



Emma Wanjiru Gachomo (Autor)

# **Studies of the life cycle of *Diplocarpon rosae* Wolf on roses and the effectiveness of fungicides on pathogenesis**

Emma Wanjiru Gachomo

---

**Studies of the life cycle of *Diplocarpon rosae*  
Wolf on roses and the effectiveness of  
fungicides on pathogenesis**

---



Cuvillier Verlag Göttingen

<https://cuvillier.de/de/shop/publications/2575>

Copyright:

Cuvillier Verlag, Inhaberin Annette Jentzsch-Cuvillier, Nonnenstieg 8, 37075 Göttingen,  
Germany

Telefon: +49 (0)551 54724-0, E-Mail: [info@cuvillier.de](mailto:info@cuvillier.de), Website: <https://cuvillier.de>

<b>Table of contents.....</b>	<b>Page</b>
<b>1 Introduction.....</b>	<b>1</b>
<b>2 Materials and Methods.....</b>	<b>10</b>
2.1 Research organisms.....	10
2.1.1 Pathogen.....	10
2.1.2 Plants.....	10
2.2 Plant cultivation.....	10
2.3 Pathogen isolation and inoculum harvesting.....	11
2.4 Inoculation of plants.....	11
2.5 Disease assessment.....	12
2.6 Maintenance of the pathogen and multiplication of the inoculum.....	14
2.7 Evaluation of the fungal growth <i>in vitro</i> .....	14
2.7.1 Preparation of growth media.....	14
2.7.2 Incubation on growth media.....	15
2.8 Microscopical investigations.....	15
2.8.1 Light microscopy.....	16
2.8.2 Transmission electron microscopy.....	16
2.8.3 Specimen preparation techniques.....	16
2.8.3.1 Fresh specimen.....	16
2.8.3.2 Whole specimen.....	17
2.8.3.2.1 Fixation with chloral hydrate.....	17
2.8.3.3 Sectioned specimen.....	17
2.8.3.3.1 Embedding in ERL-resin.....	18
2.8.3.3.2 Sectioning.....	19
2.8.3.3.3 Contrasting.....	20
2.8.4 Staining techniques.....	21

2.4.8.1 Blancophor.....	21
2.8.4.2 Diethanol (Uvitex 2b).....	21
2.8.4.3 Fuchsin acid.....	22
2.8.4.4 Aniline blue.....	23
2.8.4.5 Toluidine blue.....	23
2.9 Estimation of reactive oxygen species (ROS) production in leaf tissue.....	24
2.9.1 Superoxide anion ( $O_2^-$ ).....	24
2.9.2 Hydrogen peroxide ( $H_2O_2$ ).....	25
2.10 Fungicide tests.....	25
2.10.1 <i>In vitro</i> tests.....	25
2.10.2 <i>In planta</i> tests.....	26
2.10.2.1 Protective activity tests.....	26
2.10.2.2 Curative activity tests.....	27
2.11 Statistical analysis and software programmes.....	29
<b>3 Results.....</b>	<b>30</b>
3.1 Symptomatology of the blackspot disease caused by <i>Diplocarpon rosae</i> on roses.....	30
3.1.1 Symptoms on inoculated plants.....	30
3.1.2 Disease scoring.....	32
3.2 Characterization of <i>D. rosae</i> isolates.....	32
3.2.1 Germination rate.....	32
3.2.2 Process of symptom development.....	33
3.2.2.1 Effect of different temperatures.....	35
3.2.3 Assessment of the aggressiveness <i>in vivo</i> : accumulation of reactive oxygen species.....	37
3.2.3.1 Superoxide anion ( $O_2^-$ ) in the leaf tissue.....	37

3.2.3 2 Hydrogen peroxide ( $H_2O_2$ ) in the leaf tissue.....	38
3.3 Microscopical studies of the infection process.....	39
3.3.1 Growth on the host leaf surface.....	39
3.3.1.1 Germination of conidia.....	39
3.3.1.2 Appressorium Formation.....	40
3.3.2 Penetration of the host cuticle and growth in the host tissue.....	41
3.3.3 Subcuticular growth.....	42
3.3.4 Invasion of the host tissue.....	44
3.3.4.1 Intercellular hyphae.....	44
3.3.4.2 Intramural mycelia.....	45
3.3.4.3 Haustorium.....	46
3.3.4.4 Intracellular hyphae.....	49
3.3.5 Fructification.....	50
3.3.6 Growth through the lower side of leaf.....	52
3.3.7 Overwintering.....	53
3.3.7.1 On fallen leaves.....	53
3.3.7.2 On the canes.....	54
3.4 Investigations of the effect of nutrient source on the fungal development.....	55
3.4.1 Development of <i>D. rosae</i> on artificial growth media.....	55
3.4.2 Fungus development in detached leaves and intact plants.....	69
3.5 Investigations into the effectiveness of fungicides on the development of <i>D. rosae</i> .....	70
3.5.1 Effect on symptom development.....	70
3.5.2 Effect on pre-infection structures.....	76
3.5.2.1 <i>In vitro</i> analysis.....	76

3.5.2.2 <i>In vivo</i> analysis.....	76
3.5.3 Effectiveness on the different fungal structures	
after curative applications.....	78
3.5.3.1 Effectiveness of application on the 3 <sup>rd</sup> dpi.....	78
3.5.3.2 Effectiveness of application on the 7 <sup>th</sup> dpi.....	88
3.5.3.3 Effectiveness of application at the 10 <sup>th</sup> dpi.....	92
<b>4 Discussion.....</b>	<b>96</b>
<b>5 References.....</b>	<b>127</b>