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Microalgas em cultura de arroz: influência de diferentes manejos de adubação em áreas com residual de herbicidas (imidazolinonas)

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Recebido em 11.X.2012. Aceito em 18.XII.2013

RESUMO – O trabalho objetivou avaliar a influência de diferentes formas de adubação em arroz irrigado sobre a composição das comunidades de algas epifíticas e fitoplânctônicas em áreas com residual de herbicidas do grupo químico das imidazolinonas. O delineamento experimental abrangeu dez tratamentos que consistiram em diferentes formas de adubação e testemunho, em duas cultivares de arroz, sendo uma delas não tolerante ao herbicida. Ao total foram identificadas de 93 espécies para o epifiton e 151 para o fitoplâncton. Não foram observadas diferenças na composição taxonômica e densidades celulares entre os diferentes manejos de adubação, porém houve aumento da densidade celular no epifiton e no fitoplâncton com a maior incidência de radiação solar devido ao menor desenvolvimento da cultivar não tolerante ao residual do herbicida.

Palavras chave: agroecossistema, epifiton, fitoplâncton.

ABSTRACT – **Microalgae in paddy rice fields: influence of different fertilization treatments in areas with residual herbicides of the imidazolinone group.** The study evaluated the effect of different fertilization treatments in paddy rice fields on the community composition of epiphytic and planktonic algae in areas with residual herbicides of the imidazolinone group. The experiment consisted of ten treatments of different forms of fertilization and a control in two rice cultivars, one tolerant and one non-tolerant to the herbicide. Were identified 93 species for the epiphyton and 151 for the phytoplankton. No significant differences were found in taxonomic composition and cell density among the treatments, but there was an increase in epiphytic and phytoplanktonic cell density with increased solar radiation towards the end of the crop cycle due to slower growth of the non-tolerant cultivar residual herbicides.

Keywords: agroecosystems, epiphyton, phytoplankton.

INTRODUÇÃO

A lavoura de arroz pode ser considerada um agroecossistema temporariamente aquático, pois passa grande parte de seu cultivo inundada, sendo drenada na colheita e entressafra e devido às práticas agrícolas, passa por rápidas transformações físicas, químicas e biológicas (Roger, 1996). Serve como habitat temporário para bactérias, algas, plantas aquáticas e diversas espécies de vertebrados e

invertebrados constituindo uma grande diversidade biológica (Bambaradeniya *et al.* 2004).

Dentre as práticas aplicadas no cultivo, encontra-se a adubação com minerais, que possui o objetivo de fornecer às plantas, nutrientes que o ambiente não disponibiliza o suficiente para elevar a produtividade, além do uso de herbicidas, em especial do grupo químico das Imidazolinonas, que controlam um grande número de espécies de plantas daninhas em arroz irrigado. No entanto, após o

cultivo pode haver persistência destes herbicidas no solo, causando fitotoxicidade em culturas sucessoras (Kraemer *et al.* 2009). Essa redução no crescimento das plantas pode proporcionar maior penetração de radiação solar no interior do dossel, o que segundo Mustow (2002), favorece o desenvolvimento de diversos tipos de algas na cultura. Algas filamentosas e epifíticas podem ser prejudiciais na fase de germinação devido à competição por luz, no entanto, as microalgas juntamente com as macrófitas, participam da biomassa fotossintética aquática desempenhando um papel importante na ciclagem de nutrientes, no fornecimento de matéria orgânica para o solo nesses agroecossistemas, além de promover a fixação biológica de nitrogênio realizada pelas cianobactérias. A composição e estrutura destas comunidades aquáticas são fortemente influenciadas por fatores climáticos como luz e temperatura, fatores do solo como pH, fatores bióticos como pressão de pastejo, além das práticas agronômicas como uso de fertilizantes e agrotóxicos (Roger, 1996).

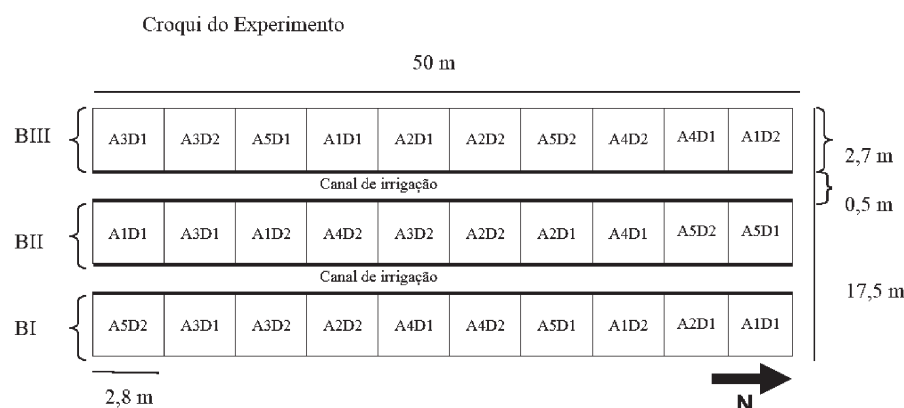
A biodiversidade de algas em lavouras de arroz foi estudada por Roger, 1996; Pereira *et al.*, 2000; Bambaradeniya *et al.*, 2004 e Alves da Silva & Tamanaha, 2007. Da mesma forma, o efeito das diferentes práticas agrícolas em comunidades planctônicas e vertebrados, como peixes, é pouco

conhecido podendo-se destacar os trabalhos de Furtado & Lucca, 2003; Resgalla Jr. *et al.*, 2007, Rodrigues *et al.*, 2011, Das *et al.*, 2011 e Mondol *et al.*, 2013.

O trabalho objetivou testar a hipótese de que existem alterações na composição e densidade de comunidades epifíticas e fitoplanctônicas da cultura de arroz irrigado em áreas com residual de imidazolinonas em manejos de adubação onde maiores quantidades de nutrientes estejam disponibilizadas.

MATERIAL E MÉTODOS

O experimento foi realizado em área de várzea com residual de herbicidas do grupo químico das imidazolinonas, no campo experimental do Departamento de Fitotecnia da Universidade Federal de Santa Maria, RS, Brasil (latitude: 29° 43'23" S, longitude: 53° 43'15" W e altitude: 95m), durante os anos agrícolas de 2009 e 2010. O delineamento experimental constituiu-se de blocos ao acaso em esquema bifatorial (5x2) com três repetições, sendo que as unidades experimentais mediram 10,8m², contendo dez tratamentos separados por taipas de 2 m de distância (Fig.1).



Amostragem de microalgas

	Data de coleta	Nº de amostras de Fitoplâncton	Nº de amostras de Epifiton
15° DAI	22 de janeiro de 2010	10 amostras X 3 Blocos	10 amostras X 1 Bloco
21° DAI	29 de janeiro de 2010	10 amostras X 3 Blocos	10 amostras X 1 Bloco
84° DAI	01 de abril de 2010	10 amostras X 3 Blocos	10 amostras X 1 Bloco

* DAI: dia após a irrigação.

Fig. 1. Delineamento experimental nos anos de 2009 e 2010, Santa Maria, RS. Fator A – Adubação; A1: adubação de base N, P e K na linha incorporada ao solo no momento da semeadura e com nitrogênio por ocasião da entrada da água; A2: adubação em superfície aplicada ao solo no momento da semeadura e com nitrogênio por ocasião da entrada da água; A3: adubação em superfície aplicada por ocasião da entrada da água; A4: sem adubação e com nitrogênio por ocasião da entrada da água e A5: sem adubação e sem nitrogênio (testemunho). Fator D – Cultivar; D1 – Cultivar IRGA 417; D2 – Cultivar IRGA 422 CL. Cada parcela foi circundada por taipas de 2 m de largura e 50 cm de altura.

A semeadura foi realizada no dia 09 de dezembro de 2009, em linhas com espaçamento de 0,17 m, utilizando 90 kg/ha de sementes tratadas com inseticida Fipronil e fungicida composto por Carboxina e Tiram nas doses 125 e 250 ml para 100 Kg de semente, respectivamente. A quantidade de adubo NPK aplicada foi 300 kg/ha da fórmula 5-20-30, sendo N = 15 Kg/ha, P = 60 Kg/ha e K = 90 Kg/ha. A adubação na linha foi realizada com uma semeadora adubadora de parcelas por ocasião da semeadura e em superfície a adubação foi aplicada a lanço. A adubação nitrogenada em cobertura foi de 70kg/ha na entrada da água (7 de janeiro de 2010) no estágio da cultura V3/V4 e 35kg/ha no estágio de iniciação do primórdio floral (R0) segundo escala de Counce *et al.* (2000). O controle de plantas daninhas foi realizado antes da entrada da água com o herbicida Bispyribac-sodium na dose de 125 ml/ha.

A radiação solar incidente no dossel da cultura foi avaliada através de Sensor de Radiação Solar (modelo LI-191 Line Quantum Sensor) e os dados registrados em *data logger* (modelo LI -1400).

Para as avaliações de fitoplâncton e epifiton foram realizadas amostragens de material em três datas (Fig.1) em função dos efeitos fitotóxicos do residual dos herbicidas nas plantas de arroz. De acordo com Kraemer *et al.* (2009), esse efeito ocorre em torno de até três a quatro semanas após a irrigação da área. Nesse sentido, foram escolhidas duas datas (15 e 21 DAI - dias após a irrigação) para verificar a influência da fitotoxicidade causada pelo herbicida na estrutura e composição das comunidades de algas. Além disso, aos 15° e 21° DAI as plantas se encontravam nos estádios iniciais de desenvolvimento, o que favorece maior incidência de radiação solar no interior do dossel podendo interferir também na estrutura e composição das algas. A terceira amostragem foi realizada no 84° DAI quando as plantas se encontram na fase de antese, um estágio mais avançado do seu desenvolvimento onde os efeitos da toxicidade já não se fazem presentes, permitindo a comparação com as épocas anteriores de maior suscetibilidade aos efeitos tóxicos dos residuais.

A amostragem do epifiton foi realizada em apenas um bloco experimental, através da retirada de folhas e colmos submersos da planta do arroz (*Oryza sativa* Linnaeus), usada como substrato natural, da qual o material aderido foi raspado com pinça e jatos de água destilada e a área superficial colonizada foi medida. As coletas de fitoplâncton consistiram na passagem de um frasco de 100 ml na sub-superfície da coluna d'água dos três blocos. Ambas as amostras

foram fixadas após a coleta com formalina 4%.

As análises quantitativas do perifiton e do fitoplâncton foram realizadas utilizando a técnica de Utermöhl (1958), através de microscópio invertido Motoc, modelo AE21, com aumento de 400 vezes. O número de campos foi definido pela contagem de ao menos 100 células da espécie mais abundante. As espécies abundantes e dominantes foram determinadas conforme o critério de Lobo & Leighton (1986), a identificação taxonômica a nível genérico seguiu Bicudo & Menezes (2006) e para espécie foram utilizadas bibliografias específicas. O material examinado foi tombado no Herbário da Universidade Federal de Santa Maria (SMDB) pertencente ao Departamento de Biologia em Santa Maria, RS, Brasil.

Os resultados de radiação solar incidente e densidade de células foram submetidos à análise de variância (ANOVA) e as médias foram comparadas pelo teste de Tukey a 5% de probabilidade de erro. As análises foram realizadas no software Action versão 2.3.

Com o objetivo de produzir uma representação gráfica das relações de abundância entre as espécies identificadas e os diferentes tratamentos, os dados foram ordenados através da Análise de Correspondência Destendenciada (DCA – Detrended Correspondence Analysis) (Hill & Gauch, 1980). O programa utilizado foi PC-ORD, versão 4.0 para Windows (McCune & Mefford, 1999). Os dados de densidades de células da comunidade fitoplanctônica e epifítica foram transformados por log (x+1), no programa FITOPAC, (Shepherd, 1996).

RESULTADOS E DISCUSSÃO

As coletas de microalgas na área experimental resultaram em um total de 120 amostras, sendo 30 epifíticas e 90 fitoplanctônicas. A incidência de radiação solar, apresentada na Tabela 1, mostrou um decréscimo durante o ciclo da cultura devido ao sombreamento causado pela ampliação da cobertura (dossel) do arroz.

Quando comparadas as datas de coleta, o 15° DAI apresentou os maiores índices de radiação solar para todos os tratamentos em relação às demais datas. Também foram observados maiores valores de incidência para a cultivar IRGA 417 em todas as épocas de avaliação devido ao menor desenvolvimento das plantas, resultado da fitotoxicidade causada pelo residual das imidazolinonas (Kraemer *et al.*, 2009), já que esta cultivar não é tolerante a este

grupo de herbicidas. Entre os manejos de adubação houve maior incidência de radiação no tratamento A5 (testemunha) provavelmente devido ao menor desenvolvimento da cultura pela falta de nutrientes (Tab.1). Em cultivo de arroz associado à criação de peixe em águas costeiras salobras, na Índia, a

quantidade de luz incidente também foi o fator que mais influenciou no crescimento de algas nas zonas entre marés, já que essa em movimento mantinha o ambiente exposto à luz. A maior concentração de algas promoveu consequentemente a melhora na produção de peixes (Bhaumik *et al.*, 2013).

Radiação solar no interior do dossel, expressa em % da quantidade incidente na superfície do dossel das plantas de arroz irrigado. Santa Maria, RS. 2012.

Tratamentos	Radiação solar incidente % ²		
	15° DAI	21° DAI	84° DAI
A1*	56,0 b ¹	43,4 b	5,7 ab
A2	62,4 b	39,0 bc	3,8 b
A3	61,1 b	36,7 bc	3,8 b
A4	66,9 b	29,6 c	4,7 b
A5	90,6 a	60,9 a	7,7 a
		Cultivar	
IRGA 417	85 a	62 a	7 a
IRGA 422 CL	50 b	22 b	4 b
Média			
CV (%)	14	21,9	35

*A1: adubação de base N, P e K na linha incorporada ao solo no momento da semeadura e com nitrogênio por ocasião da entrada da água,

A2: adubação em superfície aplicada ao solo no momento da semeadura e com nitrogênio por ocasião da entrada da água,

A3: adubação em superfície aplicada ao solo por ocasião da entrada da água e com nitrogênio,

A4: sem adubação e com nitrogênio por ocasião da entrada da água,

A5: sem adubação e sem nitrogênio (testemunha).

¹Médias seguidas da mesma letra minúscula na vertical não diferem entre si pelo teste Tukey $p \leq 0,05$.

²Avaliação realizada na entre linha da semeadura, na altura de 10 cm a cima da lâmina de irrigação.

Na figura 2, encontram-se as densidades de células cm^{-2} do epifiton. Na figura 3 encontram-se a média da densidade dos três blocos do fitoplâncton (células mL^{-1}). Embora estejam em diferentes unidades é possível perceber que há aumento na densidade total das comunidades epifítica e fitoplancônica para a cultivar IRGA 417 na maioria dos tratamentos, no entanto os valores de cada tratamento não mostraram diferença significativa quando testados pelo teste de Friedman ($p > 0,05$). Mustow, 2002 também observou aumento da produtividade primária aquática quando ampliada a área de incidência de radiação solar devido ao aumento do espaçamento entre as plantas de arroz, confirmando assim, a interferência do

sombreamento para as comunidades de microalgas. Desta forma as parcelas que continham a cultivar IRGA 422CL, resistente ao residual do herbicida, mostraram condições menos favoráveis ao aumento da densidade celular. A testemunha mostrou-se como o segundo tratamento com os maiores valores de densidade nas três datas do fitoplâncton, o que pode ser resultado da maior incidência de radiação solar (Tab.1). Este resultado é similar ao descrito por Sartori *et al.* (2011), cujo trabalho demonstrou que a quantidade de nutrientes disponível na água das parcelas testemunhas é suficiente para o desenvolvimento das algas.

