Plant-Plant Allelopathic Interactions II

Udo Blum

Plant-Plant Allelopathic Interactions II

Laboratory Bioassays for Water-Soluble Compounds with an Emphasis on Phenolic Acids



Udo Blum Raleigh North Carolina USA

ISBN 978-3-319-04731-7 ISBN 978-3-319-04732-4 (eBook) DOI 10.1007/978-3-319-04732-4 Springer Cham Heidelberg New York Dordrecht London

Library of Congress Control Number: 2011922311

© Springer International Publishing Switzerland 2014

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed. Exempted from this legal reservation are brief excerpts in connection with reviews or scholarly analysis or material supplied specifically for the purpose of being entered and executed on a computer system, for exclusive use by the purchaser of the work. Duplication of this publication or parts thereof is permitted only under the provisions of the Copyright Law of the Publisher's location, in its current version, and permission for use must always be obtained from Springer. Permissions for use may be obtained through RightsLink at the Copyright Clearance Center. Violations are liable to prosecution under the respective Copyright Law.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

While the advice and information in this book are believed to be true and accurate at the date of publication, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

This volume is dedicated to researchers and their graduate students who are interested in studying plant-plant allelopathic interactions.

Preface

In a previous volume (Blum 2011) the author suggested that we could improve our understanding of plant-plant allelopathic interactions in the field by making laboratory bioassays more holistic. The comments and suggestions in that volume as to how to go about that were rudimentary at best. Reflections after the volume was published lead the author to conclude that a more detailed analysis of the factors making up laboratory bioassays was needed in the hope that such an analysis would provide clearer and more useful directions on how to design more holistic or more relevant laboratory bioassay systems. The more holistic being a theoretical goal and the more relevant being a more pragmatic goal.

More specifically this volume presents a detailed description and discussion of the underlying features, issues, and suppositions associated with seed and seedling laboratory bioassays presented in the earlier volume. It also continues the retrospective analysis of seed and seedling laboratory bioassays begun in the previous volume. It is, however, broader in scope and substance in that the information provided is relevant to all water-soluble compounds released to soil by putative allelopathic living plants and their litter and residues. It is ultimately an attempt to update and expand the practical guidelines for designing laboratory bioassays that have previously been provided in the literature with the hope that the designs of future seed and seedling laboratory bioassays will become more relevant to field systems. This volume like its predecessor does not provide a comprehensive review of the literature. The literature about designing and implementing laboratory bioassays of water-soluble allelopathic compounds is much too extensive for that. Standard references have been included to provide background and additional details.

Chapter 1 provides a general introduction to this volume, discusses the nature of plant-plant allelopathic interactions, describes the nature and sources of allelopathic compounds in soils, discusses the concepts of holism and reductionism as it relates to laboratory bioassays, provides a listing of benefits, limits, and common pit falls for laboratory bioassays, and answers or sets the stage for answering the following questions: (a) Why is it important to design laboratory bioassays that are more holistic or, stated in a more pragmatic way, more relevant to field environments? (b) What can be done to make laboratory bioassays more relevant to field environments? and (c) Is it always necessary to make laboratory bioassays relevant

to field environments? Chapter 2 describes and provides comments on the following basic features of laboratory bioassays: (a) biotic and physicochemical factors, (b) test materials, (c) measurements, hypotheses, experimental designs, and data analyses, and (d) basic information that should be provided for all laboratory bioassays. Chapter 3 discusses a number of issues and challenges associated with creating more relevant model laboratory bioassays including the following: treatment concentrations, mobility and transport, species density, symbiotic relationships, microorganisms, controls or references, and measurements among others. Chapter 4 describes a set of standard hypothetical laboratory bioassays that may be used to screen for stimulatory or inhibitory effects of identified putative allelopathic compounds, leachates, exudates, litter, residues, and soils. Comments regarding potential benefits and limitations of these bioassays are provided. Chapter 5 provides an abridged version of the known effects, the physicochemical and biotic factors that modify effects, and the modes of action of allelopathic compounds using phenolic acids as the model compound. Chapter 6 describes a number of standard laboratory bioassays for identifying and characterizing the modes of action by which identified putative allelopathic compounds, mixtures of allelopathic compounds, mixtures of organic and/or inorganic compounds and residues may stimulate or inhibit sensitive weed species. Five different approaches will be described: (a) bioassays for simple mixtures of identified putative allelopathic compounds, (b) bioassays for residue leachates plus or minus XAD-4 resin, (c) the application of regression analysis to data from residues and soil extract bioassays, (d) bioassays for determining the role of treatment surface area, and (e) using omics methods as tools to determine modes of action. Chapter 7 compares field systems with past and present laboratory bioassay systems, provides some thoughts on ways to minimize the impacts of atypical factors in seed and seedlings laboratory bioassays, points out which factors limit our ability to design field-relevant model systems, suggests future directions for laboratory and field research on plant-plant allelopathic interactions in a question format, and outlines the central tenets (i.e., opinions, doctrines, or principles) articulated in this volume.

