

Catalina Acuña-Gutiérrez

**Optical detection of microbial
infestation and mycotoxins in
beans (*Phaseolus vulgaris* L.)**

Optical detection of microbial infestation and mycotoxins in beans (*Phaseolus vulgaris* L.)

**Dissertation to obtain the doctoral degree of
Agricultural Sciences (Dr. sc. agr.)**

Faculty of Agricultural Sciences

University of Hohenheim

Institute of Agricultural Engineering 440e

Tropics and Subtropics Group

submitted by

Catalina Alejandra Acuña-Gutiérrez

from San José, Costa Rica

2023

This thesis was accepted as a doctoral dissertation in fulfillment of the requirements for the degree “Doktor der Agrarwissenschaften” (Dr. sc. agr. / Ph.D. in Agricultural Sciences) by the Faculty of Agricultural Sciences at University of Hohenheim on 26.04.2023.

Date of oral examination: 16.10.2023

Examination Committee:

Prof. Dr. Joachim Müller	(Supervisor and reviewer)
Prof. Dr. Víctor M. Jiménez	(Co-reviewer)
Prof. Dr. Thomas Miedaner	(Additional examiner)
Prof. Dr. Uwe Ludewig	(Head of examination committee)

Schriftenreihe des Lehrstuhls für Agrartechnik in den Tropen und
Subtropen der Universität Hohenheim
herausgegeben von Prof. Dr. Joachim Müller

Band 2024/30

Catalina Acuña-Gutiérrez

**Optical detection of microbial infestation and
mycotoxins in beans (*Phaseolus vulgaris* L.)**

D 100 (Diss. Universität Hohenheim)

Shaker Verlag
Düren 2024

Bibliographic information published by the Deutsche Nationalbibliothek

The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available in the Internet at <http://dnb.d-nb.de>.

Zugl.: Hohenheim, Univ., Diss., 2023

Copyright Shaker Verlag 2024

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission of the publishers.

Printed in Germany.

ISBN 978-3-8440-9349-0

ISSN 1867-4631

Shaker Verlag GmbH • Am Langen Graben 15a • 52353 Düren

Phone: 0049/2421/99011-0 • Telefax: 0049/2421/99011-9

Internet: www.shaker.de • e-mail: info@shaker.de

Acknowledgements

I am grateful for all instances that made possible the development of this research. I am deeply grateful to the Deutscher Akademischer Austausch Dienst (DAAD) for granting me a scholarship and to the University of Costa Rica for its academic support. Moreover, this research was made possible through financial support from Fiat Panis (research grant No. 07/2019) and QS Qualität und Sicherheit.

First and foremost, I want to deeply thank my supervisor, Prof. Dr. Joachim Müller, for accepting me into his team. His guidance, supervision, and teachings enabled my scientific growth and could only motivate me further in this journey. I would also like to give my most profound appreciation to my mentor Prof. Dr. Víctor Jiménez, who, from a very early point in my career, always motivated and encouraged me to get out of my comfort zone and participate in academic activities that helped to enrich my knowledge. For the support provided during these years, I will be forever indebted.

I cannot express enough my gratitude to my office mates, Iris, Steffen, and Janvier, for always providing an excellent working atmosphere, even through hard times, like during the pandemic.

A special thanks to my colleagues at the Grains and Seeds Research Center (CIGRAS) of the University of Costa Rica, especially to Dr. María Viñas, Dr. Andrea Irias, Diego Bogantes, and Danilo Alvarado, for your help and collaboration at different points of my research.

I want to acknowledge the team at the Agricultural Engineering in the Tropics and Subtropics (ATS), Mrs. Ute Kayser, Olga Gotra, Sarah Fleischman, Stephanie Tutsch, and Ute Waldeck for always supporting my work along the way. A special thanks to Mrs. Sabine Nugent for proofreading all my work these past years.

I would like to acknowledge the Core Facility (640) of the University of Hohenheim, especially Dipl. Ing. Edeltrud Könzen and Claudia Molitor for their help and willingness to support me in my experiments. Moreover, thank you to the staff at the Department of Nutritional Crop Physiology (340h) for opening your doors to me to perform my experiments and for always providing me with a welcoming environment in which to do my work.

Acknowledgements

To my colleagues and friends at the ATS group, Farah Marabet, Ziba Barati, Alice Reineke, Joevin Bonzi, Adnan Mukhtar, Bilhate Chala, Shamaïla Kan, Sebastian Romuli, Klaus Meissner, Klaus Spohrer, Haimanot Ayele, Sawittree Chai, Supaporn Klaykruey, Selamawit Debele, Sreymey Ngoun, Zhangkai Wu, Boris Mandrapa, Leon Oehme, and Ana Salvatierra-Rojas, I appreciate every conversation we had during our breaks and scientific presentations that inspired new ideas to my research. My most sincere gratitude for your support throughout these years.