Densidade total do Epifiton

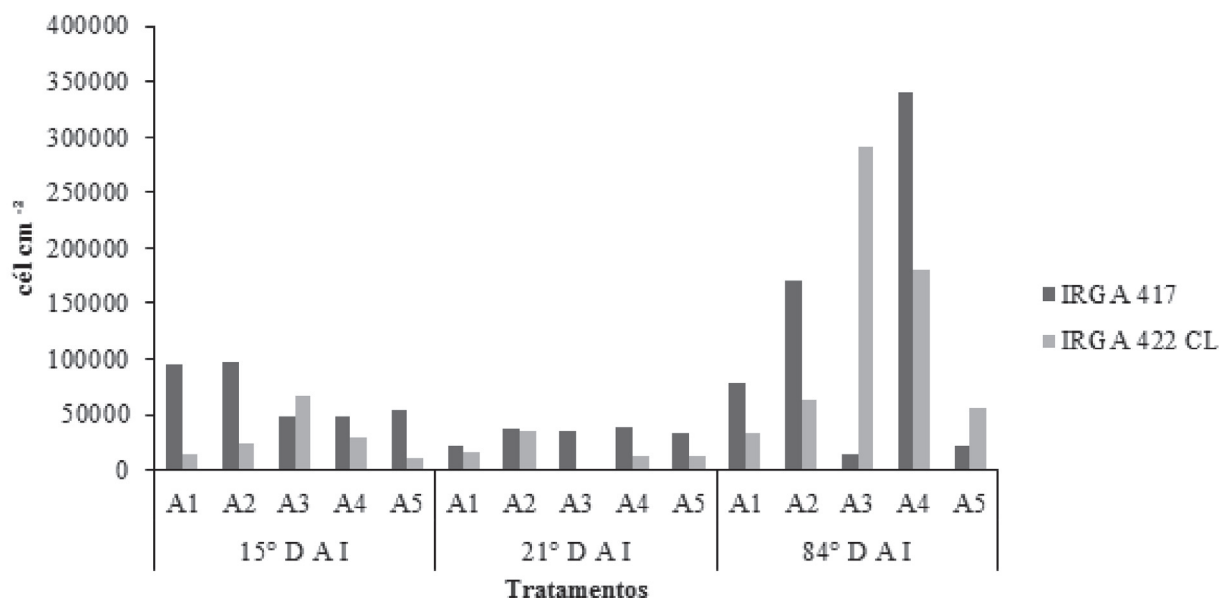


Fig. 2. Comparação dos tratamentos (sem diferença significativa $p < 0,05$) entre as densidades totais dos blocos da comunidade epifítica entre os manejos (células cm^{-2}) em arroz irrigado, Santa Maria, RS, 2012. A1: adubação de base N, P e K na linha incorporada ao solo no momento da semeadura e com nitrogênio por ocasião da entrada da água; A2: adubação em superfície aplicada ao solo no momento da semeadura e com nitrogênio por ocasião da entrada da água; A3: adubação em superfície aplicada ao solo por ocasião da entrada da água e com nitrogênio; A4: sem adubação e com nitrogênio por ocasião da entrada da água e A5: sem adubação e sem nitrogênio (testemunha).

Densidade Total do Fitoplâncton

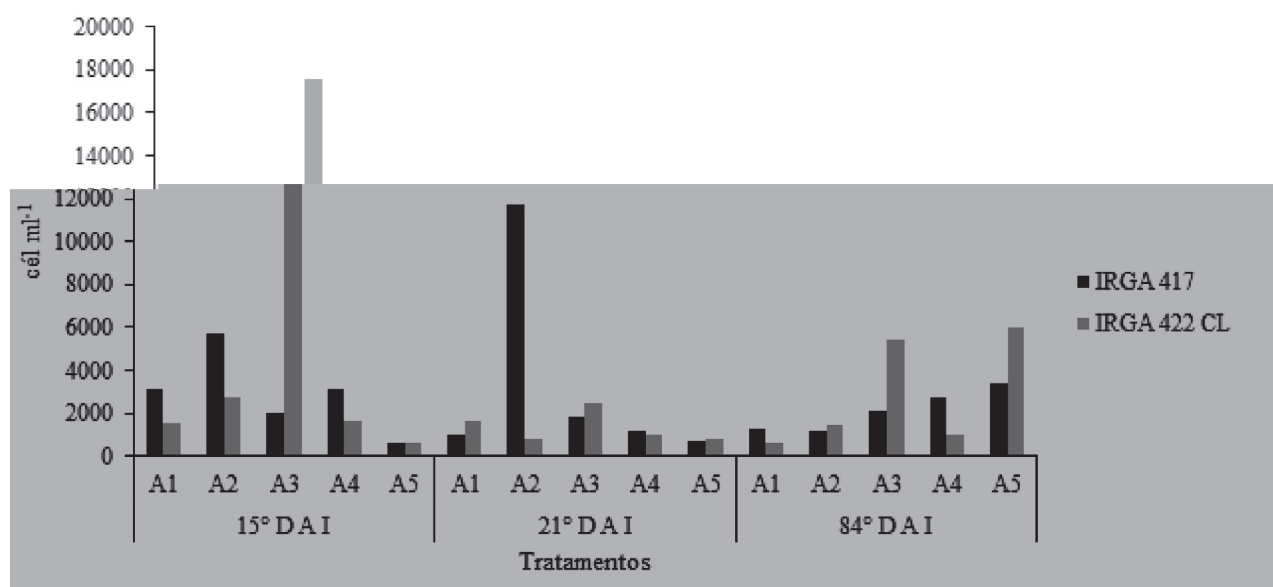


Fig. 3. Comparação dos tratamentos (sem diferença significativa $p < 0,05$) entre a média da densidade total nos três blocos onde foram amostrados o fitoplâncton (células ml^{-1}) em arroz irrigado, Santa Maria, RS, 2012. A1: adubação de base N, P e K na linha incorporada ao solo no momento da semeadura e com nitrogênio por ocasião da entrada da água; A2: adubação em superfície aplicada ao solo no momento da semeadura e com nitrogênio por ocasião da entrada da água; A3: adubação em superfície aplicada ao solo por ocasião da entrada da água e com nitrogênio; A4: sem adubação e com nitrogênio por ocasião da entrada da água e A5: sem adubação e sem nitrogênio (testemunha).

A figura 4 apresenta a riqueza de espécies de microalgas. Dentre as classes encontradas no presente estudo, *Chlorophyceae* foi a que apresentou a maior riqueza específica, tanto para o epifiton quanto para o fitoplâncton. O estudo de Mondol *et al.* (2013) também descreveu as classes *Chlorophyceae* seguida de *Bacillariophyceae* e *Euglenophyceae* como os grupos mais dominantes em lavouras de arroz na Índia.

A análise qualitativa do material epifítico revelou a presença de 93 espécies, distribuídas em oito classes (Fig. 4). Os resultados de densidade do epifiton do 15° DAI mostram números elevados para o gênero *Oedogonium* Link *ex* Hirn em todos os tratamentos se comparados à densidade das demais espécies, na qual *Oedogonium* sp. foi abundante em todas as amostras e dominante em 50% delas. A espécie *Ulothrix* sp. também foi abundante em 60% das amostras exceto no tratamento A1.

No 21° DAI, *Oedogonium* sp. continua como abundante em todos os tratamentos dominando em 90% das amostras. Segundo Schneider & Lindstrøm (2011) o gênero *Oedogonium* está associado a ambientes eutrofizados e a lavoura de arroz é passível de eutrofização devido à grande carga de nutrientes empregados na cultura, além do grande fluxo de matéria orgânica alóctone e autóctone (Rodrigues *et al.*, 2011).

Em arrozais do Chile, Pereira *et al.* (2000) também encontraram algas filamentosas como *Oedogonium* e

Spirogyra Link, as quais foram consideradas como prejudiciais à cultura do cereal, juntamente com *Nitella acuminata* A. Braun *ex* Wallman e *Chara braunii* C.C. Gmelin, pois ao proliferarem competem por nutrientes e luz com as plantas da cultura.

A pequena contribuição em densidade das diatomáceas no epifiton pode ter sido devida à baixa turbulência presente no agroecossistema, uma vez que estas estão associadas à maior correnteza do ambiente (Tundisi & Matsumura-Tundisi, 2008). Crossetti *et al.* (2007) também relatam a maior riqueza de diatomáceas em períodos de maior turbulência no sistema lagoa banhado do Taim, RS e cianobactérias em períodos de águas mais calmas.

Os resultados do 84° DAI mostram uma redução nas densidades de espécies do gênero *Oedogonium*, pois estas passam a ser abundantes em 50% das amostras, não sendo apenas no tratamento A4. *Spirogyra* sp. foi abundante em 80% das amostras e dominante em 20%, enquanto que *Dolichospermum* sp. foi abundante em 60% e dominante também em 20% delas. O desenvolvimento de *Spirogyra* e *Dolichospermum* (Ralfs *ex* Bornet & Flahault) P. Wacklin, L. Hoffmann & J. Komárek caracteriza a associação do pseudoperifiton, uma vez que esses organismos estão frouxamente aderidos à comunidade epifítica vivendo sem se fixar ao substrato (Bellinger & Sigeer, 2010).

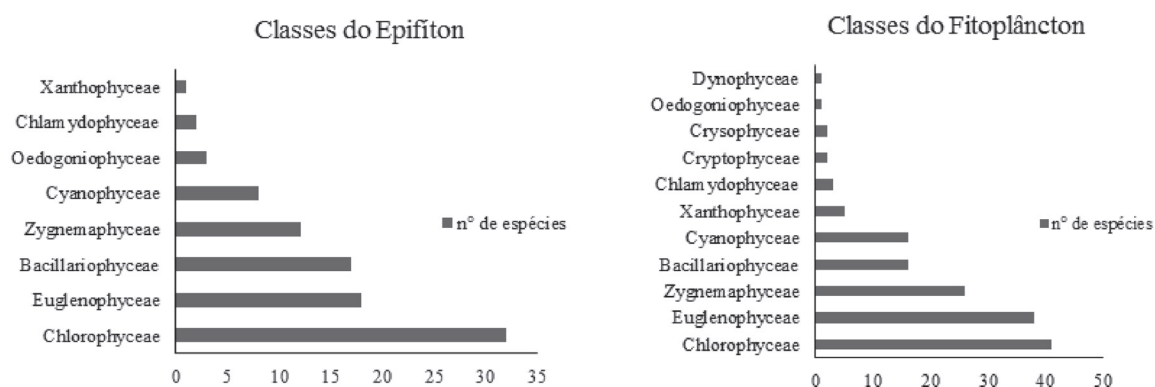


Fig. 4. Número de táxons presentes em cada classe na comunidade epifítica e na comunidade fitoplânctônica na cultura do arroz irrigado (Santa Maria, RS, 2012).

A Análise de Correspondência DCA para a comunidade epifítica (Fig. 5) mostrou que não houve influência dos manejos de adubação na composição da comunidade. Foi possível observar uma forte influência da escala temporal na ordenação das

unidades amostrais, uma vez que estão ordenadas pela data de coleta. À direita do eixo 1 estão reunidas as amostras do 84° DAI associadas às espécies das classes *Chlorophyceae*, *Zygnemaphyceae* e *Euglenophyceae* que obtiveram os maiores valores

Segundo Reynolds (2006) os organismos especialistas possuem adaptações específicas para utilizar os recursos do ambiente, estando elas principalmente na forma de armazenar, na persistência e na mobilidade, o que lhes proporciona vantagens na utilização dos recursos que talvez estivessem indisponíveis a outras espécies. Dessa forma os gêneros *Eudorina* Ehrenberg e *Pandorina* Bory de St. Vincent parecem favorecidos pela mobilidade proporcionada pelos flagelos que possibilitam o deslocamento na coluna de água de acordo com a radiação solar. Além disso, Reynolds *et al.* (2002) associa a presença do gênero *Eudorina* a ambientes com grande carga de nutrientes. Esta situação concorda com os resultados de Furtado & Lucca (2003) e Sartori *et al.* (2011), os quais demonstraram maiores níveis de nitrogênio e ortofosfato na água, nos primeiros dias da adubação, seguidos de um decréscimo ao longo do ciclo da cultura. Rodrigues *et al.* (2011), também observaram declínio dos níveis de nutrientes em duas áreas de arrozais no município de Santa Vitória do Palmar, RS e associam o fato à incorporação destes pelas plantas e/ou por sedimentos e ainda devido ao metabolismo do fitoplâncton.

No 21° DAI, *Cryptomonas* sp. ocorreu em elevada densidade, sendo abundante em 79% e dominante em 31% das amostras, não sendo apenas no tratamento A5. *Eudorina elegans* foi abundante em 52%, *Trachelomonas volvocina* em 48% e *Pandorina morum* em 38% das amostras. Segundo Reynolds (2006) o gênero *Cryptomonas* Ehrenberg também é encontrado em ambientes rasos e enriquecidos, como a lavoura de arroz. Além disso, Bicudo *et al.* (2009) e Taniguchi *et al.* (2005) relatam que as *Cryptophyceae* são oportunistas, pois aumentam sua densidade em períodos de declínio de outras espécies.

No 84°DAI relata-se a elevada densidade das classes *Cyanophyceae*, *Zygnemaphyceae*, *Euglenophyceae* e *Chlorophyceae* com a ordem *Chlorococcales* bem representada. *Spirogyra* sp. foi espécie abundante em 63% e dominante em apenas uma amostra, *Nephrocytium* sp. foi abundante em 60%, *Chroococcus* sp. em 50%, *Trachelomonas volvocina* em 43% e *Dictyosphaerium* sp., *Crucigeniella* sp. e *Ankistrodesmus* sp. em 40% das amostras. Estes gêneros também foram citados como os mais abundantes na água de irrigação e os mais consumidos por peixes em rizipiscicultura na Índia (Mondol *et al.* 2013).

De outro modo a análise do fitoplâncton permitiu identificar um predomínio de formas flageladas no período inicial da cultura (15° e 21° DAI) e uma substituição para formas não-flageladas no período final amostrado (84° DAI). Bambaradeniya *et al.*,

2004 sugere que organismos flagelados sejam melhores adaptados a ambientes com bruscas alterações físicas e químicas como a lavoura de arroz e dessa forma seriam considerados os colonizadores iniciais favorecidos pelo curto ciclo de vida e rápida colonização sendo capazes de reagir de forma fisiológica ou comportamental.

Em uma análise temporal da comunidade fitoplanctônica Furtado & Lucca (2003) indicaram decréscimo na população de *Chlorophyta*, *Bacillariophyta*, *Cryptophyta* e *Dinophyta*, ao longo do ciclo e aumento de *Cyanophyta* e *Euglenophyta*, além de uma melhora na qualidade da água com o maior tempo de irrigação.

A classe *Euglenophyceae* foi a segunda mais rica na comunidade fitoplanctônica com 38 táxons. Limnologicamente a lavoura de arroz é caracterizada por grande quantidade de matéria orgânica alóctone e autóctone (Rodríguez *et al.*, 2011) o que pode ter favorecido a riqueza desta classe. Alves-da-Silva & Tamanaha (2008) também relatam 48 morfoespécies de *Euglenophyceae* em arroz irrigado associado à criação de carpa comum (*Cyprinus carpio* Linnaeus).

A análise de correspondência (DCA) para a comunidade fitoplanctônica (Fig.6) também não mostrou influência dos diferentes manejos de adubação na comunidade fitoplanctônica. A análise permitiu identificar que a ordenação das unidades amostrais se deu a favor de um gradiente de escala temporal, bem como foi identificado na comunidade epifítica. À direita do eixo 1 encontram-se as amostras do 15° DAI associadas à espécie *Eudorina elegans*; já à esquerda do mesmo eixo encontram-se agrupadas as amostras do 84° DAI associadas às classes *Zygnemaphyceae*, *Chlorophyceae* e *Bacillariophyceae* que obtiveram elevados valores de densidade nestas datas. Na parte superior do eixo 2 estão as amostras do 21° DAI associadas com a espécie *Cryptomonas* sp. Este resultado confirma que ao comparar os tratamentos e testemunhas nas comunidades em estudo não foram observadas diferenças entre a composição e a abundância de espécies na mesma data de coleta, apenas entre elas. Possivelmente os níveis de nutrientes presentes na água de irrigação foram mantidos acima da demanda biológica, não chegando a serem limitantes para as comunidades, concordando com Sartori *et al.* (2011). Furtado & Lucca (2003) também observaram a separação do fitoplâncton na cultura de arroz irrigado pelas datas de coleta além das formas de plantio e ainda Das *et al.* (2011) também observam flutuações mensais na abundância do fitoplâncton em arroz irrigado associado à criação de peixes.

