given to one of us (W.E.S.) by the Natural Sciences and Engineering Research Council of Canada. We are also grateful to Dr. J.A. Hoes for supplying sunflower seed and the Morden isolate of *P. halstedii,* and to Juan Gonzalez for assistance in producing infected plants.

LITERATURE CITED

COHEN, Y. and SACKSTON, W.E. 1973. Factors affecting infection of sunflowers by *Plasmopara halstedii. Canadian Journal of Botany* 51:15 — 22.

FICK, G.N. and AUWATER, G.E. 1981. A new race of downy mildew affecting sunflower. Proceedings Sunflower Forum and Research Workshop January 27 — 28, 1981. Fargo, N.D. pages 15 — 16. Sunflower Association of America.

SACKSTON, W.E. 1981. Downy mildew of sunflower. *In* Spencer, D.M. (Editor). The Downy Mildews, *pp* 545 — 575. Academic Press, London.

VOLLER, A., BARTLETT, B., BIDWELL, D.E., CLARK, M.F., and ADAMS, A.N. 1976 (a). The detection of virus by enzyme-linked immunosorbent assay (ELISA). *Journal of General Virology* 33:165 — 167.

VOLLER, A., BIDWELL, D.E., and BARTLETT, A. 1976 (b). Enzyme immunoassays in diagnostic medicine. *Bulletin of the World Health Organization* 53:55 — 56.

WENDLER, P.B., GOTLIEB, A.R. and BERGDAHL, D.R. 1980. A newly discovered serotype of *Gremmeniella abietina. Canadian Journal of Botany* 58:1923 — 1928.

RESISTANCE TO A NEW RACE OF SUNFLOWER DOWNY MILDEW.

G.N. FICK and G.E. AUWARTER
SIGCO Research, Inc. Breckenridge, U.S.A. MN 56520.

ABSTRACT

A new race of downy mildew discovered in the U.S. in 1980 was found to be widespread. Selected lines with known genes for resistance that have been used throughout the world in breeding resistant cultivars were all susceptible to the new race. Three sources of resistance were identified among more than 400 diverse sources of germplasm. Resistance to the new race was due to a single dominant gene that was designated Pl_5. It is suggested that the new race be referred to as race 3 and that the previously identified European and Red River races be known as race 1 and 2, respectively.

INTRODUCTION

Downy mildew incited by *Plasmopara halstedii* (Farl.) Berl. et de Toni has been a serious disease of sunflower *(Helianthus annuus* L.*)* in the United States. During the early 1970's fields with as high as 90 percent infected plants and with yield losses exceeding 50 percent were observed (Fick and Zimmer, 1974; Zimmer, 1971).

Downy mildew has been controlled effectively in recent years by resistant hybrids. These hybrids possess the Pl_2 gene for resistance to the Red River race which occurs widely in the major production area of the U.S. (Fick 1978; Fick and Zimmer, 1974).

In 1980 we reported on the discovery of a new race of downy mildew to which all hybrids grown currently in the U.S. were susceptible (Fick and Auwarter, 1981). In this paper we report on the widespread occurrence of this new race and of our progress in identifying sources of resistance.

MATERIALS AND METHODS

Information on the occurrence of the new race of downy mildew was obtained from field surveys and reports by industry representatives. Fields with 10 percent or more infected plants were assumed to be infected with the new race as past experience has shown that most hybrid seed lots have zero to five percent susceptible plants when tested for resistance to the Red River race. The occurrence of the new race was verified in some but not all fields by collecting viable spores and inoculating plants in greenhouse trials.

More than 400 diverse sources of germplasm consisting of inbred lines, hybrids and populations from our breeding program were screened for resistance to the new race. Most sources originated from high oil Soviet cultivars or from crosses of Soviet cultivars with wild *H. annuus.* A few sources were from crosses of cultivated sunflower with *H. tuberosus, H. petiolaris* or *H. argophyllus.* Appropriate testcross, F_1 and F_2 populations involving resistant lines were also evaluated following initial screening results.

