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Conidial Germination and Infection by Diplocarpon Rosae on Susceptible and Resistant Rose Species

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Conidial germination and infection by *Diplocarpon rosae* on susceptible and resistant rose species

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Abstract: Conidial germination and infection by *Diplocarpon rosae,* the causal organism of rose blackspot, were examined on two resistant species of roses, *Rosa roxburghii* and *R. wichuraiana,* and two susceptible hybrid tea roses (*R. hybrida* cv. Chicago Peace and Garden Party). Fungal conidia germinated and gave rise to subcuticular mycelium that formed haustoria within epidermal cells of all four roses tested. On the resistant rose species, epidermal cells associated with the infection site became necrotic, indicating that a hypersensitive response is involved in conferring their resistance to *D. rosae.*

Key Words: blackspot, host resistance, hypersensitive response, *Rosa*

INTRODUCTION

The fungal disease blackspot of roses, caused by *Diplocarpon rosae* Wolf (ascomycetous affinity; anamorph *Marssonina rosae* (Lib.) Lind), is a devastating foliar disease. It is easily recognized by the appearance of irregularly shaped black lesions on the adaxial leaf surfaces. A severe infection can result in partial to complete defoliation of the plant or, in some very susceptible varieties, death (Black et al., 1994).

Resistance to blackspot is known to vary widely among rose varieties. The commonly grown hybrid tea roses, hybrid perpetuals, and polyanthas are generally susceptible to some degree (Horst, 1983), while species roses (e.g., *Rosa roxburghii* Tratt. and *Rosa wichuraiana* Crép.) are often resistant (Castledine et al., 1981).

The basis of resistance to *D. rosae* appears to vary among resistant roses. Some researchers (Dodge, 1931; Castledine et al., 1981) noted that blackspot infections could be established in resistant roses by abrading the cuticle prior to inoculation, suggesting that the cuticle serves as a primary barrier to infection. Other studies have indicated that germination of conidia is reduced on some resistant plants (Saunders, 1970; Knight and Wheeler, 1978). Recently, Reddy et al. (1992) reported that *D. rosae* conidia failed to germinate on the resistant species roses *R. roxburghii* and *R. wichuraiana.* Still other researchers have documented hypersensitive responses to *D. rosae* in a diploid rose hybrid (Svejda and Bolton, 1980) and in the floribunda cultivar Allgold (Knight and Wheeler, 1978).

This study was conducted to compare conidial germination and post-penetration events on resistant and susceptible roses in order to better understand potential resistance mechanisms to *D. rosae* in *R. roxburghii* and *R. wichuraiana.*

MATERIALS AND METHODS

Plant specimens.—*R. roxburghii* and *R. wichuraiana,* the resistant roses used in this study, were graciously provided by the Antique Rose Emporium (Brenham, Texas). The hybrid teas (*R. hybrida*) Chicago Peace and Garden Party represented the susceptible cultivars.

Inoculation procedure.—Diseased leaflets from a local garden were the source of *D. rosae* conidia. The spores were collected by placing drops of sterile water onto blackspot lesions displaying open acervuli (Palmer et al., 1966). The resulting conidial suspension was collected and placed drop-wise onto detached leaflets (one drop per leaflet) of resistant and susceptible plants. The leaflets were incubated inside sealed Petri dishes at room temperature and normal room lighting conditions for 3 d to monitor germination and for 5 d to study post-penetration events. At the end of the incubation period, the tissue underneath each drop of inoculum was excised and prepared for microscopic examination.

Epifluorescence light microscopy.—Fungal conidia and germ tubes were visualized by staining with a 0.1% solution of Calcofluor® (SIGMA Chemical Company) in 100 mM Tris-HCl buffer, pH 8.5 (Kuck et al., 1981; Butt et al., 1989). After 1 to 2 min in the dye solution, samples were briefly rinsed in water and mounted on glass slides. Leaflets were examined with ultraviolet