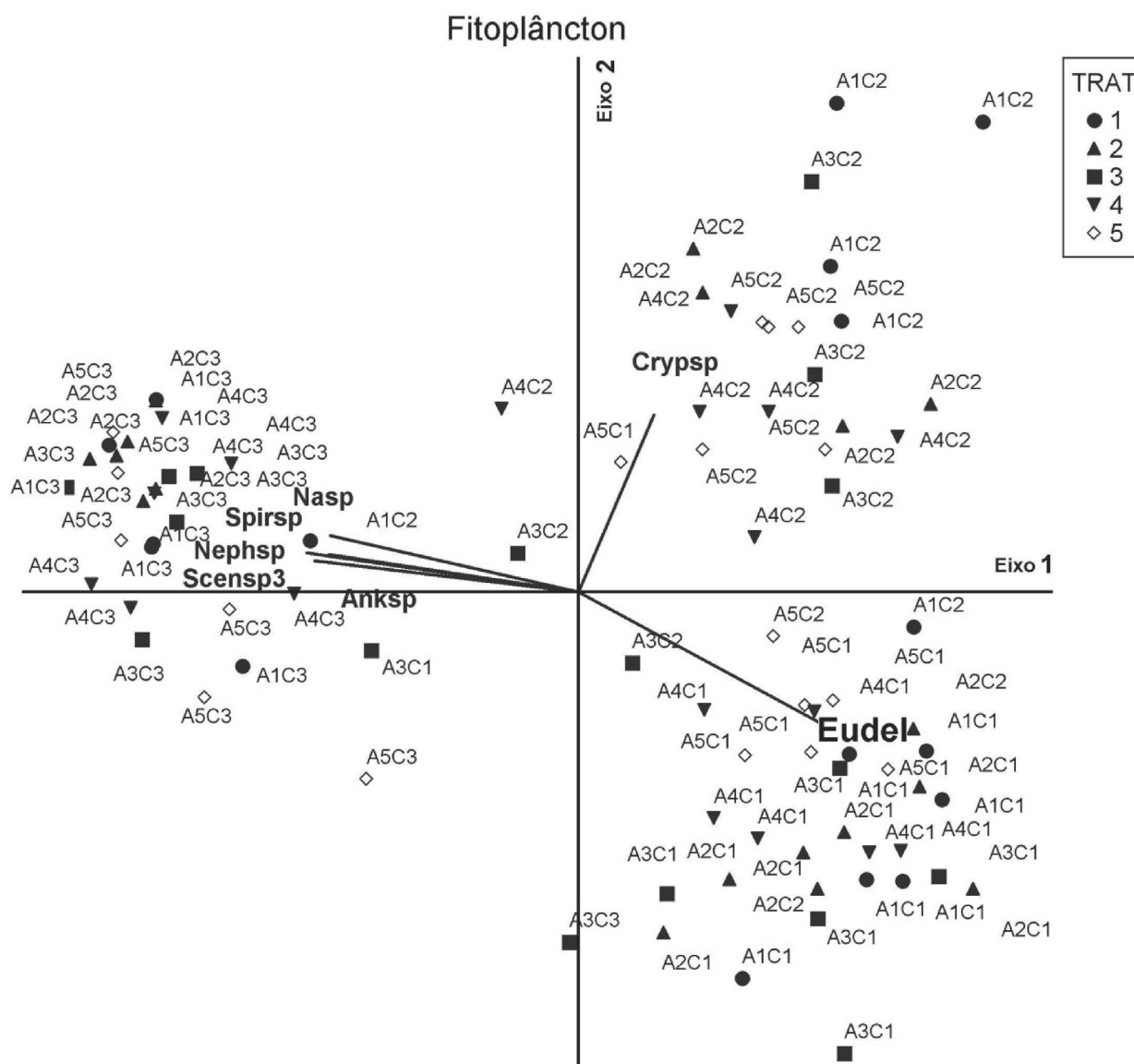


Fig. 6. Diagrama de dispersão (DCA) mostrando a relação das espécies fitoplanctônicas com os tratamentos testados na cultura do arroz irrigado, Santa Maria, RS, 2012. Eudel: *Eudorina elegans*; Crypsp: *Cryptomonas* sp.; Nephsp: *Nephrocystium* sp.; Anksp: *Ankistrodesmus* sp.; Nasp: *Navicula* sp.; Spirsp: *Spirogyra* sp.; Scensp3: *Scenedesmus* sp.3. A1: adubação de base N, P e K na linha incorporada ao solo no momento da semeadura e com nitrogênio por ocasião da entrada da água; A2: adubação em superfície aplicada ao solo no momento da semeadura e com nitrogênio por ocasião da entrada da água; A3: adubação em superfície aplicada ao solo por ocasião da entrada da água e com nitrogênio; A4: sem adubação e com nitrogênio por ocasião da entrada da água e A5: sem adubação e sem nitrogênio (testemunha). C1: 15° DAI; C2: 21° DAI e C3: 84° DAI.

CONCLUSÃO

A partir dos dados coletados pode-se inferir que não houve influência dos diferentes manejos de adubação sobre a composição das comunidades epifíticas e fitoplanctônicas. Observou-se que a fitotoxicidade, sobre as cultivares, causada pelo residual das imidazolinonas, permitiu maior incidência de radiação solar sobre a água das parcelas contendo a cultivar não tolerante, o que promoveu condições mais favoráveis ao desenvolvimento algal.

Isto foi verificado através de maior densidade celular de fitoplâncton e do epifiton nesta cultivar. Ainda, durante o período de cultivo predominaram formas filamentosas (*Oedogonium* e *Spirogyra*) no epifiton e no fitoplâncton foi observada uma substituição de formas flageladas para não-flageladas ao longo do período amostral.

Dessa forma, não houve alteração das comunidades de algas testadas sob o efeito dos diferentes manejos de adubação e sim um aumento na densidade do epifiton e do fitoplâncton pela maior

disponibilidade de luz para a cultivar não tolerante ao residual do herbicida.

AGRADECIMENTOS

Os autores agradecem ao Grupo de Pesquisas em Arroz Irrigado e Uso Alternativo de Várzeas da Universidade Federal de Santa Maria pela área experimental cedida à realização do estudo.

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Suficiência amostral para estudos de impacto ambiental sobre a comunidade de macroinvertebrados bentônicos em arrozais irrigados

Sample sufficiency for environmental impact studies on benthic macroinvertebrate community in irrigated rice fields

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— NOTA —

RESUMO

O objetivo deste estudo foi determinar a suficiência amostral para estudos de impacto ambiental sobre a comunidade de macroinvertebrados bentônicos em arrozais irrigados (*Oryza sativa* L.). Amostragens de solo foram realizadas com auxílio de coletor cilíndrico, nos anos agrícolas 2007/08 e 2012/13. As amostras foram triadas e os espécimes identificados até o nível de família. Os dados foram submetidos à análise da curva do coletor e os índices de diversidade de Shannon e equabilidade de Pielou foram calculados para o total de amostras e o número de amostras definido pela curva do coletor, em cada ano amostral. Considerando o método de amostragem verificado no presente trabalho, indica-se um mínimo de 12 réplicas para se obter o maior número de famílias sem perda de informação, quando em estudos de impacto ambiental sobre a comunidade de macroinvertebrados bentônicos em área de cultivo de arroz irrigado.

Palavras-chave: curva do coletor, amostragem, índices de diversidade, invertebrados aquáticos.

ABSTRACT

The objective of this study was to determine the sample sufficiency in environmental impact studies on the benthic macroinvertebrate community in irrigated rice fields (*Oryza sativa* L.). Soil samplings were conducted with a cylindrical collector in the agricultural years of 2007/08 and 2012/13. Individuals were sorted out and identified up to family level. Data were analyzed through the collector curve, Shannon's Diversity and Pielou's Evenness Index were calculated for the total number of samples and the number of samples defined by the collector curve in each year. Considering the results observed in this study, we indicate a minimum of 12 replicates to obtain the largest number of families

without loss of information, in environmental impact studies on benthic macroinvertebrate community in irrigated rice fields.

Key words: collector curve, sampling, diversity indices, aquatic invertebrates.

Macroinvertebrados bentônicos são organismos que habitam os substratos de fundo nos habitats de água doce, ao menos parte do seu ciclo de vida, e que podem ser retidos em peneira de malha ≥ 200 a $500\mu\text{m}$ (ROSENBERG & RESH, 1993), representados por espécies de *Insecta*, *Annelida*, *Nemertinea*, *Crustacea*, *Mollusca* e alguns *Turbellaria* e *Bryozoa* (KUHLMANN et al., 2012). Tais organismos ocupam importante posição na fauna aquática dos arrozais irrigados (BARBOUR et al., 1999), auxiliando na fertilidade do solo da lavoura, na ciclagem de nutrientes, além do controle biológico de insetos pragas da cultura (ROGER et al., 1991).

Estudos de impacto ambiental em comunidades aquáticas requerem métodos apropriados para se obter informações precisas sobre mudanças na diversidade e estrutura das comunidades biológicas. A amostragem é necessária porque não é possível acessar a totalidade de um dado universo amostral, sendo necessário avaliar se o tamanho da amostra é suficiente para uma dada precisão requerida

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e, portanto, a avaliação de suficiência amostral é uma ferramenta importante para este estudo (PILLAR, 2004). Segundo CAIN (1938), a suficiência amostral é atingida quando um incremento de 10% no tamanho da amostra corresponde a um incremento menor ou igual a 10% no número de espécies levantadas.

Segundo SILVA (2012), poucos trabalhos têm sido feitos para determinar a suficiência amostral em estudos com invertebrados do solo. Considerando a necessidade de pesquisas sobre o esforço amostral requerido para estudos de impacto ambiental, o objetivo do presente trabalho é definir a suficiência amostral requerida para ensaios com macroinvertebrados bentônicos em área de cultivo de arroz irrigado.

O estudo foi realizado em área experimental de várzea, Universidade Federal de Santa Maria, nos anos agrícolas de 2007/08 e 2012/13. Ensaios a campo foram instalados a fim de simular o ambiente da lavoura de arroz irrigado. As parcelas experimentais apresentaram área de 48m² (2007/08) e 10m² (2012/13), onde foram semeadas as cultivares 'IRGA 422 CL' (2007/08) e 'PUITA INTA CL' (2012/13), respectivamente. Amostras de solo foram coletadas após o terceiro dia de entrada de água nas parcelas, as quais permaneceram com lâmina de água de 10cm.

As coletas de solo seguiram dois transectos que se cruzavam no meio das parcelas, sendo que, em cada transecto, foram coletadas 15 amostras a uma distância de aproximadamente 75cm (2007/08) e 10 amostras a uma distância de aproximadamente 48cm (2012/13). As amostragens foram realizadas com auxílio de um cilindro coletor com área de 0,01m² a uma profundidade de 10cm. Após as coletas, as amostras foram lavadas em tamises de 0,25 (2007/08) e 0,50mm (2012/13) e acondicionadas em frascos plásticos, coradas com "rosa de Bengala" e fixadas com álcool etílico absoluto. No laboratório, o material foi triado e identificado até o nível taxonômico de família (FERNÁNDEZ & DOMÍNGUEZ, 2001).

A suficiência amostral foi avaliada pela construção da curva do coletor, utilizando-se 100 randomizações. Cada ponto foi considerado uma unidade amostral (n1=30 amostras; n2=20 amostras). A análise foi realizada no programa estatístico EstimateS 8.2 (COLWELL, 2009). A diversidade e a abundância proporcional dos organismos foram avaliadas para o número total de amostras coletadas e o número de amostras obtido na análise de suficiência amostral, através dos índices de Shannon (H') e equabilidade de Pielou (J').

Um total de 3443 indivíduos foi coletado nos dois anos amostrais, distribuídos nos filos

Arthropoda, Mollusca, Annelida e Nematoda. Na safra 2007/08, o filo mais abundante foi Arthropoda, que compôs cerca de 60% da densidade total encontrada. Na safra 2012/13, aproximadamente 89% dos organismos corresponderam ao Filo Anellida.

Segundo SILVEIRA (2010), a melhor forma de se avaliar o esforço de amostragem é através da construção da curva do coletor. Este método tem sido muito utilizado no Brasil para indicar a suficiência amostral, um conceito quantitativo para informar se a amostra utilizada é representativa da comunidade em estudo. Quanto maior o tamanho da amostra, maior o número de espécies que poderá ser encontrado, mas a uma taxa decrescente, até o ponto em que a curva estabiliza e torna-se horizontal (SCHILLING & BATISTA, 2008). Uma vez que a assíntota da curva é atingida, nenhuma amostragem adicional produzirá adição de espécies (GOTELLI & COLWELL, 2010).

Com os resultados obtidos, identificou-se que a suficiência amostral para coleta de macroinvertebrados bentônicos foi atingida após a 11ª amostra no primeiro ano de estudo (2007/08) e 10ª amostra no segundo ano de estudo (2012/13), quando já haviam aparecido os 15 e 12 táxons identificados nas amostras, respectivamente (Figura 1).

A diversidade é um dos conceitos fundamentais no estudo de comunidades e diversos métodos para sua mensuração estão disponíveis, dentre eles o uso dos índices de diversidade que combinam dois atributos de uma comunidade biológica: o número de espécies e sua equabilidade (MELO et al., 2008).

O índice de diversidade de Shannon (H') e o índice de equabilidade de Pielou (J') apresentaram valores próximos para o número de amostras totais e a suficiência amostral, para cada ano estudado, indicando que a análise da suficiência amostral foi eficaz para uma representação significativa da comunidade de macroinvertebrados bentônicos. Para o ano de 2007/08, o índice de Shannon para amostras totais foi de 1,188 e 1,156 para a suficiência amostral. O índice de equabilidade de Pielou foi 0,438 para o número de amostras totais e 0,427 para a suficiência amostral. Para o ano de 2012/13, o índice de Shannon foi de 0,496 para o número de amostras totais e 0,413 para a suficiência amostral e o índice de equabilidade de Pielou apresentou o valor de 0,199 para o número de amostras totais e 0,166 para a suficiência amostral.

As curvas de acumulação de espécies, bem como os estimadores de riqueza, fornecem subsídios para a tomada de decisões em relação à riqueza taxonômica e a conservação da biodiversidade, sendo

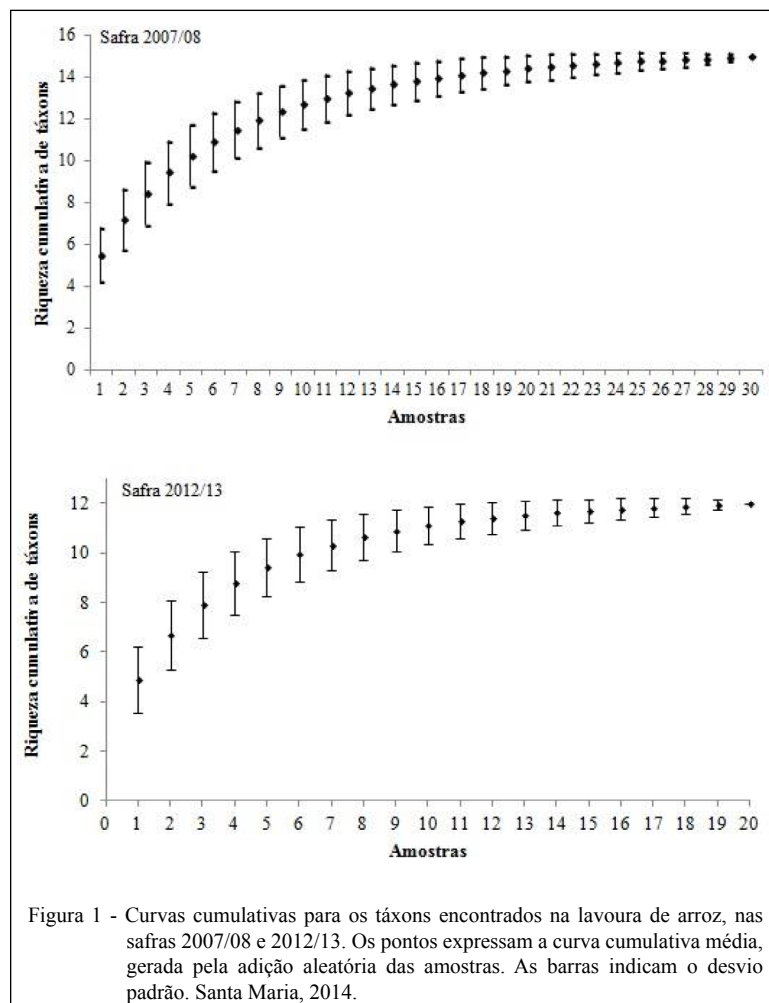


Figura 1 - Curvas cumulativas para os táxons encontrados na lavoura de arroz, nas safras 2007/08 e 2012/13. Os pontos expressam a curva cumulativa média, gerada pela adição aleatória das amostras. As barras indicam o desvio padrão. Santa Maria, 2014.

ferramentas importantes na adequação do esforço amostral (SILVA, 2012). Considerando o método de amostragem utilizado no presente trabalho, indica-se um número de 12 réplicas para se obter o maior número de famílias sem perda de informação, como suficiência amostral para estudos de impacto ambiental sobre a comunidade de macroinvertebrados bentônicos em áreas de cultivo de arroz irrigado.

