Resistance in Chickpea to *Phytophthora megasperma* f. sp. *medicaginis*

R. B. BRINSMEAD and M. L. RETTKE, Department of Primary Industries, Hermitage Research Station, Warwick 4370; J. A. G. IRWIN, Botany Department, University of Queensland, St. Lucia 4067; M. J. RYLEY, Department of Primary Industries, Tor Street, Toowoomba 4350; and P. W. LANGDON, Department of Primary Industries, Meiers Road, Indooroopilly 4068, Australia

ABSTRACT

Brinsmead, R. B., Rettke, M. L., Irwin, J. A. G., Ryley, M. J., and Langdon, P. W. 1985. Resistance in chickpea to *Phytophthora megasperma* f. sp. *medicaginis*. Plant Disease 69:504-506.

In 1981, more than 200 chickpea lines (*Cicer arietinum*) were screened for field resistance to *Phytophthora megasperma* f. sp. *medicaginis* in two trials on land known to be naturally infested with the pathogen. Twenty of the most promising lines were tested again in three trials in 1982. Several of these lines were shown to have significantly superior field resistance compared with the commercial cultivars Tyson (known as C235 elsewhere) and Opal. This field resistance was confirmed in a controlled-environment inoculation test, which showed that a glasshouse screening technique could be readily developed provided inoculum levels were strictly controlled.

Commercial production of chickpeas (*Cicer arietinum* L.) has recently begun in the eastern Australian wheat belt. Cultivars Tyson and Opal have been released for farmer use. Tyson is a desi line known as C235 on the Indian subcontinent; Opal is a medium to large white-seeded kabuli line of Cuban origin.

Root rot of chickpea, a previously undescribed disease, was first reported by Vock et al (4) in a commercial crop of the chickpea cultivar Tyson growing near Toowoomba, southern Queensland, in July 1979. About 70% of plants were affected by the disease. At that time, the pathogen was identified as *Phytophthora megasperma* Drechs. var. *sojae* A. A. Hildeb. (4).

Later studies involving cross-inoculation and electrophoretic protein patterns (1) showed conclusively that the causal agent of the chickpea root rot was identical to *P. megasperma* Drechs. f. sp. *medicaginis* Kuan & Erwin (Pmm) (3), which also causes root rot of lucerne (*Medicago sativa* L.) in Queensland. The disease has now been observed at most localities in Queensland where chickpeas are grown, and generally, losses have been severe.

There have been no previous reports on the availability of resistance in chickpea to Pmm. Resistance offers the only means of effective economic control of the disease. This paper reports the results of experiments designed to identify field resistance to Pmm in a wide range of chickpea lines and the development of a glasshouse screening assay with quantified inoculum levels.

Accepted for publication 16 October 1984.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Field experimentation. In 1981, more than 200 lines from the Australian chickpea collection were screened for field resistance to the disease. Two trials were established on land known to be naturally infested with Pmm at the Hermitage Research Station in southern Queensland (28° S, 152° E). A grid-plot, randomized block design with two replicates was used. Highly susceptible ICC 6334 was the reference cultivar in the grid plots. One hundred fifty seeds per plot were sown in single rows 5 m long spaced 70 cm apart. Seedling and final plant counts (i.e., surviving plants at maturity) and seed yields were recorded.

In the 1982 season, three trials were conducted at *Phytophthora*-infested sites using the most promising lines identified in 1981 and controls of Tyson, Opal, and ICC 6334. Two trials were conducted on the Hermitage Research Station and one in the Bongeen district (27° S, 152° E) of the Darling Downs. The experimental design of each trial was identical to those in 1981, except four replicates were used. In addition, the yield of most of these lines was ascertained at an uninfested site at Bongeen. For this trial, a randomized block design with four replicates was used. Plots consisted of rows 4 × 6 m spaced 70 cm apart, and the datum was 5.5 m of each of the two middle rows.

Soils on which the trials were done are alkaline, heavy cracking, montmorillonitic clays. The poor internal drainage characteristics of the soil and the cool, moist conditions that usually occur during the early growing period of the chickpea crop in this region are conducive to development of the disease.