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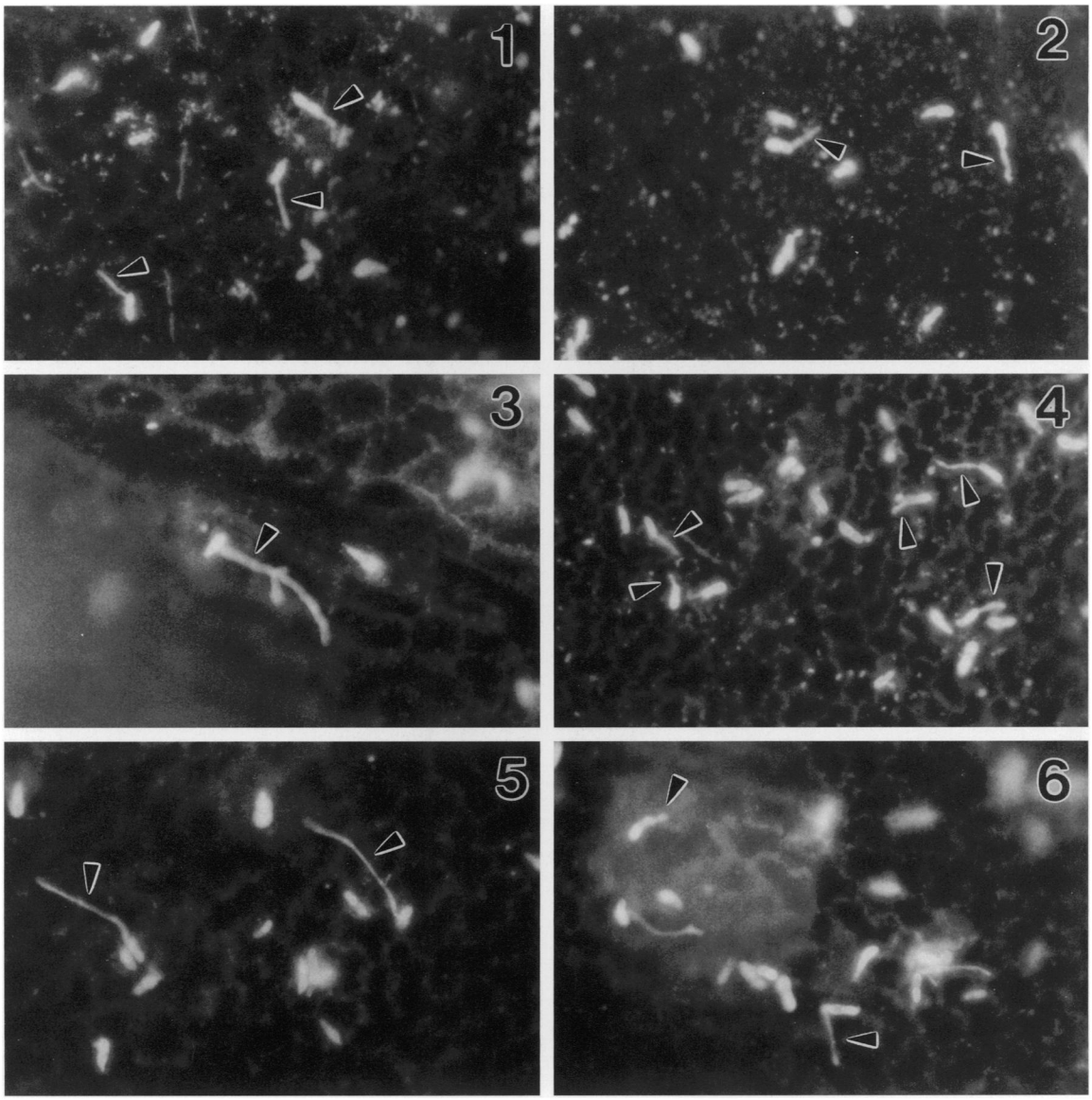
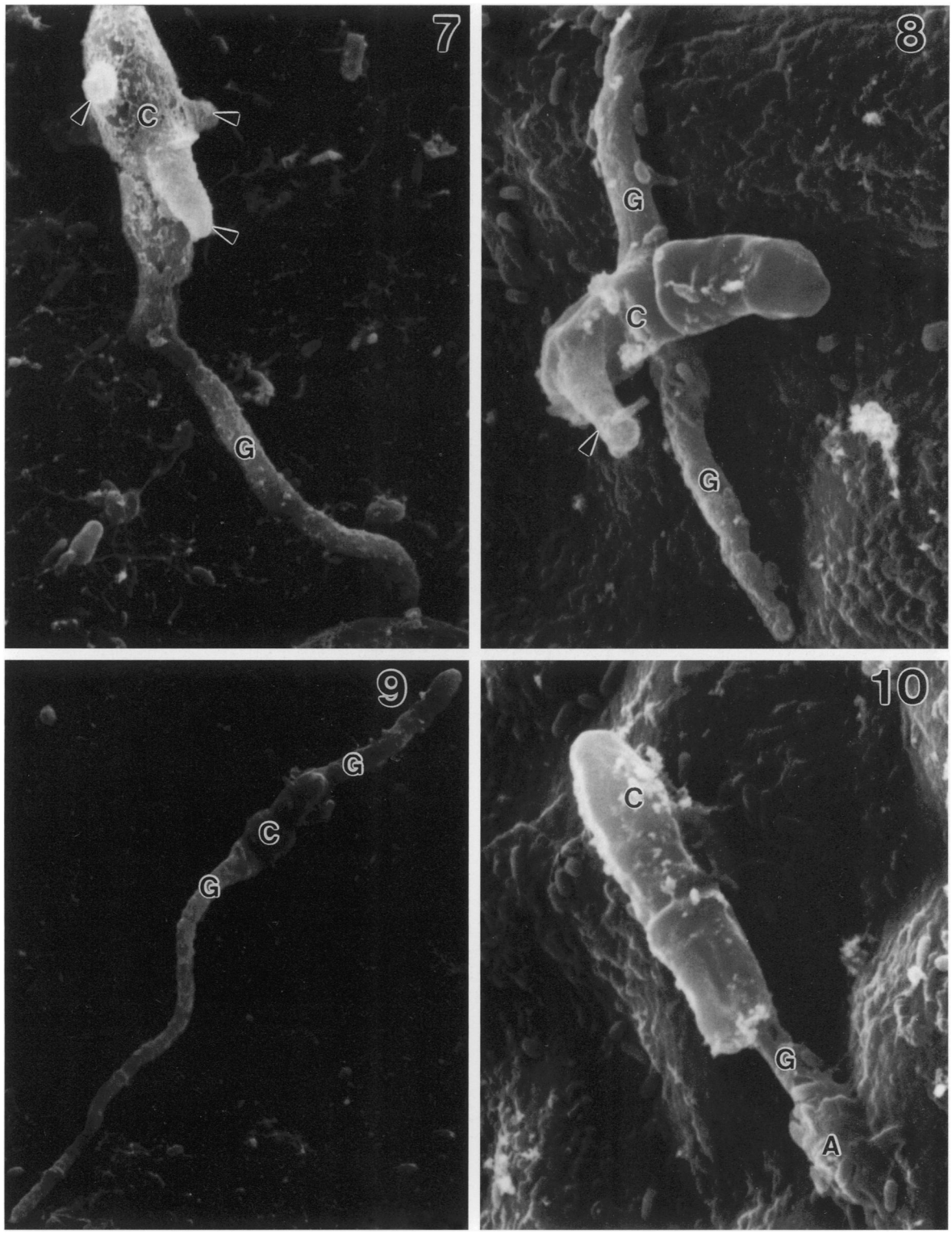