AGRADECIMENTOS

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (Edital Universal – MCTI/CNPq n. 14/2012) e à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pelo auxílio financeiro.

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Phytoremediation of lowland soil contaminated with a formulated mixture of Imazethapyr and Imazapic¹

Fitorremediação de solo de terras baixas contaminado com mistura formulada de imazetapir + imazapique

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ABSTRACT - The use of plants to decontaminate water and soil contaminated with both organic and inorganic pollutants is a promising technology for sustainable agriculture. The aim of this work was to evaluate the efficiency of plant species in the remediation of formulated mixtures of imazethapyr and imazapic, using the irrigated rice cultivar IRGA 417 as bioindicator. The treatments consisted of the combination of 13 plant species with seven rates of a formulated mixture of imazethapyr and Imazapic (75+25 g e.a. L⁻¹ respectively): 0, 200, 300, 400, 500, 1000 and 4000 mL ha⁻¹. To evaluate the potential for phytoremediation in these species, symptoms of injury and plant height were measured in rice plants at 7, 14, 21 and 28 days after emergence and shoot dry weight at 28 days after emergence. *Glycine max*, *Lolium multiflorum* and *Lotus corniculatus* are potentially promising species in the phytoremediation of soils contaminated with the herbicide imazethapyr and imazapic (up to 4000 mL ha⁻¹), due to being more adapted to hydromorphic environments, which is a feature found in soils cultivated with irrigated rice. *Crotalaria juncea*, *Canavalia ensiformis*, *Stizolobium aterrimum*, *Vicia sativa*, *Raphanus sativus* and *Triticum aestivum* are species capable of the phytoremediation of soils contaminated with imazethapyr + imazapic, however the occurrence of anoxia in hydromorphic soils reduce the establishment and development of these plants.

Key words: Rice. Phytoremediation. Soil decontamination.

RESUMO - O uso de plantas para descontaminar água e solo contaminados com poluentes orgânicos e inorgânicos é uma tecnologia promissora para a agricultura sustentável. Este trabalho teve como objetivo avaliar a eficiência de espécies vegetais na remediação da mistura formulada de imazetapir + imazapique, utilizando a cultivar de arroz irrigado IRGA 417 como planta bioindicadora. Os tratamentos foram compostos pela combinação entre 13 espécies vegetais e sete doses da mistura formulada de imazetapir + imazapique (75 + 25 g e.a. L⁻¹, respectivamente): zero, 200; 300; 400; 500; 1.000 e 4.000 mL ha⁻¹. Para a avaliação do pontencial fitorremediador dessas espécies foram avaliadas a altura e sintomas de fitointoxicação nas plantas de arroz irrigado, aos 7; 14; 21 e 28 dias após a emergência, e massa seca da parte aérea aos 28 dias após a emergência. *Glycine max*, *Lolium multiflorum* e *Lotus corniculatus* por serem mais adaptadas a ambientes hidromórficos, característica essa encontrada em solos cultivados com arroz irrigado, são espécies potencialmente promissoras em fitorremediar solos contaminados com o herbicida imazetapir + imazapique (até 4.000 mL ha⁻¹). *Crotalaria juncea*, *Canavalia ensiformis*, *Stizolobium aterrimum*, *Vicia sativa*, *Raphanus sativus* e *Triticum aestivum* são espécies capazes de fitorremediar solos contaminados com imazetapir + imazapique, porém a ocorrência de anoxia em solos hidromórficos dificultam o estabelecimento e desenvolvimento das plantas.

Palavras-chave: Arroz. Fitorremediação. Descontaminação de solo.

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¹Recebido em 07/11/2012; aprovado em 09/11/2014

Trabalho realizado como parte da Dissertação de Mestrado da primeira autora, apresentado ao programa de Pós-graduação em Agronomia da Universidade Federal de Santa Maria, Santa Maria- RS, Brasil

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INTRODUCTION

Long-term persistence of herbicides in the soil can limit the rotation of crops and potentiate environmental contamination (DAN *et al.*, 2011). Among these compounds are the herbicides imazethapyr and imazapic, which belong to the imidazolinone group of chemicals, widely used in irrigated rice fields as the main alternative in controlling red rice. The herbicide imazethapyr has a pKa of 2.1 and 3.9 and imazapic of 2.0, 3.9 and 11.1, which characterises them as weak acids (SENSEMAN, 2007), having a half-life ($t_{1/2}$) of from 60 to 90 days, and 120 days respectively (SENSEMAN, 2007). They also have high solubility in water, 1400 mg L⁻¹ for imazethapyr and 2200 mg L⁻¹ for imazapic. These characteristics among others, cause the compounds to be highly influenced by the surrounding environment.

One of the characteristics most looked for in a herbicide is that it remain active in the environment long enough for effective weed control; however, the presence of the herbicide in the soil can become undesirable, and may result in problems with non-tolerant crops that may be grown later (PROCÓPIO *et al.*, 2008). For herbicides of the imidazolinone group of chemicals, research shows that their continuing presence in the soil causes a smaller stand of plants in non-tolerant rice due to the residual effect of the association of imazethapyr with imazapic, but without any effect on productivity (VILLA *et al.*, 2006). Marchesan *et al.* (2011) found however that the residual effect of these herbicides caused losses in production of 19% for imazethapyr and 30% for imazapic in genotypes of sensitive rice.

Studies show that cultivation of certain plant species can decontaminate areas where xenobiotics are present, such as herbicides with a long residual effect on the soil (D'ANTONINO *et al.*, 2009). This process is known as phytoremediation (SULMON *et al.*, 2007) and can shorten the time needed to free the area for the cultivation of plant species which are non-tolerant to the more persistent herbicides.

Compared with the selection of plants in the decontamination of trace elements, the limitations encountered in selecting plant species for the remediation of herbicides are complex, as these display great molecular diversity in the face of the constant changes they are subject to (PIRES *et al.*, 2003). Furthermore, there is the drawback of the contaminant (herbicide) having been developed to control the contaminator, in this case, the weeds. Given that not all plant species are able to develop in contaminated environments, the first step is the identification of those species which are not only suitable for the local conditions, but are also tolerant of the contaminant (MARQUES *et al.*, 2011).

The next step is to evaluate the capacity of the tolerant plant to promote decontamination of the soil. Efficiency of the phytoremediation process is generally measured by the reduction of the contaminant in the soil to concentrations below the required reference values and time (MARQUES *et al.*, 2011). When a plant is used which is capable of reducing the concentration of herbicides in the soil, what is achieved is to bring forward the production of crops of economic interest which were previously not able to be grown in certain areas (DAN *et al.*, 2011). In view of the above, the aim of this work was to evaluate the efficiency of plant species in the remediation of lowland soil contaminated with a formulated mixture of imazethapyr and imazapic (75+25 g a.e. L⁻¹).

MATERIAL AND METHODS

The experiments were carried out in a plastic greenhouse with no air conditioning, at two times, according to the growing season (autumn-winter and spring-summer), in the crop years of 2009/2010. The A-horizon of the soil was used as substrate, classified as a hydromorphic eutrophic arenic Planosol (EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA, 2006), from the experimental area of the Palma Agricultural Centre, at the Federal University of Pelotas (UFPel), Capão-do-Leão Campus, in the state of Rio Grande do Sul, Brazil (RS). The soil is of a silty-loam texture with the following characteristics: pH water (1:1) = 6.2, clay content = 200 g kg⁻¹, organic matter content = 32 g kg⁻¹, P = 16.2 mg dm⁻³, K = 180 mg dm⁻³, Ca = 5.5 cmol_c dm⁻³, Mg cmol_c = 3.2 dm⁻³, Al = 0.0 cmol_c dm⁻³ and SMP index = 6.6.

The autumn-winter experiment was carried out from April to July of 2009 in a randomised block design with an 8 x 7 factorial scheme and four replications. The treatments consisted of a combination of cool-season plant species: *Avena strigosa* (oat), *Lolium multiflorum* (ryegrass), *Lotus corniculatus* (birdsfoot trefoil), *Raphanus sativus* (wild radish), *Secale cereale* (rye), *Triticum aestivum* (wheat) and *Vicia sativa* (common vetch), and a control treatment with no prior cultivation; seven doses of herbicide, made up from the formulated mixture of imazethapyr and imazapic (75+25 g a.e. L⁻¹) at rates of 0, 200, 300, 400, 500, 1000 and 4000 mL ha⁻¹ in polyethylene pots, 13 cm in diameter and 9.5 cm in height, at the density recommended for each crop for a soil volume of 1.0 dm³. Choice of the species and rates of the herbicide were based on preliminary studies, carried out by the authors, of the tolerance of cultivated plants to the herbicide.

Application was performed 48 hours before seeding, using a precision pipette to add the solutions with the herbicide to the soil surface. After application, the soil was turned, and then received a sufficient volume of water to reach field capacity, thereby allowing homogeneous distribution of the herbicide throughout the experimental unit. The pots were maintained under daily irrigation, in order to keep the soil moisture at around 80% of field capacity.

At 60 days after emergence, the plants were removed from the pots by cutting the stems at surface height, without removing the roots. The genotype IRGA 417, not resistant to the herbicide, was sown in each pot and used as a test with the intention of evaluating the potential for phytoremediation of each species previously grown in the soil contaminated by the herbicide.

After emergence of the rice, the plants were thinned, leaving four plants per pot. To determine the potential for phytoremediation of each species, injury (PT) was evaluated and the plant height (ALT) and dry weight (DM) of the shoots were determined. Symptoms of injury were evaluated by comparison with the control treatment, assigning scores from 0 to 100, corresponding respectively to the absence of symptoms to death of the plant (FRANS *et al.*, 1986). The plant height was evaluated by measuring the plants from the surface of the soil to the insertion point of the highest leaf. Both assessments were carried out at 7, 14, 21 and 28 days after emergence (DAE), when the rice plants were cut close to the ground to determine the dry weight, obtained by weighing the harvested material after drying in a forced circulation oven (70 ± 2 °C) for 72 hours.

The spring-summer experiment was carried out from October of 2009 to January of 2010 in an experimental design of randomised blocks in a 5 x 7 factorial scheme with four replications. The treatments consisted of a combination of warm-season plant species: *Canavalia ensiformis* (jack bean), *Crotalaria juncea* (sunn hemp), *Glycine max* (soybean) and *Stizolobium aterrimum* (velvet bean), plus a control treatment with no prior cultivation; seven doses of herbicide, made up from the formulated mixture of imazethapyr and imazapic ($75+25$ g a.e. L⁻¹) at dosages of 0, 200, 300, 400, 500, 1000 and 4000 mL ha⁻¹, following the same methodology used in the autumn-winter experiment.

The data were tested for assumptions for the mathematical model and subjected to procedures of variance analysis and polynomial regression, using the SAS software for statistical analysis and SigmaPlot® for elaboration of the dose-response curves, determined by the three-parameter log-logistic model (SEEFELDT; JENSEN; FUERST, 1995), as below (Equation 1):

$$y = \frac{a}{1 + \left(\frac{x}{x_0} \right)^b} \quad (1)$$

where: y = percentage of control; x = herbicide dosage; and a, x₀ and b = parameters of the equation, with a being the difference between the maximum and minimum points of the curve, x₀ being the dosage giving a 50% response for the variable, and b the slope of the curve.

The logistic model has advantages, since one of the terms of the equation is an estimate of the value of GR₅₀ (growth reduction by 50%), which is the dosage of herbicide which gives a biological effect of 50% (CHRISTOFFOLETI; LOPEZ-OVEJERO, 2004). From the values for GR₅₀, the remediation factor (FRem) was obtained for each combination of species being studied.

The FRem was calculated by dividing the values for GR₅₀ (PT₅₀, ALT₅₀, DM₅₀) of the irrigated rice plants, grown in soil with residual herbicide which received the potentially phytoremediative plant species, by those obtained in soil that received no plant species during crop succession and that also received no applications of herbicide (control), according to a methodology adapted from Hall, Strome and Horsman (1998).

RESULTS AND DISCUSSION

Through the analysis of the dose-response curves of the species under study, an interaction was seen between the factors plant species and dosages of the herbicide mixture, a significant effect being noted ($P \leq 0.05$) from the residual of the formulated mixture of imazethapyr and imazapic ($75+25$ g a.e. L⁻¹) on the variables plant height, injury and dry weight, analysed in the non-tolerant rice plants, IRGA 417, sown in succession to the species *A. strigosa*, *L. multiflorum*, *S. cereale*, *L. corniculatus*, *C. juncea*, *V. sativa*, *C. ensiformis*, *S. aterrimum*, *R. sativus*, *G. max*, *T. aestivum*, and also when there was no previous crop (control) (Table 1).

It was seen that an increase in herbicide dosage resulted in plants of lesser height (Figure 1) and shoot dry weight (Figure 3) and more intense symptoms of injury (Figure 2), varying with the species being tested. Similar results were found by Madalão *et al.* (2013) with *Crotalaria juncea*, *Canavalia ensiformis* and *Stizolobium aterrimum* grown in five dosages of the herbicide sulfentrazone. The evaluation carried out at 21 DAE was chosen for construction of the dose-response curves, as the recovery capacity of rice plants from that stage of development is known (WEBSTER; MASSON, 2001).