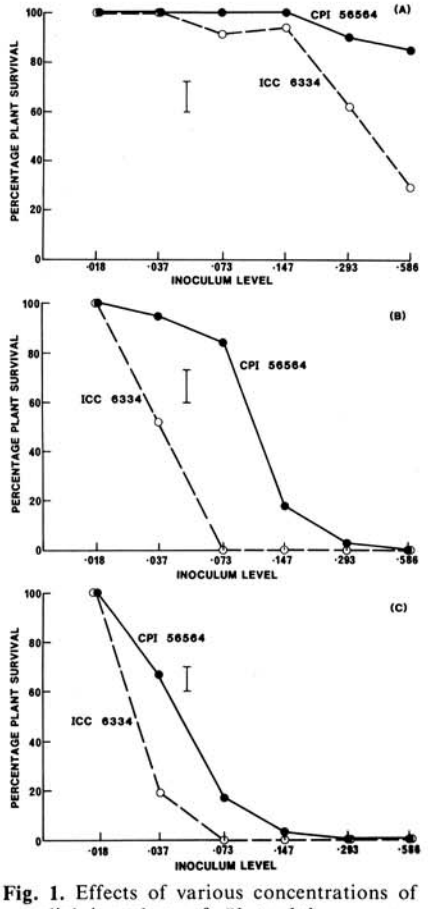

Fig. 1. Effects of various concentrations of mycelial inoculum of *Phytophthora megasperma* f. sp. *medicaginis* (grams dry weight of mycelium per kilogram dry weight of peat-sand [1:1, v/v]) on disease development in chickpea seedlings of CPI 56564 and ICC 6334 **(A)** 4 days, **(B)** 6 days, and **(C)** 8 days after saturation. Saturation began 6 days after sowing when >90% of seedlings had emerged. Bar = LSD ($P = 0.05$).

Development of glasshouse screening technique. *Production of inoculum.* Three weeks before the studies began, a culture of Pmm was obtained by direct isolation from naturally infected root tissue of lucerne collected at Gatton in southeastern Queensland. Inoculum was prepared by growing the fungus as still culture in 100 ml of liquid V-8 juice medium (100 ml of Campbell's V-8 juice plus 2 g of calcium carbonate in 1 L of distilled water) in 500-ml Erlenmeyer flasks at 25 ± 2 C on the laboratory bench. After 8 days, mats were harvested and dry weight determinations made on a subsample as described previously (2).

Influence of inoculum level on disease development. A heat-pasteurized planting mix, peat-sand (1:1, v/v), as described previously (2), was used. The experiment was conducted in 900-ml watertight plastic cups, each cup containing 590 g dry weight of planting mix. A twofold serial dilution series, beginning with a concentration of 0.586 g dry weight of fungus per kilogram dry weight of mix, through to a 1:128 dilution was prepared. An uninoculated control treatment was included. The inoculum was incorporated into the mix by hand, and water was added to give a final moisture content of 35%. In each cup, 12 seeds of either ICC 6334 (field susceptible) or CPI 56564 (field resistant), both with germination >90%, were uniformly distributed over the surface of the mix and covered with 75 g of infested mix. After sowing, the cups were covered with plastic bags and placed in a naturally illuminated growth chamber at 23–26 C. Plastic bags were removed 6 days after sowing, when most (>90%) of the seedlings had emerged. Daily saturation (water was added until 0.5–1.00 mm of free water remained on the surface) of the soil was begun and continued for 8 days.

Total emergence was determined 6 days after sowing, then daily counts were made of the number of surviving seedlings in each cup. Seedlings were classified as surviving when they were turgid and the hypocotyl showed no discoloration or collapse.

RESULTS AND DISCUSSION

Field studies. Plant survival, seed yield, and country of origin for the more promising lines and commercial cultivars in the 1981 trials and all entries in the 1982 trials are given in Table 1. The 1981 trials indicated that appreciable differences in field reaction to Pmm (as measured by plant survival and grain yield) existed between several cultivars, but these differences were not significant ($P < 0.05$). The large variation and limited replication produced high least significant difference (LSD) values. This was largely overcome in the 1982 trials by omitting highly susceptible lines identified in the 1981 trials and increasing the replication.

In 1982, there were differences in disease severity between sites where disease developed; this was shown most consistently in the susceptible check cultivars Opal, Tyson, and ICC 6334. Degree of disease severity was as follows: July-sown Bongeen < June-sown Hermitage < July-sown Hermitage. No lines were completely disease-free over this range, but CPI 56564 always showed high levels of field resistance. Several other lines, notably K736, CPI 71180, and CPI 71175 from Ethiopia and the USSR × Ethiopia cross 76.8.1.1, generally had superior resistance to the commercial cultivars Tyson and Opal where the disease was not severe. However, the grain yields of all these resistant lines were significantly lower than those of Tyson and Opal at the disease-free site (Bongeen, May-sown). The resistance identified in the USSR and Ethiopian lines may be useful in a breeding program aimed at transferring it to more agronomically suitable cultivars for Queensland conditions.