FIGS. 1–6. Conidia and germ tubes (arrowheads) of *D. rosae* observed with epifluorescence light microscopy. Spores germinated on the resistant rose species. 1, 2. *R. roxburghii*. 3, 4. *R. wichuraiana*. Spores germinated on the susceptible *R. hybrida* cultivars. 5. Chicago Peace. 6. Garden Party. × 620.

epi-illumination (excitation 330–385 nm, mirror 400 nm, barrier 420 nm; Olympus model BX50) that resulted in bright blue fluorescence of the walls of fungal conidia and germ tubes. A total of 100 conidia on two leaflets of each rose species or cultivar were scored as germinated or ungerminated.

Scanning electron microscopy.—Inoculated leaf pieces were fixed overnight at 4 C in 2.5% glutaraldehyde in 100 mM potassium phosphate buffer, pH 6.8. The tissue was then rinsed in 50 mM buffer and post-fixed in 1% OsO_4 in 100 mM potassium phosphate buffer for 2 h at 4 C (Mims, 1981). Following thorough rinsing in distilled water, specimens were dehydrated in a graded ethanol series to 100%, critical point dried using carbon dioxide as the transition fluid, mounted on specimen stubs using double sided tape, and sputter coated with gold-palladium. Conidia and germ tubes on leaflet surfaces were examined with a Hitachi S-405A scanning electron microscope operating at 15 kV.

Nomarski differential interference contrast light microscopy.—Samples were prepared following the procedure of Stumpf and Heath (1985). Inoculated leaflet sections were decolorized by boiling in 100% ethanol until white, then cleared to transparency by transferring into a saturated chloral hydrate solution near its



FIGS. 7–10. Scanning electron micrographs of *D. rosae* conidial germination on the resistant rose species. 7. Germ tube (G) emerging from the terminal end of a conidium (C) on *R. wichuraiana*. Several short germ tubes (arrowheads) appear to be emerging from the same spore. × 3300. 8. Two germ tubes (G) emerging from the septal area of a conidium (C) on *R. roxburghii*. A third, shorter germ tube (arrowhead) has grown from the larger cell of the two-celled spore. × 3300. 9. Germ tubes (G) emerging from either end of a conidium (C) on *R. wichuraiana*. × 1500. 10. Short germ tube (G) produced by a conidium (C) on *R. roxburghii*. The terminal end of the germ tube appears to have formed an appressorium-like enlargement (A) that is closely appressed to the host cuticle. × 3300.

TABLE I. Germination rates of *D. rosae* conidia on leaflets of resistant and susceptible roses. Percentages are based on the count of 100 conidia

Rose	Germination rate		
	Leaflet #1	Leaflet #2	Average
R. wichuraiana	70%	65%	67.5%
R. roxburghii	46%	34%	40%
R. hybrida			
Garden Party	36%	36%	36%
Chicago Peace	45%	37%	41%

boiling point. Tissues were stored in this solution for at least three d, then mounted on glass slides with the inoculated surface up. Subcuticular hyphae, haustoria, and host cell responses to these fungal structures were examined using the 40× oil immersion objective of an Olympus BX50 microscope equipped with Nomarski optics.

RESULTS

D. rosae conidia formed germ tubes on all four roses tested (FIGS. 1–10). Germ tubes most frequently emerged from one end of the spore (FIGS. 1–6, 7, 10), but occasionally appeared to grow from both ends (FIG. 9) or from the area near the conidial septum (FIG. 8). With scanning electron microscopy, several short extensions from the spores in addition to the primary germ tube(s) were frequently observed (FIGS. 7, 8). Germ tubes of *D. rosae* varied tremendously in length. Some were extremely short and rapidly became appressed against the cuticle upon emergence from the conidium (FIG. 4). Other germ tubes extended over several epidermal cells during their growth (FIG. 1–3, 5, 6). Typical appressoria were not formed, although a slight swelling at the tip of the germ tube was sometimes observed (FIG. 10).

Quantification of germination demonstrated that conidia on the resistant roses germinated at rates equal to or greater than that on the susceptible cultivars (TABLE I). Spore germination was highest on *R. wichuraiana* and lowest on Garden Party.

Microscopic examination of cleared leaf pieces revealed that germ tubes penetrated the cuticle to form subcuticular mycelium and that haustoria were produced in epidermal cells of both resistant (FIGS. 11–14) and susceptible (FIG. 15, 16) roses. Haustoria of *D. rosae* were long and spindle shaped with thin haustorial necks. It was not uncommon for a single epidermal cell of the susceptible cultivars to contain two or more haustoria by five d post-inoculation (FIG. 16). Whether these haustoria formed from one conidium or several was not apparent.

During the course of the detached leaflet incubation, an intense browning was noted under the inoculum drops on almost all leaflets of *R. roxburghii* and *R. wichuraiana*. When observed microscopically, many of the resistant rose's epidermal cells appeared necrotic, as evidenced by their brown, extremely granular cytoplasm (FIG. 11). The epidermal cells exhibiting this behavior were associated with subcuticular hyphae of *D. rosae*. It is likely that these darkened cells were penetrated, but haustoria were difficult to distinguish inside the necrotic cytoplasm.