Table 1 - Variance analysis of the variables plant height, injury and dry weight, analysed in the non-tolerant rice plants, IRGA 417, sown in succession to the species under test

Sources of variation	GL	-----Square Mean-----		
		Plant Height	Injury	Dry Weight
Blocks	3	43.27 (0.0001)*	53.14 (0.0842)	553.02 (0.0000)
Crops	7	333.28 (0.0000)	2,955.45 (0.0000)	5,615.90 (0.0000)
Rates	12	4,624.99 (0.0000)	60,626.07 (0.0000)	50,879.06 (0.0000)
Rates x crops	72	42.35 (0.0000)	389.98 (0.0000)	457.80 (0.0000)
Residual	269	5.65 (0.0000)	23.73 (0.0000)	23.98 (0.0000)
CV (%)		13.01	11.74	10.13

*significance level of 5% by F-test

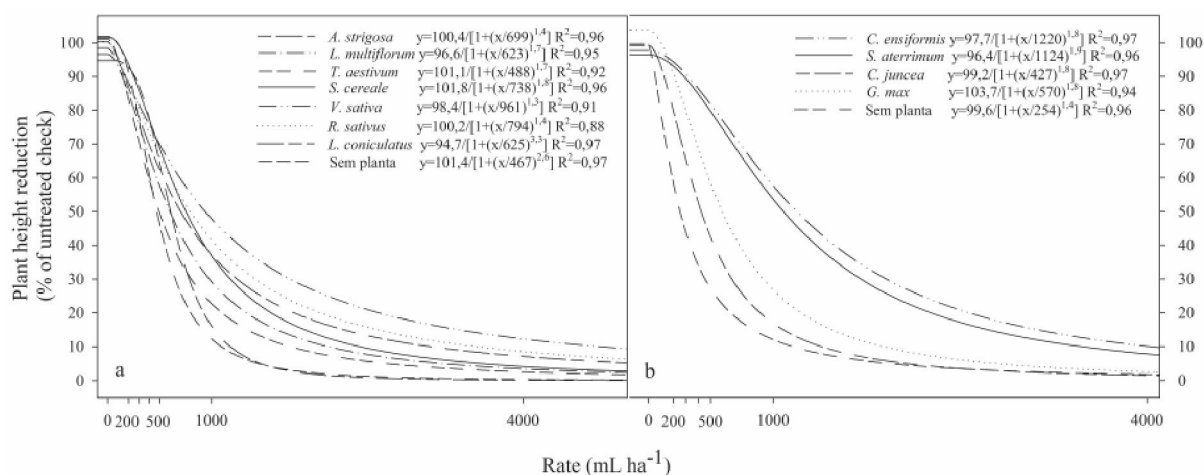
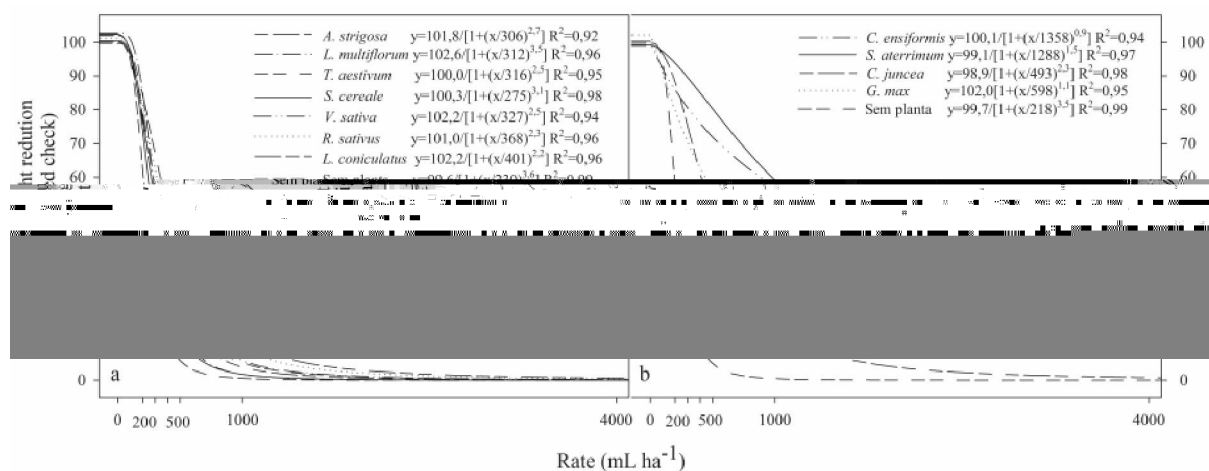
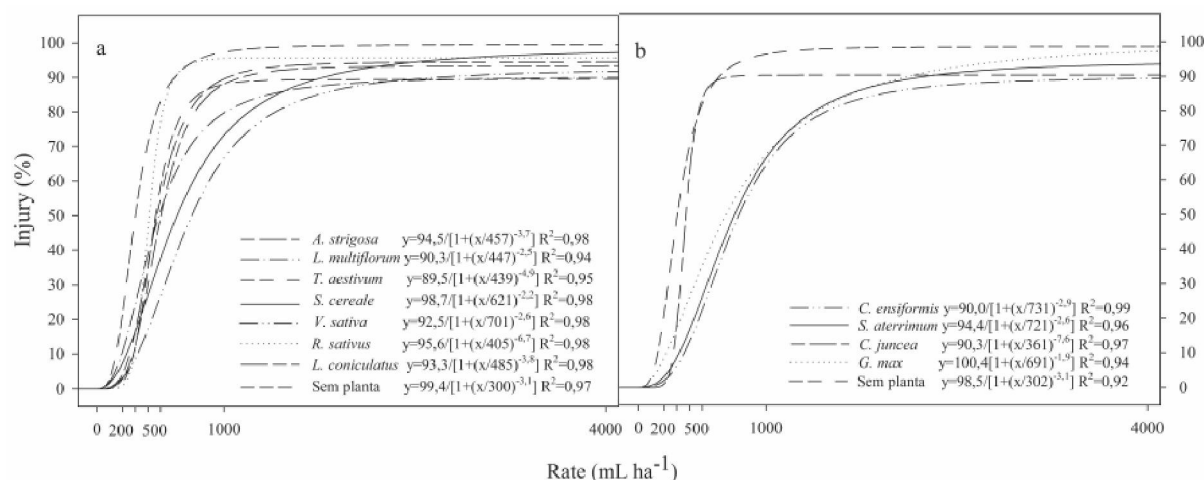
Figure 1 - Dose-response curves of cool-season (a) and warm-season species (b) to a formulated mixture of imazethapyr and imazapic (75+25 g a.e. L⁻¹), evaluated through the reduction in plant height of non-tolerant rice (IRGA 417) at 21 days after emergence (DAE), compared to the control. Santa Maria, RS**Figure 2** - Dose-response curves of cool-season (a) and warm-season species (b) to a formulated mixture of imazethapyr and imazapic (75+25 g a.e. L⁻¹), evaluated through the injury of non-tolerant rice plants (IRGA 417) at 21 days after emergence (DAE), compared to the control. Santa Maria, RS

Figure 3 - Dose-response curves of cool-season (a) and warm-season species (b) to a formulated mixture of imazethapyr and imazapic (75+25 g a.e. L⁻¹), evaluated through the shoot dry matter of non-tolerant rice plants (IRGA 417) at 21 days after emergence (DAE), compared to the control. Santa Maria, RS



As different species are high lighted for each dependent variable analysed, results were evaluated for the remediation factor of each species on the dependent variable shoot dry weight (DM₅₀). This variable was used as a basis because rice-grain production is related to the production of shoot dry weight, through organic farming and the harvest index, HI (YOSHIDA, 1981).

The estimated values of GR₅₀ for shoot dry weight therefore show that the cool-season species, *L. corniculatus*, *L. multiflorum*, *A. sativum*, *V. sativa* and *T. aestivum*, present a potential for the phytoremediation of soil contaminated with the herbicide mixture employed, since the values observed for GR₅₀ were significantly higher than those obtained with the treatment with no prior cultivation in the winter (control), there being no overlap between the confidence intervals, demonstrating the effect of these species in reducing the residual herbicide.

Similarly, the warm-season species, *C. juncea*, *C. ensiformis*, *S. atterrimum* and *G. max*, demonstrated a potential for the phytoremediation of the herbicide employed, since values for GR₅₀ for shoot dry weight were seen which were higher than the control (without cultivation in the summer); whereas the species *A. strigosa* and *S. cereale* did not differ significantly from the treatment used as control (soil without cultivation in the winter) (Table 2). In general, the results make it possible to infer that *C. juncea*, *C. ensiformis*, *S. atterrimum*, *V. sativa*, *R. sativus* and *T. aestivum* are species which are potentially capable of the phytoremediation of soils contaminated with imazethapyr and imazapic, however the anoxia occurring in hydromorphic soils impedes the establishment and development of plants. Similar results were found by Procópio *et al.* (2005a), where the species,

V. sativa, exposed to treatment with the herbicide, trifloxysulfuron-sodium, despite not having provided a shoot dry weight in beans equal to that of *S. atterrimum* and *C. ensiformes*, was superior to the other treatments.

The results seen in this study also confirm the findings of Correa *et al.* (2008), who claim that the species *C. ensiformis* and *S. atterrimum* are plants with a potential for the phytoremediation of areas treated with the herbicide diclosulam. For Procópio *et al.* (2005a), prior cultivation with *S. atterrimum* at any of the tested population densities, prevented reduction in the shoot dry weight of bean plants, caused by the presence of trifloxysulfuron-sodium in the cultivated soil.

The values for MS₅₀ ranged from 275 to 1,358 mg ha⁻¹, demonstrating that the rate required to cause a 50% reduction in the dry matter of rice plants was from one to six times higher when compared to the soil with no previous cultivation (Table 2). According to Spilborghs and Casarini (1998), places with vegetation exhibit complete and more rapid biodegradability compared to non-vegetated areas, due to the expansion of the active population of soil microorganisms (rhizosphere) which use the fraction exuded from the roots (rhizodeposition) as a source of food. Also according to the same authors, the increase in the biodegradability of herbicide molecules can take place because some plants have the ability to produce enzymes that metabolically convert organic contaminants, contributing to their faster oxidation by the microorganisms present in the soil.

According to Pires *et al.* (2003), the use of phytoremediation is based on the natural or developed

tolerance that some species exhibit to certain types of compounds or action mechanisms. It can thus be inferred that the results obtained in this study may be explained by the capacity the plants have to metabolise (phytodegradation) the herbicide in question into compounds which are non-toxic (or less toxic) to the plant and the environment, or simply their ability to compartmentalise the molecules of the herbicide. As the mass of the shoots of previously grown plants was removed, the herbicide could have been removed too, either compartmentalised in some reserve-storage organ, or adhering to the lignin of the cell wall, thus allowing the normal development of the rice plants.

Another possibility is phytostimulation, in which there is stimulation of microbial activity promoted by the release of root exudates that act by degrading the compound in the soil, which in some plants characterizes a rhizospheric aptitude for bioremediation (PIRES *et al.*, 2003). Some studies indicate that *S. atterrimum* and *C. ensiformis* are effective in decontaminating soils containing residues of the herbicides, trifloxysulfuron-sodium and tebuthiuron; and that the probable mechanism involved in decontamination is the interaction between phytostimulation and phytodegradation (PROCÓPIO *et al.*, 2005b).

In another study in an area contaminated with sodium trifloxysulfuron, maintaining or removing the

shoots of *C. ensiformis* and *S. atterrimum* after the period of phytoremediation, did not affect the subsequent development of bean plants, indicating a possible internal degradation of the product in the tissues (phytodegradation), or desactivation by other rhizospheric mechanisms, probably phytostimulation of the microbiota associated with the rhizosphere (PROCÓPIO *et al.*, 2006). Souto *et al.* (2013) proved that plants of *Stizolobium atterrimum* release rhizodeposits that stimulate existing microbial activity in the rhizosphere upon degrading molecules of a formulated mixture of imazethapyr and imazapic present in soil under rice cultivation.

Each species has specific characteristics for removing, immobilising or transforming certain contaminants. In the present study, it can be seen that the plants which displayed more intense remediation factors were leguminous; and probably the greater contribution of nitrogen to the soil provided by these species compared to species of grass (CARVALHO *et al.*, 2010), resulted in the better development of the bio-indicator plant. Normally leguminous plants also have a higher lignin content compared to grasses (FUKUSHIMA; SAVIOLI, 2001), and so favour sorption of the herbicides imazethapyr and imazapic in organelles which are rich in the compound, decreasing its availability in the soil.

Table 2 - Effect of the cultivation of plants in the off season, at a herbicide rate causing 50% injury (TP₅₀), 50% reduction in plant height (ALT₅₀) at 21 DAE, and 50% reduction in shoot dry weight (DM₅₀) at 28 DAE, in non-tolerant rice plants (IRGA 417), and the respective remediation factors (FRem). Santa Maria, RS

Cultivated cool-season species	TP ₅₀	FRem ¹	ALT ₅₀	FRem	MS ₅₀	FRem
After the cultivation of cool-season species						
No plants in the winter	300		467		230	
<i>Raphanus sativus</i>	405*	1,3	794*	1,7	368*	1,6
<i>Triticum aestivum</i>	440*	1,4	488 ^{ns}	1,0	316*	1,3
<i>Lolium multiflorum</i>	457*	1,5	623*	1,3	312*	1,3
<i>Avena strigosa</i>	460*	1,5	699*	1,5	306 ^{ns}	1,3
<i>Lotus corniculatus</i>	485*	1,6	625*	1,3	401*	1,7
<i>Secale cereale</i>	621*	2,0	738*	1,6	275 ^{ns}	1,2
<i>Vicia sativa</i>	701*	2,3	961*	2,0	327*	1,4
After the cultivation of warm-season species						
No plants in the summer	302		254		218	
<i>Crotalaria juncea</i>	361*	1,2	427*	1,7	494*	2,3
<i>Stizolobium atterrimum</i>	722*	2,4	1125*	4,4	1289*	5,9
<i>Canavalia ensiformis</i>	732*	2,4	1220*	4,8	1358*	6,2
<i>Glycine max</i>	691*	2,2	1142*	4,5	598*	2,7

^{ns} Value for GR₅₀ does not differ from the control (no plant) by overlapping of the confidence intervals for the values at 95% probability. *Value for GR₅₀ differs from the control (no plant) by non-overlapping of the confidence intervals for the values at 95% probability

In experiments conducted with rice, Martini *et al.* (2011) found that after harvesting the rice, the amount of the formulated mixture of imazapic and imazethapyr (75+25 g a.e. L⁻¹) in the soil ranged from 190 to 500 mL ha⁻¹. From this it can be inferred that all species with a GR₅₀ equal to or greater than these values have the capacity for the phytoremediation of soils contaminated with these herbicides.

CONCLUSIONS

1. *Glycine max*, *Lolium multiflorum* and *Lotus corniculatus*, due to being more adapted to hydromorphic environments, a feature found in soils cultivated with irrigated rice, are potentially promising species in the phytoremediation of soils contaminated with the herbicide imazethapyr + imazapic (up to 4,000 mL ha⁻¹);
2. *Crotalaria juncea*, *Canavalia ensiformis*, *Stizolobium aterrimum*, *Vicia sativa*, *Raphanus sativus* and *Triticum aestivum* are species capable of the phytoremediation of soils contaminated with imazethapyr + imazapic, however the occurrence of anoxia in hydromorphic soils reduce the establishment and development of these plants.

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Residues of Thiamethoxam and Chlorantraniliprole in Rice Grain

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ABSTRACT: Thiamethoxam and chlorantraniliprole insecticides have been important tools for controlling pests in rice. However, food safety issues related to pesticide residues are important to consider with a food crop such as rice. Therefore, the objective of this study was to analyze thiamethoxam and chlorantraniliprole residues in rice hull, bran, and polished rice grains. The study was conducted during the 2012 cropping season at the Texas A&M Agrilife Research, David R. Wintermann Rice Research Station, near Eagle Lake, TX, USA. Rice was planted on May 5, 2012, using the cultivar 'Presidio'. Pesticide applications were performed at 5, 15, 25, and 35 days after flowering (DAF) using 1 and 2 times the recommended rate of 30 g active ingredient (ai) ha⁻¹ for thiamethoxam and 30 g ai ha⁻¹ for chlorantraniliprole. Sequentially, two treatments using the insecticides at recommended rate were applied at 5 and 25 DAF and at 5, 25, and 35 DAF. Insecticide residues were analyzed in different sample fractions: rice hull, bran, and polished rice grains. The samples were subjected to extraction using an accelerated solvent extraction (ASE) technique. Sample aliquots were analyzed using ultrahigh-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS), with a limit of quantification (LOQ) of 5×10^{-5} mg kg⁻¹. Residues of thiamethoxam and chlorantraniliprole were detected in rice hull, bran, and polished rice grains, and the quantified values were greater in hull and in rice bran.

KEYWORDS: *Oryza sativa* L., accelerated solvent extraction, rice hull, rice bran, polished rice grains

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the main components of a basic daily diet throughout the world, the third most popular grain worldwide and a major food crop for >60% of the world's population,¹ and its consumption has increased over recent decades.² Thus, the quest for high grain yield and quality through management practices and technology is a challenge for scientists and researchers.

The use of insecticides in irrigated rice has intensified in recent years due to greater insect pressure at economically important levels; thus, efficiency in controlling insecticide use in irrigated rice is a pressing issue. Lepidoptera^{3,4} and heteroptera^{5–7} are common insects in irrigated rice crops, and their attack can occur in both the vegetative and reproductive phases of the rice plants. In many cases, the occurrence of pests, which cause crop injury, has forced farmers to apply pesticides near harvest. Therefore, the use of insecticides is characterized as an essential management practice to ensure adequate agricultural yield and maintenance of the crops yield potential⁸ as well as quality of the rice grain.^{9,10}

Insecticides from the neonicotinoid and anthranilicdiamide chemical groups are recommended for use on irrigated rice crops,^{11–13} providing higher efficiency in the control of insects due to their wide-spectrum control.