To my husband, brainstorming partner, and colleague, Andrés Hernández-Pridybailo, my most profound appreciation for jumping into this journey with me. Thank you for the love, support, and endless conversations we have had about our dissertations, where new and exciting ideas arose. Moreover, special thanks to Galina Pridybailo, Antonio Hernández, Guido Hernández, and my grandparents Carmen Martin and Alfredo Acuña for your encouragement and support in this process.

Finally, I dedicate this work to my parents, Damaris Gutiérrez and Alfredo Acuña, and my brother Alejandro Acuña. I cannot express the words to thank all the love and support you have shown in supporting my career and always encouraging me to follow my dreams, especially my father, who always encouraged my scientific curiosity from a very early age. Without your support, I do not think I would have ever taken this path.

Catalina Acuña-Gutiérrez

Table of contents

Acknowledgements	i
Table of contents	iii
List of figures	vi
List of tables	viii
1 General introduction.....	1
1.1 Common beans: nutritional benefits, production, and consumption.....	1
1.2 Fungal infections and mycotoxins: their association with common bean.....	3
1.3 Mycotoxin detection.....	4
1.3.1 Optical detection methods: Near-infrared spectrometry (NIRS) and Hyperspectral imaging (HSI)	5
1.3.1.1 Generalities	5
1.3.1.2 Optical detection methods and their application in pulses	6
1.4 Prospects and challenges.....	7
1.5 Objectives and structure of the research	7
1.6 References	8
2 Occurrence of mycotoxins in pulses	13
2.1 Abstract	13
2.2 Introduction	14
2.3 Methodology	17
2.4 Reports on fungi and mycotoxin incidence in pulses.....	18
2.4.1 Overview of the research conducted to date in pulses	19
2.4.2 Techniques used for the detection of mycotoxins in pulses.....	24
2.5 Effect of climatic conditions on mycoflora development and its relationship to mycotoxin production in pulses	27
2.6 (Absence of) Association between infection grade and accumulation of mycotoxins...	28
2.7 Potential effect of phenolic compounds of mycotoxin production in pulses	30
2.8 Phomopsins – An under-studied mycotoxin that primarily affects pulses	32
2.9 Physiological effects of (myco)toxins on pulse seeds and seedlings	33
2.10 Conclusion and future perspectives.....	34
2.11 References	36

3	Comparison of near-infrared spectroscopy (NIRS) and hyperspectral imaging (HSI) for the early detection of <i>Fusarium verticillioides</i> contamination in common black beans (<i>Phaseolus vulgaris</i> L.).....	47
3.1	Abstract	47
3.2	Introduction	48
3.3	Materials and methods	49
3.3.1	Minimally invasive disinfection and moistening of grains	49
3.3.2	Inoculation with <i>F. verticillioides</i> isolates, sampling, handling, and processing of beans	51
3.3.3	Detection of <i>F. verticillioides</i> infection by HSI.....	52
3.3.4	Detection of <i>F. verticillioides</i> infection by NIRS	53
3.3.5	Quantification of <i>F. verticillioides</i> infection by qPCR (reference method).....	53
3.3.6	Data analysis and chemometrics	54
3.4	Results	55
3.4.1	Quantification of <i>F. verticillioides</i> infection by qPCR (reference method).....	55
3.4.2	Comparing calibration models based on optical detection methods	55
3.5	Discussion	58
3.6	Conclusions	61
3.7	References	65
4	Detecting fumonisin B ₁ in black beans (<i>Phaseolus vulgaris</i> L.) by near-infrared spectroscopy (NIRS).....	69
4.1	Abstract	69
4.2	Introduction	70
4.3	Material and methods	71
4.3.1	Determination of initial mycotoxin content	72
4.3.2	Sample contamination with FB ₁	73
4.3.3	Reference method.....	74
4.3.4	NIR measurements	75
4.3.5	Data analysis and chemometrics.....	75
4.4	Results	76
4.4.1	Effectiveness of the artificial contamination method	76
4.4.2	Data processing and calibration model	78
4.4.2.1	Data pretreatment	78
4.4.2.2	PCA results	78

4.4.2.3	NIRS calibration model.....	79
4.5	Discussion.....	82
4.6	Conclusions	85
4.7	References	88
5	General discussion.....	93
5.1	State-of-the-art in the investigation of mycotoxins in pulses.....	93
5.2	Detection of <i>Fusarium verticillioides</i> by optical methods	94
5.3	Detection of fumonisin B ₁ in common black beans by near-infrared spectroscopy (NIRS) 95	
5.4	Conclusions	97
5.5	Outlook.....	98
5.6	References	99
6	Summary	101
7	Zusammenfassung.....	104
	Curriculum vitae	¡Error! Marcador no definido.