Finally, this volume has been written specifically for researchers and their graduate students who are interested in studying plant-plant allelopathic interactions. The author hopes that this retrospective and at times critical analysis of past standard laboratory bioassays will provide a foundation for better and more field-relevant laboratory designs in the future.

Reference

Blum U (2011) Plant-plant allelopathic interactions. Phenolic acids, cover crops, and weed emergence. Springer Science and Business Media, Dordrecht

Acknowledgments

The author wishes to thank Regina G. Belz, Mary Ann Blum, Nicole Blum, Larry F. Grand, Stephen O. Duke, Jeffrey D Weidenhamer, Leslie A Weston, A Doug Worsham, and Deyu Xie for editing, reviewing, and thoughtful and constructive comments, Amy Blum Grady for the following illustrations: Fig. 1.1, 2.1, 3.1, 4.4, and 4.5, and the Department of Plant and Microbial Biology at North Carolina State University for their support. I would like to especially acknowledge the contribution of mv wife, Mary Ann, and our two daughters, Amy and Nicole, for their continued support throughout the years and for their contributions to this volume. The author also wishes to acknowledge the contributions of faculty, students, and technicians at North Carolina State University and researchers world-wide who over the years contributed to the research upon which this volume is based. Writing this volume was truly a cooperative venture. Finally, in the previous volume (see Blum 2011) under acknowledgements the author neglected to specifically acknowledge the contributions of TM Gerig, C Brownie, and JO Rawlings for statistical analysis and modeling of data described in that volume and to include FL Booker under the list of faculty members who influenced, shaped, and reshaped the author's research program in allelopathy.

Reference

Blum U (2011) Plant-plant allelopathic interactions: Phenolic acids, cover crops, and weed emergence. Springer Science and Business Media, Dordrecht.

Contents

1	Bac	kgroun	d for Designing Laboratory Bioassays]
	1.1	Introd	uction	
	1.2		pathic Interactions	2
	1.3	Nature	e of Allelopathic Compounds	4
	1.4	Source	es of Allelopathic Compounds and Modifiers in Soils	7
	1.5	1.5 Holism and Reductionism		11
		1.5.1	Why is It Important to Design Laboratory	
			Bioassays that are More Holistic?	14
		1.5.2	What can be Done to Make Laboratory Bioassays	
			More Relevant to Field Environments?	15
		1.5.3	Is It Always Necessary to Make Laboratory	
			Bioassays Relevant to Field Environments?	16
	1.6	Benef	its and Limits of Laboratory Bioassay	16
	1.7	False	Assumptions and Misconceptions	
		for Laboratory Bioassays		18
	Refe	References		
2	Introduction to the Fundamentals of Laboratory Bioassays			3
	2.1	Factor	rs of Bioassay Systems	31
		2.1.1	Biotic Factors	31
		2.1.2	Physicochemical Factors	35
		2.1.3	Test Materials	4(
		2.1.4	Measurements	6
		2.1.5	Hypotheses, Experimental Designs, and Data Analyses	63
	2.2	Basic	Information Required for All Bioassay Systems	64
	Refe	References		
•	C			7
3		8 8 8 V V		
	3.1			
	3.2			
		3.2.1	Minimum Concentrations	79
		3.2.2	Modifiers of Active/Effective Concentrations	81