The insecticide thiamethoxam [3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine] represents the fastest growing class of insecticides called

neonicotinoids.¹⁴ Neonicotinoid insecticides act selectively on the central nervous system of insects with minimal effects on beneficial insects and with low toxicity toward mammals without causing either teratogenic or mutagenic effects,¹⁵ which are active against many sucking and biting insects, including aphids, whiteflies, and some lepidoptera species.¹⁶

Thiamethoxam is a versatile insecticide, which may be applied to the aerial parts of plants or to seeds with relatively low rate. It belongs to the systemic insecticide class because of its high solubility in water and low partition coefficient (K_{ow}),¹⁷ being characterized as a polar compound that tends to ascend via xylem.¹⁸ Thiamethoxam is highly efficient in controlling insects, and there are no records of cross resistance with other classes of insecticides.¹⁹ The use of neonicotinoid insecticides at various crop stages and during postharvest storage plays an important role in food security and quality preservation.

Chlorantraniliprole {3-bromo-*N*-[4-chloro-2-methyl-6-[(methylamino)carbonyl] phenyl]-1-(3-chloro-2-pyridinyl)-1*H*-pyrazole-5-carboxamide} is a new insecticide belonging to the anthranilicdiamide class.²⁰ Chlorantraniliprole selectively binds to ryanodine receptors (RyR) in insect muscles, resulting

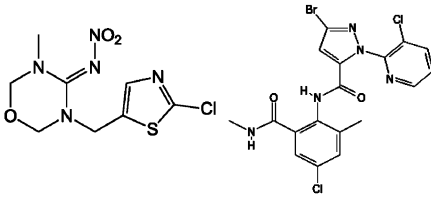
Received: September 12, 2014

Revised: January 27, 2015

Accepted: January 27, 2015

Published: January 27, 2015

Table 1. Characteristics of the Insecticides Thiamethoxam and Chlorantraniliprole and Maximum Limit of Residues (MRL) Permitted for Rice Grains in Different Countries

parameters	thiamethoxam	chlorantraniliprole
chemical group ^a	neonicotinoid	anthranilicdiamide
molecular formula	C ₈ H ₁₀ ClN ₅ O ₃ S	C ₁₈ H ₁₄ BrCl ₂ N ₅ O ₂
molecular Structure		
molecular weight (g mol ⁻¹)	291.7	483.1
water solubility at 20 °C (mg L ⁻¹)	4.1	0.8
log K _{ow}	-0.13	2.86
dissociation constant (pK _a) at 25 °C	Not applicable	10.8
vapor pressure at 25 °C (mPa)	6.6 × 10 ⁻⁶	6.3 × 10 ⁻⁹
henry's law constant at 25 °C (Pa m ⁻³ mol ⁻¹)	4.7 × 10 ⁻¹⁰	3.2 × 10 ⁻⁹
maximum residue limits (MRL) permitted to rice grain (mg kg ⁻¹)		
codex alimentarius ^b	---	0.02
United States ^b	0.02	0.15
European Union ^b	0.6	0.4
Brazil – ANVISA ^c	1.0	0.2
safety interval-ANVISA ^{c**}	21 days	15 days

^aIUPAC agrochemical information.¹⁶ ^bU.S. Department of Agriculture.⁶⁶ ^cANVISA, Brazilian Health Surveillance Agency.⁶⁷ ^{**}Not registered for rice production. ^{***}Safety interval between application of the pesticide and harvesting of rice grains in Brazil.

in an uncontrolled release of calcium from internal stores in the sarcoplasmic reticulum,²¹ causing impaired regulation of muscle contraction and leading to feeding cessation, lethargy, paralysis, and eventual death of target organisms.²² Its use provides good lepidoptera control;^{23,24} it is a systemic insecticide that tends to be translocated long distances ascendingly³ in the plant and is recommended for and widely used in irrigated rice crops by foliar application.^{11,12} The insecticide moves within the foliar tissue, where it is protected from leaching while remaining available to chewing insects in both top and bottom surfaces of the leaves. This transfoliar activity associated with its insecticide potential, resistance, and photodegradation are the bases for efficient protection in the tomato plant. It is noteworthy that this insecticide presents low toxicity to nontarget organisms such as mammals, birds, fishes, and others,^{25,26} a good option for pest control with low environmental impact. The anthranilicdiamide class has no cross-resistance with other insecticide chemical groups²⁷ and is an efficient alternative in the insecticide rotation.²⁸

In rice crops the increasing application of pesticides directly on the organ of the plant that is used for consumption and in association with the fact that rice does not receive intensive industrial processing, the analysis of residues of insecticides on the grain becomes essential as a way to ensure food safety, due to the fact that insecticide residues can persist until the harvest stage, resulting in the contamination of the rice grain.^{29–31}

However, pesticides may also affect human life due to pesticide buildup throughout the food chain may lead to an ecologic phenomenon called biomagnification, which is the bioaccumulation of a pesticide through an ecological food chain by transfer of residues from the diet into body tissues. The tissue concentration increases at each trophic level in the food web when there is efficient uptake and slow elimination.³² However, knowledge pertaining to the long-term effects of pesticides in human health is still very restricted,³³ but indicates that its effects may be associated with several health problems.^{34–37} The literature presents several studies related to pesticide residues in food; however, there is little information on the use of insecticides in rice fields and its persistence in the grains. It is important to analyze pesticide residues not only on the rice grain itself but also on rice subproducts such as rice bran, which can be used in whole rice³⁸ and animal feed.^{39,40} Overall, the results of these studies show variation in the presence of pesticide residues in rice grain, likely due to environmental factors that can interact and interfere with the persistence of pesticides. The literature presents several hypotheses regarding what may influence the persistence of pesticides in food, and many such reasons are not fully understood due to the characteristics of the active ingredients,^{41,42} the moment of application,⁴³ solar radiation, temperature,⁴⁴ and the persistence of pesticides in plants.^{45–47}

Thus, studies analyzing pesticide residues in rice grain are pertinent and provide a better understanding of if and how

Table 2. Pesticides Analyzed by UHPLC-MS/MS Using ESI and EI Ionization, Retention Time, and Cone and Collision Energy for Each Transition Monitored in the SRM Mode

pesticide	retention time (min)	ionization mode	precursor ion (<i>m/z</i>)	cone voltage (eV)	product ion (<i>m/z</i>)	collision energy (eV)
thiamethoxam	0.423	+	291.96	20	211.0; ^a 133.1 ^b	12; 13
chlorantraniliprole	0.979	+	483.97	30	285.8; ^a 449.3 ^b	14; 29

^aIon selected for quantification. ^bIon selected for confirmation.

technologies used in commercial crops compromise grain quality. Another important issue may be related to the rate of pesticides applied to the aerial parts of plants; however, reports on the use of the recommended rate and double the recommended rate for the insecticide thiamethoxam have not been able to detect residues in polished rice grains.⁴⁶ However, it is important to analyze pesticide residues in rice as a whole because in a study with the insecticide thiamethoxam, the highest residue concentration was detected on the rice hull (62% of total detected), followed by rice bran (38% of total detected); no residues were detected on the polished rice grains.⁴⁸

With regard to chlorantraniliprole in rice, residues were detected in polished rice grains varying with the applied rate [30 and 60 g active ingredient (ai) ha⁻¹]. The addition of a double rate of chlorantraniliprole presented the highest values of residues detected in polished rice grains, which was 85% higher than the recommended rate.⁴⁹

The need for information regarding the persistence of pesticides in irrigated rice is relevant because of the nutritional and toxicological issues associated with rice consumption.⁴⁸ Therefore, it is necessary to study pesticide residues in rice grains to provide basic information for the use of thiamethoxam and chlorantraniliprole in pest management strategies for rice and grain quality.

The objective of this study was to analyze residues of the insecticides thiamethoxam and chlorantraniliprole in rice hull, bran, and polished grains when applied on the aerial part of the rice plants.

MATERIALS AND METHODS

The study was conducted in two stages; the first stage was conducted in the field and the second in the laboratory.

Experimental Procedures in the Field. The first phase of the experiment was conducted during the 2012 cropping season at Texas A&M Agrilife Research, David R. Wintermann Rice Research Station, near Eagle Lake, TX, USA. The rice was seeded on May 5, 2012, using the cultivar 'Presidio', with a seeding rate of 80 kg ha⁻¹. Each plot had seven rows spaced at 0.19 m from each other and measuring 4 m long. The management practices were conducted according to the technical recommendations for rice cultivation in Texas.⁵⁰

Pesticide applications were performed at 5, 15, 25, and 35 days after flowering (DAF) using 1 and 2 times the recommended rate, that is, 30 g ai ha⁻¹ of thiamethoxam and 30 g ai ha⁻¹ of chlorantraniliprole (Table 1). Besides, two treatments using the insecticides recommended rate were applied sequentially at 5 and 25 DAF plus at 5, 25, and 35 DAF. Insecticide residues were analyzed in different sample fractions: rice hull, bran, and polished grains.

The applications were made with a CO₂ pressurized backpack sprayer calibrated to dispense a 205 L ha⁻¹ spraying volume. The system was attached to a spray boom with three nozzles operating at 276 kPa. The experiment was conducted using a randomized complete block design with four replications.

For quantification of residues in grains, rice plants were harvested when the average moisture content in the grains reached 20%, based on Texas recommendations.⁵⁰ Rice harvest was performed 40 DAF from an area totaling 3.8 m² (4.0 × 0.95 m) in each plot. After grain

harvest, a homogeneous sample of 2 kg was separated for each treatment. Subsequently, samples were cleaned and dried with forced air ventilation at 35 ± 2 °C until reaching an average moisture content of 13%. The samples were then stored at -20 °C during the 30 day period until extraction for pesticide residues.

Insecticide residues were analyzed using different fractions of rice samples including (1) rice hull, (2) rice bran, and (3) polished rice grains. These fractions were obtained by processing the samples in rice milling equipment (Zaccaria, model PAZ-1-DTA, Limeira, SP, Brazil).

Determination of the Pesticide Residues. The second stage of the study was conducted in the Department of Soil and Crop Sciences at Texas A&M University. Prior to chromatographic analysis, samples were extracted using an accelerated solvent extraction Dionex ASE 200 system (Dionex Co., Sunnyvale, CA, USA), and afterward the samples were analyzed using ultrahigh-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS).

Sample Preparation for ASE. The sample preparation proceeded with 2 g of the matrix (rice hull, bran, or polished grains), 3 g of Chem tube hydromatrix (Agilent Technologies), and 2 g of Florisil (Acros Organics). Sample preparation was carried out in a 50 mL beaker, and the contents were mixed with a spatula to obtain a free-flowing powder. The cell size used was a 22 mL stainless steel cell, using two circular cellulose filters (size = 19.8 mm in diameter) at the low end of the cell. Fortification was done by adding appropriate volumes of solution to the samples, where they were equilibrated in the extraction in cells at room temperature for 10 min.

Optimization of ASE Conditions. Optimal extraction conditions demonstrating the highest chemical recoveries included were extraction temperatures of 75 °C for rice hull and 100 °C for rice bran and polished grains, pressure of 10.3 MPa, and solvent of acetonitrile (100%) determined from a previous experiment (unpublished data). The duration of static phase was 5 min, following 1 min of preheating and 5 min of equilibration. The solvent was collected in 60 mL vials with Teflon septa. Subsequently, each extraction cell containing the same sample went through another identical extraction cycle, and the solvent was collected in the same vial.

Optimization of Evaporation Conditions. The total extracted volume of 60 mL was transferred into 15 mL volumetric tubes 10 mL at a time for sample evaporation using a TurboVap LV Evaporator (Zymark Center, Hopkinton, MA, USA). Samples were evaporated at 45 °C to dryness using a gentle stream of nitrogen. Pesticides were resuspended in 2 mL of acetonitrile and shaken in a vortex mixer (Fisher Vortex Genie2, model G-560; Fisher Scientific, Bohemia, NY, USA). The samples were stored at 4 °C during the 2 h period to complete the preparation of all samples and begin analyses by chromatograph.

Chromatography Conditions. Analyses were performed using an UHPLC-MS/MS, model Acquity TQD (Waters Corp., Milford, MA, USA). Chromatographic separation was done with an ethylene-bridged hybrid (BEH) C₁₈ column (50 × 2.1 mm; 1.7 μm) with controlled temperature of 30 °C and a 5 μL injection volume. Mobile phase was pumped from two solvent reservoirs consisting of 0.05% of formic acid in water (A) and acetonitrile (B) in an isocratic run (50 A/50 B) with a flow rate of 0.4 mL min⁻¹, and the estimated void time for this method is 0.3 min. The mass spectrometer was operated using electrospray ionization (ESI) in positive mode, capillary voltage at 2.2 kV, source and dissolution temperatures of 150 and 400 °C, and dissolution and cone gas flows of 800 and 50 L h⁻¹, respectively. Nitrogen (AOC, Bryan, TX, USA) was used as dissolution and cone gas and argon (AOC) as collision gas. The pesticides analyzed were

Table 3. Slope, Intercept, Determination Coefficient (r^2), Limits of Detection, and Limits of Quantification of Pesticides with the UHPLC-MS/MS Method

pesticide	slope	intercept	r^2	LOD (mg kg ⁻¹)	LOQ (mg kg ⁻¹)
thiamethoxam	5.2×10^{-3}	4.5×10^{-4}	0.998	0.00015	0.0005
chlorantraniliprole	3.9×10^{-3}	1.2×10^{-4}	0.996	0.00015	0.0005

confirmed using selected reaction monitoring (SRM) mode in which ionizations of the parent and one product were analyzed and monitored for each compound studied (Table 2).

Extraction Efficiency and Statistical Design. The efficiency of the extraction method was determined by sample fortification and analysis of quality control samples. Untreated rice samples were fortified to a concentration of 0.75 mg kg⁻¹. The samples were then processed according to the extraction procedure previously described, and six repetitions of each sample were analyzed.

Limit of Detection (LOD) and Limit of Quantification (LOQ). The quantification was based on a seven-point calibration curve (0.0005, 0.001, 0.002, 0.01, 0.1, 0.5, and 1.0 mg kg⁻¹). A matrix effect was obtained by comparing each response generated from calibration standards, which were prepared in pure acetonitrile and in matrix extracts. ME (%) was calculated as follows: $(B/A) \times 100$, where B is a response of matrix-matched calibration standard and A is a slope of non-matrix-matched calibration standard. Matrix effect values between -20 and +20% are considered acceptable.⁵¹

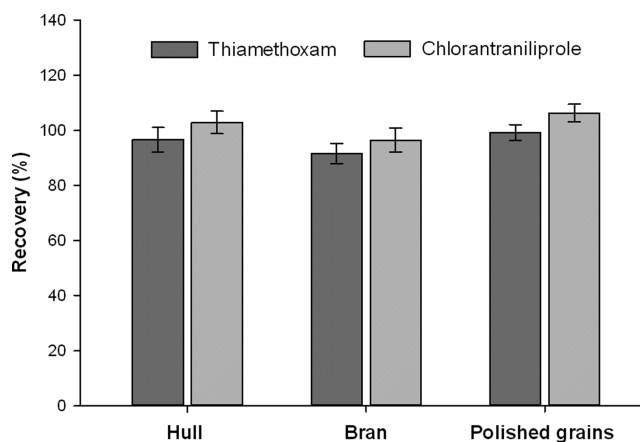
LOD and LOQ were calculated according to the peak-to-peak noise method.⁵² The baseline of the unfortified blank was magnified to obtain the instrument noise response and converted into a concentration estimate from the known concentration of the fortified extract. The LOD was estimated by multiplying the response of the method noise level by approximately 3 and then converting the total response into an estimated concentration. The LOQ was estimated by multiplying the response of method noise level by approximately 10 and then converting the total response into concentration.