Inoculations with downy mildew were made in the greenhouse according to the procedures described by Zimmer (1974). The source of inoculum was derived from downy mildew infected leaves collected in September 1980 from a field near Breckenridge, MN. The collection of zoosporangia was increased and maintained in the greenhouse on susceptible cultivars prior to use in initial tests.

Susceptibility of the seedlings was indicated by sporulation of the fungus on the cotyledons or the undersurface of the first true leaves after 16 hours in a saturated humidity chamber. Less than 10 percent of the plants of susceptible lines escaped systemic infections.

RESULTS AND DISCUSSION

Occurrence of the New Race

Observations in 1981 indicated that the new race of downy mildew was widespread. Fields with as high as 70 percent infected plants were reported from 12 counties of Minnesota and the Dakotas. Most fields were located in or near the Red River Valley, an area with climatic conditions conducive to downy mildew and a relatively long history of sunflower production.

The widespread occurrence of the new race was further substantiated by the results of an additional survey encompassing about 300 fields in a 40 county area of Minnesota and the Dakotas. (T.J. Gulya, personal communication). In a sampling of 63 of 85 fields with downy mildew infected plants over 90 percent had the new race.

Sources of Resistance

Selected lines with known genes for resistance to downy mildew were initially evaluated for resistance to both the Red River and the new race (Table 1). As expected from previous information (Zimmer, 1974), lines with the Pl_2 and Pl_4 genes were resistant to the Red River race. Each of the genes Pl_1, Pl_2 and Pl_4 confers resistance to the race of downy mildew in Europe (Zimmer, 1974). None of the lines with these genes were resistant to the new race. These results confirmed our previous observations that the new race was highly virulent and different from both the Red River and European races (Fick and Auwarter, 1981).

Table 1. Resistance genes and percentage of infected sunflower plants of selected lines inoculated with two races of downy mildew.

Entry	Resistance Gene	% Downy Mildew Red River Race (race 2)	New Race (race 3)
Peredovik	—	95	98
HA 89	—	100	90
RHA 265	Pl_1	98	100
RHA 266	Pl_1	100	100
CM 90 RR	Pl_1	93	93
HA 60	Pl_1	91	100
HA 61	Pl_2	2	100
CM 29 — 1	Pl_2	10	93
RHA 273	Pl_2	0	98
RHA 274	Pl_2	3	100
Hybrid 894	Pl_2	6	100
HIR 34	Pl_4	6	100

Data averaged over two experiments about 25 seedlings each entry per test. Resistance genes according to Zimmer (1974) and Vear (1978).

Three sources of resistance to the new race were identified from among the more than 400 germplasm sources that were subsequently evaluated. These sources originated from the Soviet cultivar Progress, an HS hybrid from Romania and a cross of an ornamental sunflower possessing red ray flowers and ligulate disc flowers.

Progress was heterogeneous for reaction to the new race of downy mildew with about 45 percent of the plants showing resistance. The other two sources were 100 percent resistant. When tested for resistance to the Red River race Progress was again mixed in reaction while selections from the HS hybrid were susceptible. The ornamental sunflower was not tested.

Progress is a cultivar with downy mildew resistance derived from *H. tuberosus* (Pustovoit *et al.*, 1976). It is reported to be 99 to 100 percent resistant to downy mildew in the Soviet Union. The fact that Progress was heterogeneous for resistance in our studies suggests that both the new and Red River races may be pathogenically distinct from the race(s) occurring in the Soviet Union. Another possibility is that the seed source of Progress that we have available has been contaminated through admixture or outcrossing during maintenance and increase.

Inheritance of Resistance

Information on the inheritance of resistance to the new race was obtained from lines selected from Progress and the HS hybrid. The F_1 plants from crosses of resistant selections with susceptible lines were resistant, thus indicating dominant gene action (Table 2). The susceptible plants in the F_1 of the cross of RHA 274 and selection PRG-040 from Progress were assumed to be due to incomplete hybridization rather than lack of resistance. Segregation ratios of resistant to susceptible plants in F_2 and test cross populations involving the HS hybrid approximated 3:1 and 1:1 ratios, respectively. These data are consistent with the hypothesis of a single dominant gene for resistance.