		3.2.3	Frequency of Treatments and/or Adjustments
		3.2.4	Concentration and Dose
		3.2.5	Serial Dilutions of Complex Mixtures
	3.3	Field I	Inputs and Laboratory Treatments of Water-Soluble
			ounds
	3.4	Mobili	ity and Proximity of Compounds in Soil Media
	3.5	Seed a	Ind Seedling Densities
	3.6	Symbi	iotic Relationships
	3.7		ficroorganisms (Microflora and Fauna)
		3.7.1	Numbers
		3.7.2	Species Diversity
		3.7.3	Biomass
		3.7.4	Distribution
		3.7.5	Function
		3.7.6	Relevance to Laboratory Bioassays
	3.8		vory and Disease
	3.9	Physic	cochemical Environments
	3.10	Refe	rences or Controls
	3.11	Meas	surements
		3.11.1	Seeds/Seedlings/Older Plants
		3.11.2	0
		3.11.3	Media
		3.11.4	
	3.12	Final	Comments
	Refe	rences	
4	Нур		al Standard Screening Bioassays
	4.1		uction
	4.2	Living	g Plants
		4.2.1	Solutions Used to Collect Leachates and "Root
			Exudates Plus"
		4.2.2	Leaf Leachates
		4.2.3	
		4.2.4	Significance
	4.3	Plant I	Litter and Residues
		4.3.1	5
		4.3.2	Test Materials
		4.3.3	Simulated-Rain Water
		4.3.4	Residue Bioassays
		4.3.5	Residue Leachates
		4.3.6	Significance
	4.4	Field S	Soils
		4.4.1	Soil
		4.4.2	Soil Plus or Minus Activated Carbon
		4.4.3	Soil Extracts

		4.4.4 Significance	175			
	4.5	Final Comments	176			
	Refe	erences	177			
5	Effe	Effects, Modifiers, and Modes of Action of Allelopathic				
	Con	npounds Using Phenolic Acids as Model Compounds	185			
	5.1	Introduction	185			
	5.2	Individual Compounds	188			
		5.2.1 Stimulatory Effects	188			
		5.2.2 Inhibitory Effects	190			
		5.2.3 Modifying Factors	192			
		5.2.4 Summary Comments	219			
	5.3	Simple Mixtures	220			
		5.3.1 Stimulation	220			
		5.3.2 Inhibition	220			
		5.3.3 Summary Comments	224			
	5.4	Complex Mixtures	225			
	5.5	Modes of Action	226			
	5.6	Final Comments	227			
		erences	228			
			-			
6	Hvp	oothetical Cause and Effect Bioassays	237			
	6.1	Introduction	237			
	6.2	Identified Putative Allelopathic (IPA) Organic Compounds	242			
	6.3	Complex Solutions	249			
	6.4	Using Regression Analyses to Relate Potential				
		Causes with Effects	253			
		6.4.1 Residues	254			
		6.4.2 Soil Extracts	256			
	6.5	Treatment Surface Areas	259			
	6.6	Using Omics Methods as Tools	264			
		erences	266			
	Reit		200			
7	Lah	oratory Model Systems and Field Systems: Some				
'		al Thoughts	273			
	7.1	Introduction	273			
	7.2	Comparison of Field and Present Laboratory Model Systems	274			
	1.2	7.2.1 Inputs of Water-Soluble Compounds	274			
		7.2.2 Losses of Water-Soluble Compounds	275			
		7.2.2 Eoses of water-soluble compounds7.2.3 Timing and Frequency of Inputs and Losses	275			
		7.2.4 Treatment Surface Areas	270			
		7.2.4 Treatment Surface Areas7.2.5 Microorganisms (Microflora and Fauna)	277			
			278			
		7.2.7 Plant Densities	278			
		7.2.8 Physicochemical Environments	279			