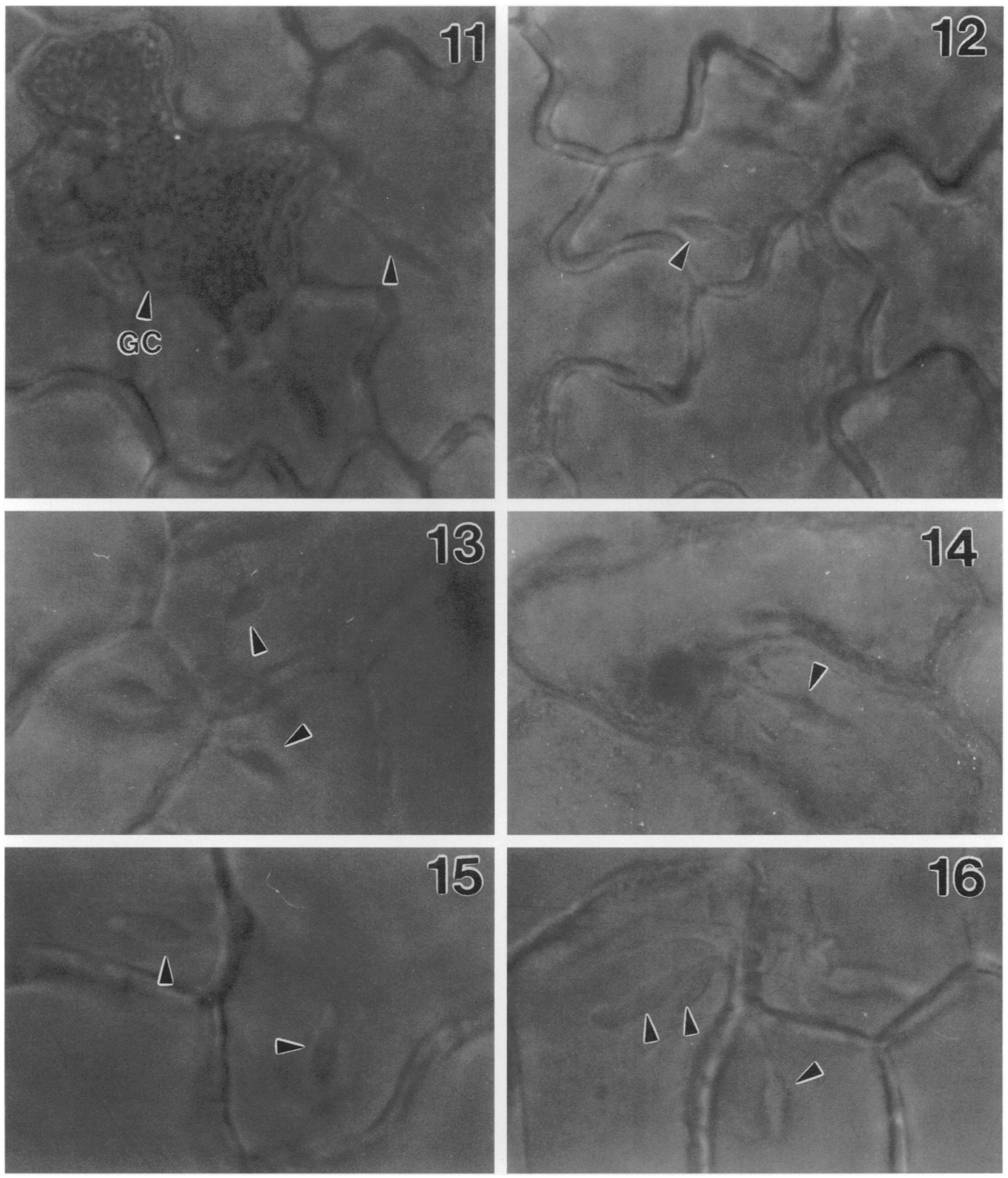
DISCUSSION

Germination of *D. rosae* conidia proceeded in a manner very similar to that described by Aronescu (1934) and Palmer et al. (1978), including the observations that germ tubes may emerge from any point along the spore surface, and that some spores germinate from both ends. Neither of these reports described the short hyphal extensions emerging from many of the conidia that were seen in this investigation using scanning electron microscopy. These structures may have been involved in penetrating the leaf cuticle and establishing infection but no visual evidence for this was seen with either scanning electron microscopy or Nomarski differential contrast microscopy.

