A new race of Diplocarpon rosae capable of causing severe black spot on Rosa rugosa hybrids'

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In 1977, severe black spot symptoms appeared at Ottawa on the highly resistant ***Rosa rugosa*** hybrid cultivar Martin Frobisher, and on an unidentified ***Rosa*** species collected at Bar Harbor, Maine. The ***Diplocarpon rosae*** Wolf isolated from Martin Frobisher was unable to infect the very susceptible cultivars Samantha and Arthur Bell. The isolate of ***D. rosae*** from the unidentified ***Rosa*** sp. was capable of producing severe symptoms on Martin Frobisher and the two susceptible cultivars. It was concluded that the isolate from Martin Frobisher constituted a new pathogenic race of the fungus. The possibility of the highly virulent isolate from the unidentified ***Rosa*** sp. constituting a pathogenic race is considered.

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En 1977 de graves symptômes de tache noire sont apparus a Ottawa sur le cultivar pourtant tres resistant de ***Rosa rugosa*** Martin Frobisher. ainsi que sur une espece non identifiee de rose obtenue de Bar Harbor, Maine. L'isolat de ***Diplocarpon rosae*** Wolf preleve sur Martin Frobisher n'a pas reussi a infecter les cultivars tres sensibles Samantha et Arthur Bell. Par contre, l'isolat obtenu sur l'espece de rose non identifiee a donne lieu a de graves symptômes de la maladie sur Martin Frobisher et sur les deux cultivars sensibles. L'isolat trouve sur Martin Frobisher serait donc un nouveau pathotype du champignon. On examine la possibilite de classer l'isolat ultravirulent trouve sur la rose non identifiee comme une pathotype distinct.

During the late summer of 1977, lesions typical of those resulting from infection by ***Diplocarpon rosae*** Wolf (black spot) were observed at Ottawa on one rose cultivar and on one species that had been considered very resistant to the disease. As the disease developed, defoliation became severe and infected leaves were brought into the laboratory and the causal fungus isolated. The monoconidial colonies produced in culture from the normally resistant cultivar, Martin Frobisher, and from the resistant species were different from one another and from the fungus obtained from two susceptible garden rose cultivars. The fungus isolated from Martin Frobisher grew very slowly while that from the unidentified ***Rosa*** sp. grew much more rapidly and resembled the *D.* ***rosae*** isolated from the susceptible floribunda cultivar Arthur Bell and an unidentified garden rose growing in the same area. The possibility of the isolates from Martin Frobisher and the resistant species constituting new races was recognized, and investigations initiated to determine if this was the case.

Materials and methods

D. ***rosae*** was isolated from 1. Martin Frobisher, an open-pollinated seedling **of** the hybrid ***Rosa rugosa*** cultivar, Schneezwerg; 2. G57, an unidentified wild species collected at Bar Harbor, Maine; 3. Arthur Bell, a floribunda cultivar; and 4. an unidentified garden rose. Isolations were made by placing leaf tissue encompassing a typical black spot lesion in petri dishes containing potato dextrose agar (PDA). When acervuli developed on the leaf surface, conidia were picked off and placed in test tubes containing sterile distilled water. The resulting suspensions were then distributed over PDA in petri dishes and single conidia picked off and placed on PDA. Several of the monoconidial colonies were selected at random **for** further testing but, since no differences in pathogenicity or morphology were found among single conidia obtained from any one cultivar, a representative monoconidial culture from each isolate was maintained for further investigations. Inoculum was prepared by growing the fungus for 14 days on PDA and then floating off the conidia with distilled water.

In preliminary experiments, four ***D. rosae*** isolates were compared; MF from Martin Frobisher, G57 from the unidentified ***Rosa*** sp., GR1 from Arthur Bell, and GR2 from the unidentified garden rose. Martin Frobisher was used as the resistant host, and Samantha, a florist cultivar was used as the susceptible host. In later experiments, only the three isolates, MF, G57, and GR1 were used, and Arthur Bell was included as a susceptible host. The cultivar Jens Munk, a newly introduced ***R. rugosa,*** with high resistance to black spot was also added to the host series. The unidentified ***Rosa*** sp. was not available for inoculation.