Statistical Analysis. The experimental design was a completely randomized factorial arrangement with eight replications in laboratory. Data were subjected to analysis of variance, and means were compared using the Scott-Knott test ($p \leq 0.05$) using the SAS program (Statistical Analysis Systems, 9.2 software, SAS Institute Inc., Cary, NC, USA). The results plotted in the figure were determined by a 95% confidence interval.

RESULTS AND DISCUSSION

Recovery and Detection Limits. The standard calibration curves of thiamethoxam and chlorantraniliprole were constructed by plotting analyzed concentrations with the peak areas. Linearity of curves for each insecticide was achieved within the range from 5×10^{-4} to 1 mg kg⁻¹ (Table 3). During the calibration step it was observed that thiamethoxam and chlorantraniliprole insecticides showed an increase or decrease effect in the detector response when the calibration solutions were prepared in matrix extracts or pure solvent. To assess the matrix effect of our extracts, the responses of calibration standard prepared in solvent and matrix extract were compared. In accordance with previously published works, soft matrix effects (suppression or enhancement of 0–20%) are negligible. Otherwise, if the pesticides suffer medium (suppression or enhancement of 20–50%) or strong (suppression or enhancement >50%) matrix effects, it is necessary to use certain calibration methods to overcome the influence of matrix. For hulls, grains, and bran extracts, thiamethoxam presented high suppression (−94, −34, and −88%, respectively). Moreover, for the same extracts chlorantraniliprole presented high suppression for hull (−75%) and bran (−59%). Otherwise, for grains the matrix effect for this pesticide was positive (31%). These results prove the importance of calculating the matrix effect.

The recovery study was conducted for hull, bran, and polished grains. Average recovery ranged from 89.6 to 109.7% with a relative standard deviation <0.05% (Figure 1).

**Figure 1.** Recovery of thiamethoxam and chlorantraniliprole from rice hull, bran, and polished grains. Bars represent 95% confidence intervals.

Determination of Pesticide Residues in Rice Grains.

Results showed thiamethoxam and chlorantraniliprole residues in rice hull, bran, and polished grains (Table 4).

Insecticide Thiamethoxam. For thiamethoxam, pesticide residues were quantified in rice hull for all analyzed treatments. The results showed that greater concentration of pesticides was observed for the sequential applications at 5, 25, and 35 DAF at the recommended rate (0.225 mg kg⁻¹); this result is 20% higher than the average of the other treatments. It is important to highlight that there was no difference regarding the recommended rate and the doubled rate.

The analysis carried out on the rice bran showed that for the insecticide thiamethoxam, the three-time sequential application using the recommended rate and the application at 35 DAF using the double rate resulted in the highest concentration of residues in rice bran. Treatments with applications at 5 and 15 DAF with the recommended rate and twice the rate had lower residues. This behavior may be related to the time between the applications of insecticides and the time of harvest, allowing the metabolism of these compounds,^{41,46,53} which includes transformation of parent molecules into metabolites.⁵⁴

Another hypothesis may be the hygroscopic behavior related to rice grains close to harvest,⁵⁵ which involves absorption and water loss to reach equilibrium with the relative moisture of the ambient air,⁵⁶ along with an increased rate of respiration of grain. This situation can facilitate penetration of insecticides into grain, associated with slow metabolism and translocation of assimilates in the rice plant.⁵⁷

With respect to the analysis conducted in polished rice grains, residues of thiamethoxam insecticide were not detected for the applications using the recommended rate performed at 5 and 15 DAF. However, for all other treatments, residues were detected with the highest values for applications containing

Table 4. Thiamethoxam and Chlorantraniliprole Residues in Rice Hull, Bran, and Polished Grains^a

rate (g ai ha ⁻¹)	application done DAF ^b	residue detected (mg kg ⁻¹)		
		hull	bran	polished grains
Thiamethoxam				
30	5	0.169 b	0.153 c	nd ^c
30	15	0.158 b	0.136 c	nd
30	25	0.195 b	0.192 b	0.0012 c
30	35	0.182 b	0.185 b	0.0022 b
30 + 30	5, 25	0.167 b	0.160 b	0.0027 b
30 + 30 + 30	5, 25, 35	0.225 a	0.244 a	0.0028 b
60	5	0.143 b	0.131 c	nd
60	15	0.187 b	0.153 c	<LOQ ^d
60	25	0.200 b	0.177 b	0.0040 a
60	35	0.204 b	0.230 a	0.0029 b
Chlorantraniliprole				
30	5	0.529 c	0.486 d	nd
30	15	0.519 c	0.510 d	0.0059 d
30	25	0.586 b	0.600 c	0.0069 c
30	35	0.531 bc	0.785 c	0.0090 c
30 + 30	5, 25	0.575 b	0.780 c	0.0074 c
30 + 30 + 30	5, 25, 35	0.707 a	1.100 a	0.0108 b
60	5	0.557 b	0.444 d	0.0015 d
60	15	0.568 b	0.657 c	0.0114 b
60	25	0.557 b	0.876 b	0.0121 a
60	35	0.601 b	1.125 a	0.0108 b

^aMeans followed by a different letter within a column are significantly different according to the Scott–Knott test ($p \leq 0.05$). Data from each insecticide were analyzed separately. ^bDAF, days after flowering. ^cnd, not detected. ^d<LOQ, below the limit of quantification of the analytical method applied.

twice the rate. Applications with double the recommended rate at 25 DAF resulted in greater concentrations of thiamethoxam residues when compared to sequential applications. This might be associated with a period of intense translocation of assimilates to the grain during rice grain filling.

In summary, the highest insecticide concentrations were observed in rice hull and rice bran, and the mean value detected in polished rice grains alone represents <1% of the total residue value detected on the sample (hull + bran + polished grain). Thus, the lipophilic composition of the hull and bran constituents may serve as a physical barrier to translocation of pesticides, lowering detected values in polished rice grains.²⁹ Similar tendencies in residue concentration were detected in rice hull, bran, and polished grains when several pesticides used in irrigated rice crops were studied, with results similar to the ones in this study when greater concentrations in hull and rice bran were detected.⁴⁸

Experiments studying the dynamics and persistence of thiamethoxam from the chemical group of neonicotinoids did not show insecticide residues in polished rice grains.⁴⁶ Because of the relationship to dissipation and degradation of thiamethoxam in rice plants, about 40–60% of the initial residue dissipated 5 days after application, which after 15 days increased to 83–88%, regardless of the applied rate (33 or 66 g ai ha⁻¹). The calculated half-life values for thiamethoxam in rice plants were found to be 5.2 days for the 33 g ai ha⁻¹ rate and 5.8 days for the double rate.⁴⁶

Insecticide Chlorantraniliprole. Chlorantraniliprole residues were detected on the rice hull; it was noted that the

applications using the recommended rate at 5–15 DAF resulted in lower pesticide concentrations. This demonstrated that applications made closer to rice flowering using the recommended rate, would favor dissipation or degradation of this insecticide. For other treatments, greater concentrations were quantified, especially for the treatment with three sequential applications that showed the highest value (0.707 mg kg⁻¹).

The literature presents several hypotheses that may influence the concentration of pesticide residues in the irrigated rice ecosystem. Many of the hypotheses are not fully understood because of the numerous factors involved. Some of the primary factors that can influence the persistence of pesticides are time of application, weather conditions, pesticide characteristics, and pesticide translocation within the plant.⁵⁸

However, the adsorption or absorption of insecticides in rice grains, specifically in rice hulls, can be directly associated with the composition of the rice hull, which consists of >90% silica,⁵⁹ characterizing the rice hull polarity and contributing to the sequestration of pesticides in rice hull.

Chlorantraniliprole residues were detected on the rice bran, and the detected values are 22% higher than the value detected on the rice hull. The results showed that greater concentration of pesticides was observed for the sequential applications at 5, 25, and 35 DAF at recommended rates and for one application at 60 DAF with double the recommended rate.

These results may be related to the characteristics of the insecticide, highlighting that this insecticide presents greater lipophilic character; thus, distribution on these high lipid content matrices is reasonable.⁴⁸ This characteristic can be directly associated with increased quantification of residues in rice bran due to the fact that rice bran has a high lipid content.⁶⁰

No residues for the 5 DAF applications with the recommended rate were detected on the polished rice grain. However, for all other treatments, residues were detected for chlorantraniliprole, with the highest values detected for applications containing double the recommended rate. Applications with double the recommended rate at 25 DAF resulted in greater concentrations of residues when compared to sequential applications. This might be associated with a period of intense translocation of assimilates to the grain during rice grain filling.

Chlorantraniliprole studies in rice plants demonstrated that there was a reduction of 98% of applied insecticide in 14 days after the application independent of the applied rate (30 or 60 g ai ha⁻¹).⁴⁹ In brown rice grains (processed grains with no polishing process), chlorantraniliprole residues were detected for the applications done at 21, 14, and 7 days before rice grain harvest for both of the applied rates. The highest values were detected for applications done 7 days before harvest (8 µg kg⁻¹, recommended rate; and 19 µg kg⁻¹, 2 times rate), showing that applications done near rice harvest had the highest residue concentration. In general, there were increases of 74 and 84% in residue concentrations for the applications made at 7 days before harvest in comparison to the application done 21 days before harvest, with the recommended rate and 2 times rate, respectively.⁴⁹

Insecticide Residue Distribution. The distribution of residues throughout the rice grain is presented in Figure 2. The highest concentrations of thiamethoxam were observed in rice hulls, except for applications done at 35 DAF, for which the highest concentration was observed in rice bran.

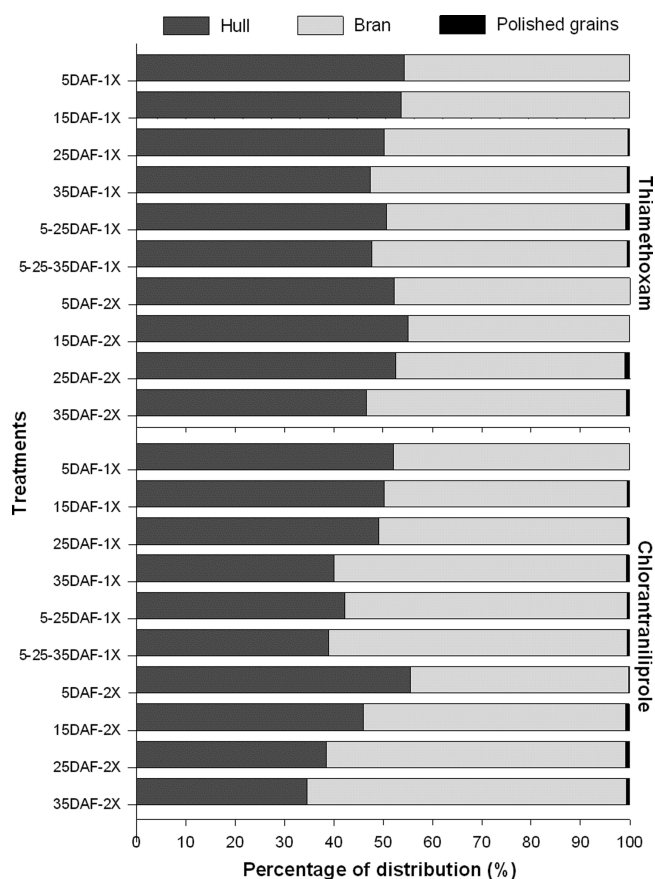


Figure 2. Percentage of thiamethoxam and chlorantraniliprole residues in rice hull, bran, and polished grains. Insecticides were applied at different timings after rice flowering using 1 and 2 times the recommended rate (30 g ai ha⁻¹ recommended rate). DAF, days after flowering.

The highest concentrations in rice bran were found for the insecticide chlorantraniliprole, except for applications done at 5 DAF characterizing different behaviors between insecticides. A possible explanation could be that this pesticide is the most lipophilic compound; thus, its distribution on these high lipid content matrices is reasonable. The highest concentration of crude fat is in rice bran,⁴⁸ associated with higher concentrations of chlorantraniliprole. The rice hull and polished rice grains presented 96 and 97% less crude fat, respectively.⁴⁸

The literature describes several factors that may influence how pesticide residues in plants may be affected by the time of application, application rate, weather conditions,⁶¹ matrix chemical composition, pesticide lipophilicity, mode of action,⁵⁹ time of application, and weather conditions.⁵⁸ The correct pesticide residue distribution tendency on the rice grain is not clear; however, higher concentrations of pesticides have been detected on the rice hull and bran.⁴⁸

For the rates applied in this study, the concentrations detected in polished rice grains were below the MRL set by Codex Alimentarius, the United States, the European Union, and the Brazilian Health Surveillance Agency (ANVISA). It is important to note that the MRLs are only for rice grain (Table 1).

It is important that management practices account for the limit of time between pesticide application and grain harvest. Overall, if the pesticides are applied according to good agricultural practices (GAP), MRLs are not exceeded; however,

the misuse of these compounds is possible and may result in significant amounts of residues found in both food and the environment.^{62,63} Corroborating this approach, there are no consolidated studies on concentrations of pesticide residues quantified in foods, especially those that exceed the maximum allowable limits for human consumption; the effects of accumulation of these compounds in humans are not yet known.^{64,65}

In conclusion, the present study demonstrates that residues of thiamethoxam and chlorantraniliprole applied to rice were detected in hull, bran, and polished grains. Insecticide concentrations were higher in hull and bran, demonstrating the importance of evaluating the destination of a pesticide and its residues from the field to the final product.

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Funding

We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing a doctorate sandwich scholarship to the first author and a postdoctoral scholarship to the fourth author. We acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the productivity and research scholarship of the third author.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the Department of Soil and Crop Sciences at Texas A&M University for the opportunity to work with the project.

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Impact of fungicide and insecticide use on non-target aquatic organisms in rice paddy fields

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ABSTRACT: *The intensive use of plant protection products in rice paddy fields (*Oryza sativa* L.) has caused concern about the environmental impact on communities of non-target organisms that are natural inhabitants in these agroecosystems. The purpose of this review is to analyze the data currently available in the literature about some important fungicides and insecticides (such as trifloxystrobin, tebuconazole, tricyclazole, lambda-cyhalothrin, and thiamethoxam), which are currently used to control pests and diseases in rice paddy fields, as well as their effects on the community of non-target aquatic organisms.*

Key words: *plant protection products, benthic insects, *Oryza sativa* L., lowlands.*

Impacto do uso de fungicidas e inseticidas sobre organismos aquáticos não alvos em lavouras de arroz irrigado

RESUMO: *O uso intensivo de produtos fitossanitários na lavoura de arroz irrigado (*Oryza sativa* L.) tem causado preocupação quanto ao impacto ambiental sobre comunidades de organismos aquáticos não alvos que são habitantes naturais nesses agroecossistemas. O objetivo da presente revisão é analisar os dados atualmente existentes na literatura sobre alguns importantes fungicidas e inseticidas (tais como trifloxistrobina, tebuconazol, triciclazol, lambda-cialotrina e tiametoxam), os quais são usados para controlar pragas e doenças na lavoura de arroz irrigado, bem como seus efeitos sobre a comunidade de organismos aquáticos não alvos.*

Palavras-chave: *produtos fitossanitários, insetos bentônicos, *Oryza sativa* L., terras baixas.*

INTRODUCTION

Rice cultivation areas are considered humid agroecosystems, which are temporarily managed by man (LUPI et al., 2013). Such environments have a higher biological diversity of water and terrestrial invertebrates compared to other agricultural areas (STENERT et al., 2012). Although invertebrates are predominant in lowland environments where irrigated rice is grown, amphibians, fish, mammals, and aquatic plants can also be present in this agroecosystem.

Rice paddy field management practices cause changes in the community of non-target aquatic organisms. RIZO-PATRÓN et al. (2013) observed that invertebrates resistant to pollution were more abundant in conventional

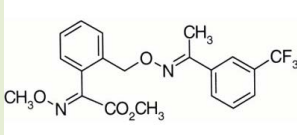
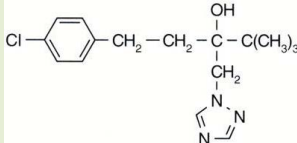
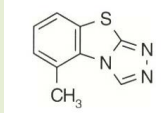
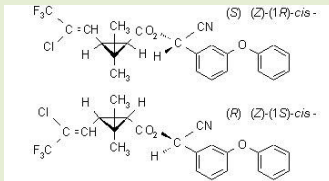
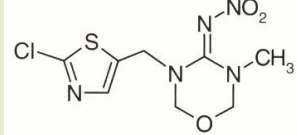
farming compared to organic farming and concluded that such organisms respond to both type of management and plant protection products applied to the crop.