	7.2.9	Available Water-Soluble Compounds	279
	7.2.10	Doses (Active Water-Soluble Compounds)	280
	7.2.11		281
	7.2.12	2 Final Comment	281
7.3	Is the	Present Criticism by Critics Regarding Plant-Plant	
	Allelo	pathic Interactions in the Field Credible?	281
7.4	Impro	wing the Value of Laboratory Bioassay Systems	282
	7.4.1		282
	7.4.2	Physicochemical Environments	283
	7.4.3		283
	7.4.4	Treatments	283
7.5	Future	e Directions: Questions that Need Answers	285
7.6	Centra	al Tenets (i.e., Opinions, Doctrines,	
	or Pri	nciples) Articulated in this Volume	287
	7.6.1	Plant-Plant Allelopathic Interactions	287
	7.6.2	Laboratory Bioassay Systems	293
	7.6.3	Personal Note	295
7.7	Final	Comments	296
Refe	rences		297
Subject	Index		301
Author	Index		315

Abbreviations

AIW	Average individual weight
AM	Arbuscular mycorrhizae
ATP	Adenosine triphosphate
BOA	Benzoxazolin-2-one
CFU	Colony-forming units
C/N	Carbon/Nitrogen ratio
DIBOA	2,4 dihydroxy-1,4-benzoxazin-3(4H)-one
ECM	Ectomycorrhizae
ED	Effective dose
EDTA	Ethylenediamine tetraacetic acid
FA	Ferulic acid
F-C	Folin-Ciocalteau
FER	Ferulic acid
GB	Glass beads
IPA compound	Identified putative allelopathic compound
MES	2(N-morpholino)-ethanesulfonic acid
NMR	Nuclear magnetic resonance
NPK	N, NO ₃ , NH ₄ , P and K unless otherwise indicated
PCO	<i>p</i> -Coumaric acid
pC-M	<i>p</i> -Coumaric acid and Methionine
pC-G	<i>p</i> -Coumaric acid and Glucose
PPFD	Photosynthetic Photon Flux Density
PVP	Polyvinylporrolidone
PVPP	Polyvinylpolyporrolidone
R	Reference
VA	Vanillic acid
XAD or XAD-4 resin	Amberlite XAD polymeric absorbent

List of Figures

Fig. 1.1 Fig. 1.2	Pathways by which organic and inorganic compounds are lost from plants. (Illustration by Amy Blum Grady, used with permission) Summary of sources, types of transport, and soil processes that determine the nature of soil solutions	8 10
Fig. 2.1	Two symbiotic relationships, nodules and mycorrhizae. (Illustration by Amy Blum Grady, used with permission)	33
Fig. 3.1	Zones for arbuscular mycorrhizae. (Illustration by Amy Blum Grady, used with permission)	107
	Field of sunflowers in Minnesota in August Petri dish system with morningglory seeds, water, and filter paper	135
-	on day one and day four Morningglory seedlings growing in soil cup system Stair-step system for studying recirculating "root exudates plus" leachates. (Illustration by Amy Blum Grady,	139 141
Fig. 4.5	used with permission) XAD-4 resin and glass bead systems for collecting "root exudates plus" leachates. A modified version of figure by Tang and Young (1982) used with permission of American Society of Plant Biologists. (Illustration by Amy Blum Grady,	145
Fig. 4.6	used with permission) Wheat and reference plots just before harvest in central North Carolina in June	151 155
Fig. 4.7	Morningglory (<i>top</i>), pigweed (<i>middle</i>), and prickly sida (<i>bottom</i>) seedlings in wheat residue field subplots in July. (<i>middle</i> and <i>bottom</i> figures taken from Blum (2011), figures used with permission of Springer Science and Business Media)	
Fig. 5.1	Some common simple plant phenolic acids, cinnamic acid derivatives on the <i>right</i> and benzoic acid derivatives on the <i>left</i> , where H equals hydrogen, OH equals hydroxy, and OMe equals methoxy. (Figure taken from Blum (2011), figure used with	
	permission of Springer Science and Business Media)	187 xvii