Glasshouse studies. Seedling emergence for both lines was not significantly reduced ($P = 0.05$) at any mycelial inoculum concentration compared with that of the uninoculated controls. The percentage of seedlings surviving after emergence was higher for CPI 56564 than for ICC 6334 only at the two highest inoculum concentrations 4 days after saturation (Fig. 1A). Six days after saturation, the percentage of surviving seedlings of both lines at every inoculum level was lower than the corresponding

Table 1. Field reactions of chickpea cultivars to *Phytophthora megasperma* f. sp. *medicaginis* (Pmm)

		Hermitage[a] (1981) sown 16 April		Hermitage[b] (1981) sown 10 June		Bongeen (1982) sown 21 July		Hermitage (1982) sown 9 June		Hermitage[c] (1982) sown 5 August	Bongeen[d] (1982) sown 4 May
Strain	Origin	Plant survival (%)	Seed yield (kg/ha)	Plant survival (%)	Seed yield (kg/ha)	Plant survival (%)	Seed yield (kg/ha)	Plant survival (%)	Seed yield (kg/ha)	Plant survival (%)	Seed yield (kg/ha)
CPI 56564	USSR	67	441	...	...	70	554	73	810	42	1,552
K736	Ethiopia	34	214	77	221	100	1,189	89	225	5	...
CPI 71180	Ethiopia	82	860	60	266	78	962	77	124	7	1,606
CPI 71175	Ethiopia	73	452	65	188	77	963	83	221	1	1,408
76.8.1.1[e]	Australia	81	999	76	338	82	770	55	66	0	1,300
CPI 56290	Ethiopia	75	1,063	66	288	79	1,044	43	87	0	...
CPI 56315	Iran	43	548	62	610	80	1,060	43	111	5	1,357
ICC 2828	Iran	56	543	54	626	63	1,204	46	134	1	2,018
K652	USSR	43	717	65	316	39	757	68	310	0	1,842
ICC 4931	India	67	572	66	363	43	288	27	56	3	1,601
CPI 71195	Ethiopia	64	827	30	100	60	1,036	38	64	1	1,717
ICC 6067	India	35	758	73	491	39	641	50	150	0	1,736
ICC 2903	Iran	87	1,370	35	242	54	993	21	47	0	2,042
CPI 56317	Iran	61	817	52	294	50	698	26	44	0	1,203
CPI 71197	Ethiopia	51	958	51	549	34	611	46	90	3	1,641
CPI 71198	Ethiopia	48	755	64	562	39	459	34	77	0	1,343
76.15.2.1[f]	Australia	43	353	56	626	48	668	30	53	4	1,666
CPI 52989	Spain	56	592	56	449	22	140	36	62	0	...
K246	USSR	47	550	51	574	39	638	19	20	0	1,750
CPI 52994	Israel	50	697	39	333	12	99	29	51	0	1,801
Opal	Cuba	75	301	3	44	31	224	15	17	1	1,593
Tyson (C235)	India	32	453	30	154	37	564	16	20	0	1,793
ICC 6334	Egypt	16	66	13	89	3	77	12	29	0	1,265
C.V. (%)		56	105	56	99	35	41	34	122	260	9.8
LSD ($P = 0.05$)		50	645	34	260	26	404	21	232	10	223

[a] Total of 283 entries in trial.
[b] Total of 219 entries in trial.
[c] Seed yield not recorded because of feral animal damage to surviving plants.
[d] Yield trial conducted on site not infested with Pmm.
[e] K368 (USSR) × CPI 71180 (Ethiopia).
[f] K368 (USSR) × CPI 53008 (India) × CPI 71180 (Ethiopia).

figures recorded after 4 days. There were significant differences ($P<0.05$) between the lines only at three levels of inoculum (Fig. 1B). At the inoculum level of 0.073 g dry weight of mycelium per kilogram dry weight of mix, 84% of CPI 56564 seedlings survived, whereas all plants of ICC 6334 had succumbed. After 8 days of saturation, only 17% of plants of CPI 56564 survived at this inoculum level (Fig. 1C).

These results clearly demonstrated that chickpea lines can be screened in the early seedling stage for resistance to Pmm. Both the inoculum level and the period of exposure to the pathogen under saturated soil conditions had a marked effect on the expression of resistance.

The assay should have application in a breeding or selection program aimed at developing chickpea lines with resistance to Pmm.

ACKNOWLEDGMENTS

Statistical designs and analysis were provided by officers of the Biometrics Branch, Queensland Department of Primary Industries, Toowoomba, Queensland 4350. Part of this work was funded by the Queensland Graingrowers Association.

LITERATURE CITED

1. Irwin, J. A. G., and Dale, J. L. 1982. Relationships between *Phytophthora megasperma* isolates from chickpea, lucerne and soybean. Aust. J. Bot. 30:199-210.
2. Irwin, J. A. G., and Langdon, P. W. 1982. A laboratory procedure for determining relative levels of field resistance in soybeans to *Phytophthora megasperma* f. sp. *glycinea*. Aust. J. Agric. Res. 33:33-39.
3. Kuan, T. L., and Erwin, D. C. 1980. Formae speciales differentiation of *Phytophthora megasperma* isolates from soybean and alfalfa. Phytopathology 70:333-338.
4. Vock, N. T., Langdon, P. W., and Pegg, K. G. 1980. Root rot of chickpea caused by *Phytophthora megasperma* var. *sojae* in Queensland. Australas. Plant Pathol. 9:117.