Table 2. Segregation of sunflower plants for resistance to a new race (race 3) of downy mildew in crosses of resistant and susceptible lines.

Cross	Generation	No. of Plants Resistant	Susceptible	Expected Ratio	P
HS hybrid	F_1	33	0		
HS hybrid	F_2	59	13	3:1	.10 — .20
RHA 274 x HS hybrid	Test cross	39	34	1:1	.50 — .95
cmsHA 89 x HS hybrid	Test cross	39	39	1:1	1.00
RHA 274 x PRG-040	F_1	33	4		
RHA 274 x PRG-040	F_2	91	32	3:1	.50 — .95
cmsHA 291 x PRG-040	Test cross	8	9	1:1	.50 — .95

The F_2 and test cross involving PRG-040 also fit the expected 3:1 and 1:1 ratios assuming a single dominant gene. In a few segregating lines from Progress, however, a poor fit to a 3:1 ratio was obtained. Although more tests are required, it may be that additional genes for resistance are present in this cultivar.

Inheritance of resistance to downy mildew by a single dominant gene agrees with other investigations involving resistance derived from *H. annuus* (Fick, 1978; Zimmer and Kinman, 1972) and from *H. tuberosus* (Pogorietsky and Geshele, 1976).

We propose that the gene that confers resistance to the new race of downy mildew be designated Pl_5. We also suggest that the new race henceforth be referred to as race 3, and that the races previously known as the European and Red River races be referred to as races 1 and 2, respectively.

The presence of a gene for resistance in agronomically suitable lines suggests that it should be possible to develop hybrids resistant to race 3 in a relatively short period of time. In our program we expect to test hybrids resistant to both race 2 and 3 during the 1982 growing season. We anticipate production of seed for commercial use in 1983. We have also determined that the systemic fungicide Ridomil is effective as a seed treatment against race 3 (unpublished), thus providing an additional means of control if the need arises.

LITERATURE CITED

FICK, G.N. 1978. Breeding and genetics. *In:* J.F. Carter (ed.) *Sunflower Science and Technology.* Agronomy 19:279 — 338.

FICK, G.M. and AUWARTER, G.E. 1981. A new race of downy mildew affecting sunflower. *Proceedings of the Sunflower Forum and Research Workshop, Fargo,* ND. p. 15 16.

FICK, G.M. and ZIMMER, D.E. 1974. RHA 271, RHA 273, and RHA 274 . . . Sunflower parental lines for developing downy mildew resistant hybrids. *North Dakota Farm*

problem becomes a statistical one. It will be necessary to determine the sample size required to assure the identification of individual infected or infested seeds at various tolerance levels.

We have applied the ELISA test only to spore suspensions, young seedling tissues, and seeds. If in our pending studies we consistently obtain positive results with tissues of inoculated plants which survive infection and produce seeds, ELISA can have an immediate application under Australian conditions. Sunflower seed imported for research or other purposes must be grown for two generations under quarantine. If ELISA testing of plant tissues when the seed of the first crop is mature gives negative results, it might be accepted as evidence of freedom from *P. halstedii* infections, and might permit release of the seed lot for increase after only one generation in quarantine.

ACKNOWLEDGEMENTS

We are grateful for the financial assistance given to one of us (W.E.S.) by the Natural Sciences and Engineering Research Council of Canada. We are also grateful to Dr. J.A. Hoes for supplying sunflower seed and the Morden isolate of *P. halstedii,* and to Juan Gonzalez for assistance in producing infected plants.

LITERATURE CITED

COHEN, Y. and SACKSTON, W.E. 1973. Factors affecting infection of sunflowers by *Plasmopara halstedii. Canadian Journal of Botany* 51:15 — 22.

FICK, G.N. and AUWATER, G.E. 1981. A new race of downy mildew affecting sunflower. Proceedings Sunflower Forum and Research Workshop January 27 — 28, 1981. Fargo, N.D. pages 15 — 16. Sunflower Association of America.