Aronescu (1934) observed that a well defined appressorium did not always form at the ends of *D. rosae* germ tubes; instead, many hyphae were merely swollen at their tips. Germ tubes described by Palmer et al. (1978) and most of the germ tubes in this investigation appeared to directly penetrate the cuticle without forming an appressorium.

Aronescu (1934) also observed that collar material accumulated at host cell penetration sites and covered the mature haustoria of *D. rosae* along half their length. The collar was not described by Palmer et al. (1978) in the hybrid tea cultivar Red Radiance, but can be seen in Fig. 12 of their paper. A collar was not produced by host cells of either resistant or susceptible plants examined in this study.

There are conflicting reports as to the mechanism of resistance operating in the species roses *R. roxburghii* and *R. wichuraiana*. Reddy et al. (1992) reported that conidia of *D. rosae* failed to germinate on leaflets of these two hosts. By two d post-inoculation they observed that spores were collapsed and apparently dead, and speculated that germination inhibitors were responsible for conferring resistance in these two rose species. Other studies indicate that a post-penetration defense mechanism is operating; in the case of *R. wichuraiana*, Palmer et al. (1966) found that subcuticular hyphae and acervuli lacking conidia were produced by inocula from several



FIGS. 11–16. Post-penetration events on resistant and susceptible rose leaflets observed with Nomarski differential interference contrast light microscopy. Spindle-shaped haustoria (shown at arrowheads) formed in the epidermal cells of the resistant roses: 11, 12. *R. roxburghii*; 13, 14. *R. wichuraiana*; as well as the susceptible *R. hybrida* cultivars: 15, 16. Chicago Peace. In FIG. 11, one of the epidermal cells of *R. roxburghii* which exhibited the brown, extremely granular cytoplasm characteristic of a necrotic cell can be seen at GC. × 5700.

sources, and Castledine et al. (1981) observed limited mycelial development on both control and abraded leaf disks. Our results demonstrate that conidia germinate as well on the two resistant roses as on susceptible cultivars, and that haustoria are produced in both resistant hosts.

There are several possible reasons for this discrepancy. Reddy et al. (1992) observed germination using scanning electron microscopy. They do not make it clear as to the number of conidia that they scored; it would seem to be difficult to look at a large number of spores using this technique. In addition, several

researchers have shown that blackspot resistance in a particular rose species or cultivar can vary depending on the source of inoculum (Palmer et al., 1966; Knight and Wheeler, 1978; Svejda and Bolton, 1980). Thus, there are pathogenic races of *D. rosae* which may behave differently on different roses; in some cases they germinate very poorly (as low as 10%) on roses on which they grow poorly (Knight and Wheeler, 1978). We conclude that conidia from a polysporous inoculum collected in East Texas do germinate and infect *R. roxburghii* and *R. wichuraiana,* and that the resistance observed in these hosts to the prevailing races of *D. rosae* in the area (Black et al., 1994) is a post-penetration defense response.

Hypersensitive host cell death involves the death of only a few plant cells, limiting the progress of infection (Goodman and Novacky, 1994). Hypersensitive responses to *D. rosae* have been previously reported in the literature. Knight and Wheeler (1978) observed necrotic flecks on the floribunda cultivar Allgold in response to one of three *D. rosae* isolates that they inoculated with in their leaf disc assay. Svejda and Bolton (1980) described a hypersensitive reaction on plants of the diploid hybrid H71. When inoculated with a *D. rosae* isolate from the floribunda cultivar Arthur Bell, H71 began to drop its leaves within 12 hours post-inoculation, and newly emerging leaves showed no symptoms. H71 was susceptible to the other two fungal isolates which were tested by Svejda and Bolton (1980).