List of figures

Figure 1-1. Dry bean production by continent from 2011 to 2021. (FAO, 2023).....	2
Figure 1-2. Common steps required to detect mycotoxins in food commodities. Black arrows describe the general work flow for mycotoxin detection by traditional methods, while the dotted arrows show the alternative pathways using optical detection methods. ELISA: enzyme-linked immunosorbent assay, HPLC: High-performance liquid chromatography, HSI: hyperspectral imaging, and NIRS: near-infrared spectrometry. (Author’s own elaboration).....	5
Figure 2-1. Correspondence analysis between the research aims and their association with the country’s income level from where the samples or the study originated. Colors blue to red indicate the level of contribution of the research aim to the variability, with red representing the highest. Continents in gray are supplementary variables associated with the country’s income. Countries were categorized according to The World Bank income classification. “Market sampling” is defined as monitoring the mycotoxin status in a particular country or region, including other commodities, and “specific survey in pulses” is dedicated monitoring of the mycotoxin status in a specific country, specifically in pulses.....	20
Figure 2-2. Frequency of mentions of the different (a) pulses and (b) mycotoxins in the studies cited in Table S1.	23
Figure 2-3. Detection techniques used to determine mycotoxins in pulses grouped by continent. Rapid tests include enzyme-linked immunosorbent assay (ELISA), indirect competitive assay, lateral flow immunochromatographic assay, and AflaTest. Abbreviations: BGYF, bright greenish-yellow fluorescence; GC, gas chromatography; HPLC, high-performance liquid chromatography; NIRS, near-infrared spectrometry; TLC, thin layer chromatography.....	26
Figure 3-1. The system employed for the disinfection of black bean grains with chlorine gas (Cl ₂). Air coming from an aquarium pump (a) is forced through a rubber hose with an air stone to an Erlenmeyer flask filled with water (b) and passed through a sterile cotton filter (c). A clamp is used as a shutoff valve to facilitate the disconnection of the box without letting in air from outside (d). The air enters an 8.5 L box for sample disinfection using Cl ₂ (e). The box is supported by a lab jack (f). The air escapes then through a check valve (g) to an	

Erlenmeyer flask containing bromothymol blue solution as a pH indicator to detect Cl ₂ (h).	51
Figure 3-2. Fungal DNA concentration in common black beans sampled every 24 h after inoculation with spores of <i>F. verticillioides</i> (isolates Fv1 and Fv2 averaged together). NI = non-inoculated. Box plots show the median and interquartile range. Six replicates were used for the analysis. DM: dry matter.	55
Figure 3-3. NIRS (a) and HSI (b) prediction results of <i>F. verticillioides</i> (isolates Fv1 and Fv2) infection in common black beans calibrated with fungal DNA content quantified by qPCR. DM: dry matter.	56
Figure 3-4. The score plot obtained from the PLSR models of NIRS (a) and HSI (b) used to predict the fungal DNA content of <i>F. verticillioides</i> in common black beans (isolates Fv1, Fv2, and non-inoculated NI).....	58
Figure 4-1. Measured FB ₁ concentration vs. applied theoretical concentration for submersion contamination (full line) and spread contamination (dotted line).	77
Figure 4-2. PCA results for beans artificially contaminated with FB ₁ . (a, b) corresponds to the submersion contamination method and (c, d) to the spread one. (a, c) are measurements done with whole beans and (b, d) those with milled ones.....	79
Figure 4-3. Prediction results for the applied FB ₁ . (a,b) correspond to the submersion contamination method, and (c, d) to the spread one. (a, c) are the measurements done with whole beans, and (b, d) with milled ones.	80
Figure 4-4. Regression coefficient plot for the calibration model of milled beans with the spread contamination. (a) Corresponds to the coefficient of the factor #1 and (b) to factor #2 in the model.....	82

List of tables

Table 2-1. Complete list of plants considered as pulses matching the FAO (1994) classification (adapted from Calles, 2016)	14
Table 3-1. Partial least square regression (PLSR) results of the optical detection methods (NIRS and HSI) used to predict <i>F. verticillioides</i> (isolates Fv1 and Fv2) infection in common black beans.	57
Table 3-2. Relevant wavelengths of the optical detection methods (NIRS and HSI) for the prediction model of <i>F. verticillioides</i> infection in common black beans. Common wavelengths among both optical methods are in bold.	57
Table 4-1. Concentration of the prepared solutions to reach the desired FB ₁ concentration.	74
Table 4-2. Classification groups for the different concentrations of the FB ₁ used for the contamination.....	77
Table 4-3. Regions of the spectra used for the development of a calibration model.	78
Table 4-4. PLS regression results for two different artificial FB ₁ contamination methods on black beans.	81