SACKSTON, W.E. 1981. Downy mildew of sunflower. *In* Spencer, D.M. (Editor). The Downy Mildews, *pp* 545 — 575. Academic Press, London.

VOLLER, A., BARTLETT, B., BIDWELL, D.E., CLARK, M.F., and ADAMS, A.N. 1976 (a). The detection of virus by enzyme-linked immunosorbent assay (ELISA). *Journal of General Virology* 33:165 — 167.

VOLLER, A., BIDWELL, D.E., and BARTLETT, A. 1976 (b). Enzyme immunoassays in diagnostic medicine. *Bulletin of the World Health Organization* 53:55 — 56.

WENDLER, P.B., GOTLIEB, A.R. and BERGDAHL, D.R. 1980. A newly discovered serotype of *Gremmeniella abietina. Canadian Journal of Botany* 58:1923 — 1928.

RESISTANCE TO A NEW RACE OF SUNFLOWER DOWNY MILDEW.

G.N. FICK and G.E. AUWARTER
SIGCO Research, Inc. Breckenridge, U.S.A. MN 56520.

ABSTRACT

A new race of downy mildew discovered in the U.S. in 1980 was found to be widespread. Selected lines with known genes for resistance that have been used throughout the world in breeding resistant cultivars were all susceptible to the new race. Three sources of resistance were identified among more than 400 diverse sources of germplasm. Resistance to the new race was due to a single dominant gene that was designated Pl_5. It is suggested that the new race be referred to as race 3 and that the previously identified European and Red River races be known as race 1 and 2, respectively.

INTRODUCTION

Downy mildew incited by *Plasmopara halstedii* (Farl.) Berl. et de Toni has been a serious disease of sunflower *(Helianthus annuus* L.*)* in the United States. During the early 1970's fields with as high as 90 percent infected plants and with yield losses exceeding 50 percent were observed (Fick and Zimmer, 1974; Zimmer, 1971).

Downy mildew has been controlled effectively in recent years by resistant hybrids. These hybrids possess the Pl_2 gene for resistance to the Red River race which occurs widely in the major production area of the U.S. (Fick 1978; Fick and Zimmer, 1974).

In 1980 we reported on the discovery of a new race of downy mildew to which all hybrids grown currently in the U.S. were susceptible (Fick and Auwarter, 1981). In this paper we report on the widespread occurrence of this new race and of our progress in identifying sources of resistance.

MATERIALS AND METHODS

Information on the occurrence of the new race of downy mildew was obtained from field surveys and reports by industry representatives. Fields with 10 percent or more infected plants were assumed to be infected with the new race as past experience has shown that most hybrid seed lots have zero to five percent susceptible plants when tested for resistance to the Red River race. The occurrence of the new race was verified in some but not all fields by collecting viable spores and inoculating plants in greenhouse trials.

More than 400 diverse sources of germplasm consisting of inbred lines, hybrids and populations from our breeding program were screened for resistance to the new race. Most sources originated from high oil Soviet cultivars or from crosses of Soviet cultivars with wild *H. annuus.* A few sources were from crosses of cultivated sunflower with *H. tuberosus, H. petiolaris* or *H. argophyllus.* Appropriate testcross, F_1 and F_2 populations involving resistant lines were also evaluated following initial screening results.

Inoculations with downy mildew were made in the greenhouse according to the procedures described by Zimmer (1974). The source of inoculum was derived from downy mildew infected leaves collected in September 1980 from a field near Breckenridge, MN. The collection of zoosporangia was increased and maintained in the greenhouse on susceptible cultivars prior to use in initial tests.

Susceptibility of the seedlings was indicated by sporulation of the fungus on the cotyledons or the undersurface of the first true leaves after 16 hours in a saturated humidity chamber. Less than 10 percent of the plants of susceptible lines escaped systemic infections.

RESULTS AND DISCUSSION

Occurrence of the New Race

Observations in 1981 indicated that the new race of downy mildew was widespread. Fields with as high as 70 percent infected plants were reported from 12 counties of Minnesota and the Dakotas. Most fields were located in or near the Red River Valley, an area with climatic conditions conducive to downy mildew and a relatively long history of sunflower production.