The reactions of *R. roxburghii* and *R. wichuraiana* to *D. rosae* observed in this study are very similar to those described in the rose cultivar Queen Anne, which is resistant to the powdery mildew fungus *Sphaerotheca pannosa* (Conti et al., 1985). In both cases, conidia germinated and infected leaflets of the resistant hosts, and equal numbers of haustoria were produced in resistant and susceptible plants. Very early on, however, (within 48 hours for Queene Anne), resistant host cells surrounding each infection site began to necrose, severely restricting further pathogen development.

In the case of *R. roxburghii* and *R. wichuraiana* responding to *D. rosae,* host cell death resulted in the formation of macroscopic necrotic flecks on the detached leaflets in our study. In the field, this hypersensitivity expressed at the cellular level goes unnoticed; both species were rated as highly resistant to blackspot and had extremely low defoliation ratings in recent field trials (Black et al., 1994).

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LITERATURE CITED

Aronescu, A. 1934. *Diplocarpon rosae*: from spore germination to haustorium formation. *Bull. Torrey Bot. Club* 61: 291–329.

Black, W.A., D. H. Byrne, and H. B. Pemberton. 1994. Field study of black spot resistance in rose. *HortScience* 29: 525.

Butt, T.M., H.C. Hoch, R.C. Staples, and R.J. St. Leger. 1989. Use of fluorochromes in the study of fungal cytology and differentiation. *Exp. Mycol.* 13: 303–320.

Castledine, P., B.W.W. Grout, and A.V. Roberts. 1981. Cuticular resistance to *Diplocarpon rosae*. *Trans. Br. Mycol. Soc.* 77: 665–666.

Conti, G.G., M. Bassi, D. Maffi, and R. Bonecchi. 1985. Host-parasite relationship in a susceptible and a resistant rose cultivar inoculated with *Sphaerotheca pannosa*. I. Fungal growth, mechanical barriers and hypersensitive reaction. *Phytopath. Z.* 113: 71–80.

Dodge, B.O. 1931. A further study of the morphology and life history of the rose blackspot fungus. *Mycologia* 23: 446–462.

Goodman, R.N., and A.J. Novacky. 1994. *The hypersensitive reaction in plants to pathogens*. APS Press. The American Phytopathological Society, St. Paul, Minnesota. 244 pp.

Horst, R.K. 1983. Black spot. Pp 7–11. *In: Compendium of rose diseases*. Ed. R.K. Horst. APS Press. The American Phytopathological Society, St. Paul, Minnesota.

Knight, C., and B.E.J. Wheeler. 1978. The germination of *Diplocarpon rosae* on different rose cultivars. *Phytopath. Z.* 91: 346–354.

Kuck, K.H., R. Tiburzy, G. Hänssler, and H.J. Reisener. 1981. Visualization of rust haustoria in wheat leaves by using fluorochromes. *Physiol. Plant Pathol.* 19: 438–441.

Mims, C.W. 1981. SEM of aeciospore formation in *Puccinia bolleyana*. *Scanning Electron Microsc.* 3: 299–303.

Palmer, J.G., I.B. Sachs, and P. Semeniuk. 1978. The leafspot caused by *Marssonina rosae* observed in scanning electron and light microscopy. *Scanning Electron Micros.* 2: 1019–1026.

———, P. Semeniuk, and R.N. Stewart. 1966. Roses and blackspot I. Pathogenicity to excised leaflets of *Diplocarpon rosae* from seven geographic locations. *Phytopathology* 56: 1277–1282.

Reddy, S., J.A. Spencer, and S.E. Newman. 1992. Leaflet surfaces of blackspot-resistant and susceptible roses and their reactions to fungal invasion. *HortScience* 27: 133–135.

Saunders, P.J.W. 1970. The resistance of some cultivars and species of *Rosa* to *Diplocarpon rosae* Wolf causing blackspot disease. *Nat. Rose Soc. Rose A.* 1970: 118–128.

Stumpf, M.A., and M.C. Heath. 1985. Cytological studies of the interactions between the cowpea rust fungus and silicon-depleted French bean plants. *Physiol. Plant Pathol.* 27: 369–385.

Svedja, F.J., and A.T. Bolton. 1980. Resistance of rose hybrids to three races of *Diplocarpon rosae*. *Canad. J. Plant Pathology* 2: 23–25.