Inoculum, containing approximately 30,000 conidia/ml distilled water, was applied with an atomizer to the upper surface of the leaves of 14-week-old rooted cuttings. A minimum of four plants with an average of 10 compound leaves per plant were used for each treatment. After inoculation, plants were placed in a chamber equipped with misting nozzles and the relative humidity maintained at 95-99%. The temperature was 25-27°C. Light was provided by fluorescent tubes at

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Table 1. Comparative pathogenicity on four rose cultivars of three isolates of *D. rosae* *

Cultivar	Isolate and disease rating**		
	GR1	G57	MF
Martin Frobisher	0	4	4
Samantha	4	5	0
Arthur Bell	4	5	0
Jens Munk	0	0	0

* Average of three inoculations of Martin Frobisher and Samantha, two of Arthur Bell and Jens Munk.
** 0 = no symptoms to 5 = complete defoliation.

approximately 2000 1x. Three days after inoculation, the plants were removed to a greenhouse bench where day length was 16 h and temperature 21°C night and 24°C day. The foliage was moistened once a day with a fine spray of tap water at 22°C. Observations were made on disease development 17 days and, on number of lesions and rate of defoliation, 31 days after inoculation.

Observations on conidia germination and germ tube penetration were made by boiling the leaves in 95% ethyl alcohol for 2 min and staining in lactophenol-aniline blue for 5 min.

Results

Inoculation of the two cultivars, Martin Frobisher and Samantha, demonstrated differences in pathogenicity among the three isolates of ***D. rosae*** (Fig. 1, Table 1). Isolate MF caused lesions and rapid defoliation in Martin Frobisher, but did not produce any disease symptoms on the Samantha leaves. Isolates GR1 and GR2 produced identical symptoms on Samantha and failed to infect Martin Frobisher. In subsequent inoculations, only GR1 was used. Isolate G57 caused severe symptoms on both Martin Frobisher and Samantha. Inoculations carried out on three separate occasions were identical, except that symptoms produced on plants inoculated during the month of March were less severe than those produced in December and April.

No correlation could be found in any of the isolate-cultivar combinations between % leaf area covered by lesions and degree of defoliation.

Examination of leaves 7 days after inoculation showed differences in rate of germination of conidia of the three isolates. At this time 25% of the G57 conidia had produced germ tubes, while less than 5% of the conidia of MF and GR1 had germinated. Maximum germination was reached 10 days after inoculation at which time 32% of G57, 17% of MF, and 18% of the GR1 conidia had produced germ tubes. All three isolates germinated equally well on Samantha and Martin Frobisher. Germ tubes of isolate MF failed to penetrate leaves of Samantha and isolate GR1 did not penetrate Martin Frobisher. Isolate G57 penetrated both varieties equally.

The cultivar Arthur Bell was susceptible to both G57 and GR1 and resistant to isolate MF. Jens Munk was completely resistant to all three isolates.

Discussion

Proof of the existence of pathogenic races of *D. **rosae*** is very limited. Significant differences in degree of pathogenicity have been demonstrated. The work of Jenkins (1955) showed that differences in ability to infect several rose cultivars and species existed among isolates of the fungus. It did not, however, prove that specific races were present. The fact that his tests were conducted over a 6-month period very probably had an effect on his results. Palmer et al. (1966) reported a definite seasonal variation in host susceptibility to *D. **rosae.*** These workers tested 50 rose cultivars against the fungus isolated from roses growing in seven geographical areas in the U.S.A. None of their seven isolates infected ***R. rugosa.*** Although they refer to "locally adapted races", the differences between their isolates appear to be the result of different degrees of virulence rather than the presence of specific races. Saunders (1967) assessed the degree of resistance of several rose species and cultivars in England. He mentioned races as one of the factors causing anomalies in results, but used only a single isolate in his experiments.

Frick (1943). working in Switzerland, tested isolates from seven wild rose species and two cultivars. She observed that there was no evidence of different biological races although there were considerable differences in virulence among the nine isolates. According to Baker and Dimock (1969), the existence of races of ***D. rosae*** has not been proven.

Much of the work on variation of resistance of roses to black spot has been done using excised leaflets or parts of leaflets infected by placing a drop of conidia suspension on the surface and placing in a moist chamber for 8-17 days (Jenkins 1955, Palmer et al. 1966, Saunders 1967).

Jenkins (1955) and Saunders (1967) used size of lesion to differentiate resistance. At Ottawa, lesion size was extremely variable within each isolate and host. It was also observed that number of lesions and amount of leaf surface covered by lesions could not be correlated with degree of defoliation. In several cases, leaves dropped that had less than 10% of the surface covered by discolored tissue. Since the damage to roses from black spot results largely from premature defoliation, it is doubtful if lesion size can be used as a reliable criterion for susceptibility.