Fig. 5.2	Effects on absolute and relative rates of leaf expansion of cucumber seedlings grown in solution culture given a single 0, 0.25, 0.5 or 1 mM ferulic acid or <i>p</i> -coumaric acid treatment on day 16. All treatment solutions were replaced on day 18 with nutrient solutions. Seedlings were grown in Hoagland's nutrient solution plus or minus phenolic acids and 5 mM MES buffer. The pH values of the initial treatment solutions were 5.5, 6.25, or 7.0 (N =3). Points are connected only to aid in the visualization of patterns over time. (Figure taken from Blum et al. (1985b).	
	Plenum Publishing Corporation, figure used with permission of Springer Science and Business Media)	194
Fig. 5.3	Effects on water utilization of cucumber seedlings grown in solution culture given a single 0, 0.25, 0.5 or 1 mM ferulic acid or <i>p</i> -coumaric acid treatment on day 16. All treatment solutions were replaced on day 18 with nutrient solutions. Seedlings were grown in Hoagland's nutrient solution plus or minus phenolic acids and 5 mM MES buffer. The pH values of the initial treatment solutions were 5.5, 6.25, or 7.0 (N =3). Points are connected only to aid in the visualization of patterns over time. (Figure taken from Blum et al. (1985b). Plenum Publishing Corporation, figure used with permission of Springer Science and Business Media)	198
Fig. 5.4	Percent inhibition of net phosphorus (Pi) uptake by cucumber roots given a 5 h treatment of ferulic acid in solution culture when 12 days old. Treatment solutions contained ferulic acid (0.25, 0.5 or 0.75 mM), 0.5 mM CaSO ₄ , 5 mM MES buffer, and 0.5 mM KH ₂ PO ₄ . Regression models: pH 4.5 = not significant, pH 5.5 r^2 =0.71, and pH 6.5 r^2 =0.45. Based on figure from Lehman and Blum (1999b). (Plenum Publishing Corporation, data derived from figure used with permission of Springer Science and Business Media)	200
Fig. 5.5	Percent distribution of total ferulic acid equivalents in cucumber seedlings exposed for 5 h to 5 mM plus [U-ring ¹⁴ C] labeled ferulic acid. Equivalents were based on the specific activity of the treatment solutions. For details regarding treatment solutions see text. Mean seedling dry weights for 8 day-old seedlings were (a) roots = 30 mg, stem = 20 mg, cotyledons = 40 mg, and leaves = 110 mg and for 18 day old seedlings (b) roots = 90 mg, stem = 50 mg, cotyledons = 50 mg, and leaves = 200 mg. (Figures based on data from Shann and Blum (1987b). Pergamom Journals Ltd., data used with permission of Elsevier B.V.)	202
		202

Fig. 5.6 Effects of a 7-phenolic acid mixture modeled after phenolic acids found in no-till wheat-soybean soil extracts (pH 5) on radicle and hypocotyl lengths of crimson clover as modified by polyethylene glycol (a; $r^2 = 0.61$) and Hoagland's solution (b; $r^2 = 0.37$) based on freezing-point depression (milliosmoles, mOsm) of solutions. The 7-phenolic acid mixture was composed of 10% caffeic acid, 9% ferulic acid, 35% p-coumaric acid, 15% p-hydroxybenzoic acid, 4% sinapic acid, 10% syringic acid, and 17% vanillic acid. Figures originally based on regressions from Blum et al. (1992). Plenum Publishing Corporation, regressions used with permission of Springer Science and Business Media. (Figure taken from Blum (2011), figure used with permission of Springer Science and Business Media)..... 206 Changes in net phosphorus uptake (**a**; $r^2 = 0.52$), net water uptake Fig. 5.7 (**b**: $r^2 = 0.19$), and absolute growth rates of leaf expansion (**b**: r^2 for FER = 0.76 and PCO = 0.58) of 13–15 day-old cucumber seedlings grown in nutrient culture as the proportion of the root systems in contact with a phenolic acid was increased, where FER equals 0.5 mM ferulic acid and PCO equals 0.5 mM p-coumaric acid. Figures originally based on regressions from Lyu and Blum (1990; a, net phosphorus uptake; b, water utilization) and Lehman et al. (1994; b, leaf expansion). Plenum Publishing Corporation, regressions used with permission of Springer Science and Business Media. (Figure taken from Blum (2011), figure used with permission of Springer Science and Business Media) 208 Fig. 5.8 Percent inhibition of p-coumaric acid treatments (day 6, 8, and 10) on transpiration, water utilization, leaf area, and absolute and relative rates of leaf expansion of cucumber seedlings growing in nutrient culture (pH 5.0). The reference (or baseline) values for untreated seedlings are presented in Fig. 5.9. (Figures taken from Blum and Gerig (2005). Figures used with permission of Springer Science and Business Media)..... 214 Fig. 5.9 Transpiration (ml/cm²/hr), water utilization (ml/seedling/hr), leaf area (cm²), and absolute (cm²/seedling/24 h) and relative $(cm^2/cm^2/24 h)$ rates of leaf expansion of cucumber seedlings growing in nutrient culture (pH 5.0) in the absence of phenolic acids. Means and standard errors in the figures were derived from parsimonious models of Blum and Gerig (2005). Absence of standard error bars indicates that error bars are smaller than the symbols representing the means. Means and standard errors used with permission of Springer Science and Business Media 215