The objective of this review was to analyze the literature data on some important fungicides and insecticides, which are currently used in the control of pests and diseases in rice paddy fields, and their effects on non-target community of aquatic organisms. The data presented below are from field and laboratory studies, which were conducted in Brazil and abroad.

Trifloxystrobin

Trifloxystrobin (Table 1) is a mesostemic fungicide, which can be used as an active principle alone or in combination with other active principles.

Table 1 - Chemical and toxicological characteristics and application doses of plant protection products in rice paddy fields. Santa Maria, RS, 2015.

Active principles	Chemical groups	Structural formulas	K_{ow}^*	Toxicological classes	Doses (g a.i. ha ⁻¹)**
Trifloxystrobin	Strobilurin	251658240 	4.5	II	50
Tebuconazole	Triazole	251658240 	3.7	IV	100
Tricyclazole	Benzothiazole	251658240 	1.42	II	225
Lambda-cyhalothrin	Pyrethroid	251658240 	6.9	III	15.9
Thiamethoxam	Neonicotinoid	251658240 	-0.13	III	21.2

* K_{ow} : Octanol-water partition coefficient. **a.i.: active ingredients. Source: ANVISA (2014), SOSBAI (2012).

In rice paddy fields, the trifloxystrobin + tebuconazole commercial formulation (which is used at a dosage of 50+100g of active ingredients (a.i.) ha⁻¹, respectively) is used to control brown spot (*Bipolaris oryzae*), narrow brown leaf spot (*Cercospora janseana* = *C. oryzae*), and leaf scald (*Gerlachia oryzae* = *Rhynchosporium oryzae*) (SOSBAI, 2012).

In rice paddy fields, trifloxystrobin residues are highly correlated with ecological risk. However, how such processes occur is not yet clear. Trifloxystrobin showed a half-life in the range of 0.7-7.5 days in rice paddy fields. However, its major metabolite presented a high persistence in water, indicating that frequent application of the fungicide represent a long-term

potential risk for aquatic organisms that inhabit the rice agroecosystem (CAO et al., 2015).

Laboratory studies allowed to observe trifloxystrobin toxic effects on amphibians (JUNGES et al., 2012), crustaceans such as *Daphnia magna* (OCHOA-ACUNA et al., 2009) and *Hyalella azteca* (MORRISON et al., 2013), and fishes (USEPA, 2013). Fish may be present in rice fields by entering through the irrigation water or when they are added aiming rice-fish culture (LAWLER, 2001). Embryonic and larval development of the fish *Oryzias latipes* was changed after exposure to trifloxystrobin (ZHU et al., 2015a). LIU et al. (2013) reported that strobilurins, including trifloxystrobin, was toxic to *Ctenopharyngodon idella*, one of the most important fish species in Chinese aquaculture.

Trifloxystrobin also presented numerous toxic effects in embryos of *Gobiocypris rarus*, as observed through the increase in the number of malformations, changes in heart rate and enzyme activities, in addition to DNA damage, indicating that trifloxystrobin is highly toxic to fish embryos (ZHU et al., 2015b).

In chironomids, sediment chronic toxicity tests showed an CE_{50} (effective concentration for 50% of organisms) of $450\mu\text{g L}^{-1}$ (28 d; *Chironomus riparius*) and NOEC (highest concentration in which effects are not observed) of $200\mu\text{g L}^{-1}$ (28 d; *Chironomus riparius*). However, effects were less significant for metabolite CGA 321113, with an CE_{50} of $49200\mu\text{g L}^{-1}$ (28 d; *Chironomus riparius*) and NOEC of $25000\mu\text{g L}^{-1}$ (28 d; *Chironomus riparius*) (EUROPEAN COMMISSION, 2003). Studies conducted with the amphipod *Hyaella azteca* showed that trifloxystrobin toxicity may vary according to the environmental conditions; i.e., the presence of sediment may cause a decrease in toxicity of certain fungicide formulations (MORRISON et al., 2013).

Tebuconazole

Literature presents several laboratory studies on the effects of tebuconazole on parameters such as mortality, growth, behavior, and physiology of non-target aquatic organisms. A large part of these studies were conducted with crustaceans such as *Gammarus pulex* (ADAM et al., 2009), *Daphnia magna*, and *Americamysis bahia* (USEPA, 2013), fish such as *Rhamdia quelen* (KREUTZ et al., 2008), *Danio rerio* (ANDREU-SANCHEZ et al., 2012), *Cyprinodon variegatus*, and *Oncorhynchus mykiss* (USEPA, 2013), and mollusks such as *Crassostrea virginica* (USEPA, 2013). However, field studies testing the effects of active principles in the recommended doses were not reported in the literature.

The stress response in jundiá fingerlings of the species *Rhamdia quelen* was evaluated after acute exposure to plant protection products including tebuconazole fungicide. It was noticed that the presence of the stressful stimulus influenced the fish performance parameters more significantly than their own exposure to the fungicide (KOAKOSKI et al., 2014). Another recent study evaluated the tebuconazole toxic effects on various parameters of individuals of the species *Daphnia magna*. Results showed that the number of newborns per female was the highest sensitive parameter to tebuconazole exposure, and a seven-day recovery period in a toxicity-free medium was not enough to restore the reproduction normal parameters in daphnids pre-exposed to the fungicide (SANCHEZ et al., 2016). Tebuconazole toxic effects were observed

when amphipods of the species *Gammarus pulex* were fed with leaves exposed to tebuconazole fungicide, which caused a reduction in the organisms' feed rate (DIMITROV et al., 2014).

Enantio selectivity can contribute to the toxicity of plant protection products in the natural environment, and this phenomenon has been recently studied. Tebuconazole enantio selectivity was evaluated in three aquatic species (*Scenedesmus obliquus*, *Daphnia magna*, and *Danio rerio*) and R - (-) - tebuconazole was about 1.4 - 5.9 times more toxic than S - (+) - tebuconazole. Tebuconazole enantio selectivity showed a significant correlation with soil properties. This property may be a common phenomenon in the biodegradation of chiral triazole fungicides and aquatic toxicity, and should; therefore, be considered when the ecotoxicological risks of these compounds in the environment are assessed (LI et al., 2015). Currently, methods to determine tebuconazole enantio selectivity have been studied (LIU et al., 2015). A recent study showed that no significant enantio selective degradation of tebuconazole was observed in sterile conditions (ZHANG et al., 2015), indicating that organic matter is important in fungicide enantio selective degradation.

When plant protection products are released into the environment, highly toxic processing products can be generated. However, the occurrence of these products and their potential environmental risk are difficult to predict. Transformation products of the fungicide tebuconazole were identified in the soil during a field study, which detected 22 known and 12 still unknown transformation products (STORCK et al., 2016). This suggested that further studies on derivatives toxicity to non-target aquatic organisms after degradation of this fungicide in the environment are important.

In addition to the toxic effects on physiological functions of organisms, the effects on DNA are also important when the environmental risk to non-target organisms is considered. The genotoxic potential of active principle tebuconazole was assessed in snail embryos of the species *Cantareus aspersus*, in which individual changes were observed with tebuconazole doses starting from $50\mu\text{g L}^{-1}$ (BAURAND et al., 2015).

Tricyclazole

Tricyclazole, which is a systemic fungicide of the benzothiazole chemical group, is applied at a dose of 225g a.i. ha^{-1} in rice cultivation to control rice blast (*Pyricularia grisea*) (SOSBAI, 2012). Studies indicated that tricyclazole presents a high risk of environmental contamination, is not readily hydrolysable in the environment, and has a high capacity for soil adsorption (PADOVANI et al., 2006).

Although tricyclazole is one of the fungicides most used in rice paddy fields, there is still little information in the literature about its toxic effects on non-target aquatic organisms, and the information available refers to acute toxicity tests with bioindicators (in a few species however) in laboratory conditions. Amphibian mortality, after exposure of *Rana limnocharis* to tricyclazole, was observed by PAN & LIANG (1993), who determined a CL_{50} of $19425 \mu\text{g L}^{-1}$. The CL_{50} values were determined for the fish *Lepomis macrochirus* ($2460 (1609-3880) \mu\text{g L}^{-1}$) and *Oncorhynchus mykiss* ($1801 (1500-2200) \mu\text{g L}^{-1}$) (USEPA, 2013). Intoxication of mollusk *Crassostrea virginica* embryos was also determined in laboratory conditions ($CE_{50} = 32000 \mu\text{g L}^{-1}$) (USEPA, 2013).

Tricyclazole caused an increase in the triglyceride, cholesterol, glucose, and lactate levels in fish of the species *Danio rerio*, in addition to enzymatic disorders observed after the organisms were recovered at the end of the experiment (SANCHO et al., 2009). One of the few studies about the effects of tricyclazole on benthic macroinvertebrates was developed by ROSSARO & CORTESI (2013), who did not find significant negative effects of the fungicide in field tests. In tests for acute toxicity under laboratory conditions, tricyclazole also showed a low toxicity ($CL_{50 (48 h)} = 26000 \mu\text{g L}^{-1}$) on invertebrates.

Lambda-cyhalothrin

Lambda-cyhalothrin, which is a halogenated pyrethroid insecticide, comprises two stereoisomers, being widely used in pest control (COLOMBO et al., 2013). It is used in rice paddy fields to control small rice stink bug (*Oebalus poecilus*), in combination with insecticides of the neonicotinoids chemical group such as thiamethoxam (15.9 and $21.2 \text{ g a.i. ha}^{-1}$, respectively) (SOSBAI, 2012). Pyrethroid insecticides, such as lambda-cyhalothrin, are hydrophobic compounds that, in aquatic environments, can bind to organic matter (e.g., debris, leaves, and phytoplankton), which are important in the benthic macroinvertebrate community structure. However, pyrethroid coefficient of partition between different fractions of organic carbon depends on the bioavailability, which may influence toxicity to aquatic invertebrates (MAUL et al., 2008).

In nature, there is a range of contaminants that interact with each other, causing synergistic or antagonistic effects on species. Lambda-cyhalothrin, cadmium, and the neonicotinoid imidacloprid were tested in combination, and their toxic effects on earthworms of the species *Eisenia fetida* were analyzed. The combination of lambda-cyhalothrin and cadmium resulted in light synergistic effects on organisms; whereas,

binary mixtures with imidacloprid resulted in antagonistic effects, which were more significant in ternary mixtures with this insecticide (WANG et al., 2015).

In laboratory tests, SCHROER et al. (2004) observed that *Chaoborus obscuripes* (Diptera: *Chaoboridae*) was the species most sensitive to lambda-cyhalothrin ($CE_{50 (48 \text{ and } 96 h)} = 0.0028 \mu\text{g L}^{-1}$), followed by other insect larvae of the orders Hemiptera and Ephemeroptera and macrocrustaceans, which were relatively sensitive ($CE_{50 (48 \text{ and } 96 h)} = 0.01-0.1 \mu\text{g L}^{-1}$). The groups of microcrustaceans (Cladocera, Copepoda) and insect larvae of the orders Odonata and Chironomidae were the least sensitive ($CE_{50 (48 h)} > 0.1 \mu\text{g L}^{-1}$).

Several recent studies on the toxic effects of lambda-cyhalothrin in fish can be reported in the literature. The quality of sperm from individuals of the species *Oncorhynchus mykiss* (rainbow trout) was significantly reduced by exposure to lambda-cyhalothrin (KUTLUYER et al., 2015). In fish *Danio rerio*, lambda-cyhalothrin caused disturbance in the endocrine system, and the T3 and T4 hormones were significantly altered after exposure to the insecticide (TU et al., 2016). In another study conducted with zebrafish embryos, it was observed that synthetic pyrethroids have a high bioconcentration capacity, suggesting that pyrethroids have a highly-cumulative risk for fish (TU et al., 2014).

Recent studies showed that enantio selectivity may be another factor to be considered in the toxicity of chemicals in the environment. Bioavailability and enantio selectivity of lambda-cyhalothrin and bifenthrin was observed in earthworms of the species *Eisenia fetida*. Results showed that lambda-cyhalothrin was more easily adsorbed on the soil than bifenthrin, and bioaccumulation of both products was enantio selective (CHANG et al., 2016). Recently, WIELOGÓRSKA et al. (2015) observed that pyrethroid metabolites are concerning regarding their estrogenic activity, which is relatively higher than their parent compounds.

Thiamethoxam

Neonicotinoids are highly potent and selective systemic insecticides (VEHOVSZKY et al., 2015). They are persistent in the environment, exhibit high bleaching capacity, and are highly toxic to many species of invertebrates (MORRISSEY et al., 2015). Temporary wet areas, as is the case of rice paddy fields, are among the places of greatest risk for contamination by neonicotinoids (MAIN et al., 2016).

Imidacloprid is the neonicotinoid most studied up to now, representing 66% of the 214 toxicity tests with neonicotinoids reported in the literature. Insects belonging to the orders Ephemeroptera, Trichoptera, and Diptera appear to be the most sensitive

among the species evaluated, whereas crustaceans in general are less sensitive (MORRISSEY et al., 2015). Aquatic insects are particularly vulnerable to neonicotinoids. However, there are few studies on the biological effects of thiamethoxam in fish, amphibians, and mollusks (ANDERSON et al., 2015). Recent studies showed the toxic effects of thiamethoxam in crustaceans (*Gammarus kischineffensis*) (UĞURLU et al., 2015; DEMIRCI et al., 2015), *Daphnia magna* and *Americamysis bahia* (USEPA, 2013), fish of the species *Channa punctata* (KUMAR et al., 2010), *Cyprinodon variegatus*, *Lepomis macrochirus*, and *Oncorhynchus mykiss* and mollusks of the species *Crassostrea virginica* (USEPA, 2013). BARBEE & STOUT (2009) determined CL_{50} values for *Procambarus clarkia* (967 (879-1045) $\mu\text{g L}^{-1}$; 96h) under laboratory conditions, and insects of the genus *Chironomus* sp. presented $CL_{50}^{(48h)} = 35$ (33-38) $\mu\text{g L}^{-1}$ (USEPA, 2013). VEHOVSZKY et al. (2015) observed that neonicotinoids inhibited the cholinergic neurotransmission in the nervous system of mollusks of the species *Lymnaea stagnalis*. The authors emphasized that aquatic animals, including mollusks, are in direct contact with the contaminants present in the aquatic environment, and they can thus be a suitable model for future studies on the neuronal and behavioral consequences of neonicotinoid poisoning.

In a recent study, BREDESON et al. (2015) found clothianidin, a toxic metabolite of thiamethoxam, in aphids that were fed with wheat plants treated with thiamethoxam. This suggested that studies on the effects in herbivores of thiamethoxam residues and its metabolites are important. TAILLEBOIS et al. (2014) described the synthesis of two new fluorescent thiamethoxam derivatives and compared their toxicities on the aphid *Acyrtosiphon pisum*. Results showed that these compounds presented toxic effects, acting as agonists on insect nicotinic acetylcholine receptors.

CONCLUSION

The literature showed that the plant protection products presented here have the potential to cause negative effects on non-target aquatic organisms inhabiting rice paddy fields. Triazoles and benzothiazoles persist in the environment and may cause a negative impact on communities of non-target aquatic organisms. Strobilurins, such as trifloxystrobin, have a low persistence in the environment, although they are toxic to aquatic organisms. Pyrethroids are hydrophobic compounds that can bind to the soil organic matter. They are among the most studied chemical groups, being highly toxic to aquatic organisms. Currently, special attention has been given to neonicotinoids, as this class

of insecticides has many active principles and their risks to non-target organisms are little known. However, they persist in the environment and have a high capacity to leach and contaminate water bodies, impacting the biological communities that inhabit these ecosystems.

ACKNOWLEDGEMENTS

The authors acknowledge Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for their financial support (Projeto Universal N° 14/2012).

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