The cultivar Martin Frobisher has been tested in several locations across Canada and in the northern U.S. Over a period of several years, no evidence of susceptibility to black spot has been reported. The sudden breakdown of resistance at Ottawa indicates the presence of a new race of the fungus. The fact that isolate MF was capable of causing severe infection on the resistant cultivar sug-

gests a change in pathogenicity. The inability of the isolate to infect the generally recognized very susceptible cultivars Samantha and Arthur Bell further indicates that it is a new pathogenic race. Isolate G57, although isolated from a Rosa sp. unrelated to the R. *rugosa* hybrids, caused severe symptoms on both Martin Frobisher and the two susceptible cultivars suggesting that this isolate is a more virulent form of the fungus possibly constituting a third race.

Additional research is planned to determine if G57, in fact, also constitutes a new pathogenic race and if other pathogenic races are present in eastern Ontario.

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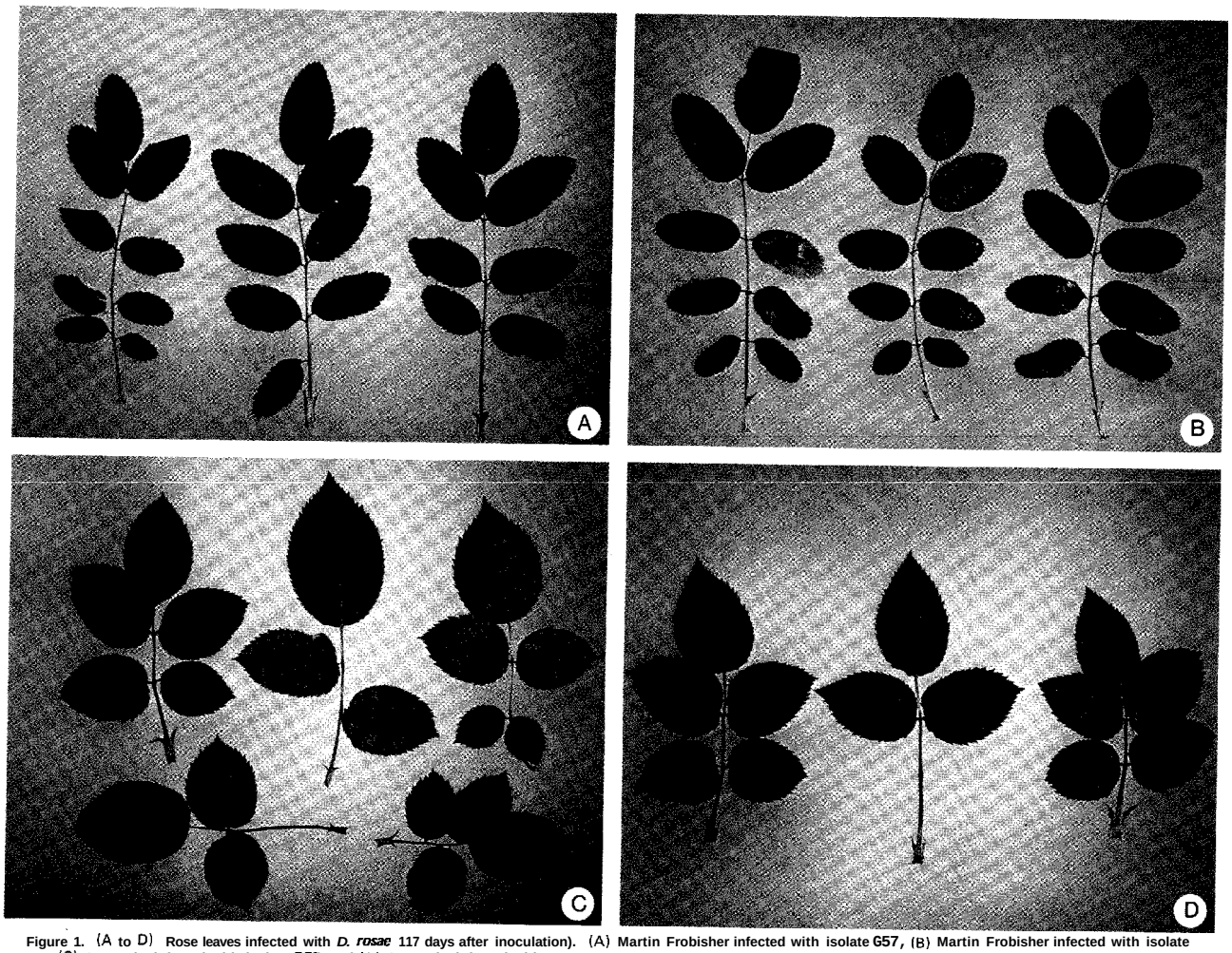

Figure 1. (A to D) Rose leaves infected with *D. rosae* 117 days after inoculation). (A) Martin Frobisher infected with isolate G57, (B) Martin Frobisher infected with isolate MF, (C) Samantha infected with isolate G57, and (D) Samantha infected with GR1.