Fig. 5.10	Percent inhibition of <i>p</i> -coumaric acid treatments (day 6, 8, and	
0	10) on shoot and root dry weights (g) of 12 day-old cucumber	
	seedlings grown in Hoagland's nutrient culture (pH 5.0). Shoot	
	and root dry weights of untreated seedlings were 0.123 ± 0.006	
	and 0.031 ± 0.002 g, respectively. Percent inhibition in figure for	
	shoot ($r^2=0.66$) and root ($r^2=0.47$) dry weight was derived from	
	parsimonious models of Blum and Gerig (2005). Models were	
	used with permission of Springer Science and Business Media	216
Fig 5 11	Concentrations for each phenolic acid (<i>one</i>), any combination	210
115.0.11	of two and three phenolic acids, and a combination of all four	
	phenolic acids required for an approximate 30% inhibition of	
	absolute rates of leaf expansion for 8–18 day-old cucumber	
	seedlings grown in Portsmouth B soil materials . (Figure taken	
	from Blum (1996). Figure used with permission of Society	
	of Nematologists)	222
Fig 5.12	Concentrations of <i>p</i> -coumaric acid and methionine (a), and	
Fig. 3.12	<i>p</i> -coumaric acid and glucose (b) required to inhibit dry weight	
	of morningglory seedlings growing in Portsmouth B and Cecil	
	B soils, respectively, by 10–50%. Figures adapted/replicated	
	from (a) Blum et al. (1993) and (b) Pue et al. (1995). Plenum	
	Publishing Corporation, figures used with permission of Springer	
	Science and Business Media. (Figure taken from Blum (2011),	
	figure used with permission of Springer Science and Business	
	• • • • •	223
Fig. 6.1	Media) An example of a nutrient-solution culture system. For this	223
rig. 0.1	· · ·	
	example, cucumber seedlings were treated with different concentrations of vanillic acid which were given 4 times on	
	e	244
Fig ()	alternate days starting with day 8	244
Fig. 6.2	Light banks with cucumber seedlings growing in nutrient- solution culture. (Figure taken from Blum (2011), figure used	
		245
Fig 6 2	with permission of Springer Science and Business Media)	243
Fig. 6.3	An example of a split-root system containing a cucumber	
	seedling. (Bottom figure taken from Blum (2011), figure used	260
	with permission of Springer Science and Business Media)	260

List of Tables

	Some examples of potential modifiers of active/effective concentrations of water-soluble identified putative allelopathic (IPA) compounds in the absence of microorganisms	83 84
Table 5.1	Relative potencies and standard errors for effects of <i>p</i> -coumaric acid, <i>p</i> -hydroxybenzoic acid, and vanillic acid compared to ferulic acid for transpiration, water utilization, leaf area, absolute and relative rates of leaf expansion, and shoot and root dry weights of cucumber seedlings growing in nutrient culture (pH 5.2)	213
Table 5.2	Relative potencies of sinapic acid, <i>p</i> -coumaric acid, vanillic acid, syringic acid, caffeic acid, <i>p</i> -hydroxybenzoic acid, and protocatechuic acid compared to ferulic acid for inhibition of absolute rates of leaf expansion of cucumber seedlings grown in Portsmouth soil B_1 soil materials (pH 5.2)	221
Table 6.1	Some examples of the application of omics methods to	
Table 6.2	plant-plant allelopathic interactions Some additional examples of how omics methods could be	265
	applied to plant-plant allelopathic interactions	265