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ACT: 519 PRESENTACION	

DIRECCIÓN DE NUEVAS CREACIONES

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Bogotá, D.C.,

Abogado(a)/Señor(a)
LUZ CLEMENCIA DE PAEZ

Asunto: Radicación: 13-40351- -2
 Trámite: 11
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Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/US2011/049480				
Fecha de Presentación Internacional	29/08/2011				

1. Documentos estudiados

Descripción:	Radicado N°13-40351-00000-00000	28/Febrero/2013
Reivindicaciones:	Radicado N°13-40351-00000-00000	28/Febrero/2013
Prioridad:	US 61/378,130	30/agosto /2010

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada.

Para el caso de la presente solicitud, al verificar la tasa pagada, todo el ultimo capitulo reivindicatorio presentado se tendrá en cuenta para realizar el examen de patentabilidad.

Título de la solicitud: “Composición herbicida sinérgica que contiene penoxsulam, y betazón”

El problema planteado en esta solicitud es la necesidad de desarrollar composiciones herbicidas con efecto sinérgico.

Para resolver este problema técnico la solicitud en estudio proporciona una composición herbicida sinérgica, debidamente definida (radicado N°13-40351-00000-00000, Pág. 2).

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A 01 N 43/54 // 43/88 // 43/90 // P 13/02.

4. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El método de las reivindicaciones 6 y 7 corresponden al uso de la composición, mencionada en la reivindicación 1 para controlar vegetación no deseable. Es evidente que la llamada etapa de “contactar con la vegetación...aplicar a...” corresponde a la forma de usar la composición, aunque en su preámbulo se reclame como método. Se le aclara al solicitante que un método cuya única etapa corresponde a una aplicación, dicha aplicación es un uso.

Por lo tanto dicho método no es patentable de acuerdo con el artículo 14.

5. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 1 no se encuentra debidamente soportadas en la descripción puesto que en las realizaciones preferidas hace referencia a la preparación y actividad de mezclas de tres componentes en proporciones como se mencionan en la tabla 1.

Se sugiere restringir la solicitud a una razonable cantidad de acuerdo al tipo de formulaciones probadas, es decir, que se encuentran debidamente soportados en el capítulo descriptivo. Teniendo en cuenta la descripción en los ejemplos se encuentra:

Tabla 1. Ejemplos de la mezcla

Componentes de la mezcla	Soporte de los ejemplos presentados (proporciones)
Penoxsulam	0,3, 7.5, 15, 23 (g /ha)
Bentazón de sodio	0, 500, 1000, 1100, 2000 (g/ha)

El solicitante debe definir específicamente la materia y el alcance de la invención a través de las características técnicas esenciales que se encuentren enteramente soportadas en la descripción.

Las reivindicaciones 1 a 3 y 5 hacen referencia a una mezcla y una composición pero no se mencionan las proporciones de los mismos, por lo tanto se sugiere mencionar cuales son los componentes y sus proporciones.

La reivindicación 4 menciona unas proporciones no muy claras puesto que sería mejor mencionar las proporciones que hacen parte de las características esenciales y por tanto deberían mencionarse en la reivindicación independiente que contiene todas las características esenciales de la invención.

En la reivindicación 5 al mencionarse los términos “adyuvante y/o portador aceptable en la agricultura” se está haciendo alusión a unos términos muy amplios, puesto que para una persona de nivel medio versada en la materia estos evocan su función y no se está indicando la denominación común de los compuestos. Se sugiere definir a qué tipo o tipos de cada uno de los anteriores términos se está haciendo referencia, pues no se puede definir sólo por su función. De acuerdo a lo sustentado en la descripción.

6. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en la Fecha de Presentación de la Solicitud de Prioridad, agosto 30 de 2010 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	US20060183637	“Penoxsulam as a turfgrass, vineyard and orchard floor herbicide”	17/Agosto/2006
D2	Damalas et Al, Weed Technology. Volume 20:992–998	“Control of Early Watergrass (Echinochloa oryzoides) and Late Watergrass (Echinochloa phyllopogon) with Cyhalofop, Clefoxydim, and Penoxsulam Applied Alone and in Mixture with Broadleaf Herbicides1”	2006
D3	Lee et Al, Korean Journal of Weed Science, Volume 26, Issue ,3, pp.246-253	“Weed Control by Herbicide Mixtures of Penoxsulam SC in Transplanted Rice Paddy Fields “	2006

El documento D1 ^[1] divulga una mezcla en la que el penoxsulam a menudo es mejor aplicarse en conjunto con uno o más herbicidas para obtener control de una amplia variedad de vegetación indeseable. El herbicida en conjunto es bentazón (Párr. [0014], Párrs. [0017] – [0021]).

El documento D2 ^[2] divulga que los experimentos fueron llevados a cabo para estudiar el efecto de la velocidad de aplicación, etapa de crecimiento y tanque de mezcla de bentazón o azimsulfuron sobre la actividad de penoxsulam, entre otros. Penoxsulam fue aplicado a 20, 30 o 40 g/ha solo o en mezcla con bentazón a 960g/ha, la proporciones empleadas entre bentazón a penoxsulam son 48:1, 32:1 y 24:1 (abstract; Pág. 994, col 1, Párr. 1; Pág. 996, col. 2; Pág. 997, tabla 4)

El documento D3 ^[3] divulga un tratamiento de penoxsulam 3%SC mezclado con bentazon 40% SL a 45 días después del trasplante, crecimiento de arroz, altura de la planta y N° de macollos fueron similares a los de la parcela de control no tratada. Además se enseña que el tratamiento 4 es penoxsulam 30g/ha+bentazon 1600g/ha en la cual la proporción de penoxsulam a bentazon es de 1:53,3 (abstract y tabla 4)

7. Examen de Patentabilidad (Art 14 D486)

Nivel Inventivo (Art18 D486)

La **reivindicación 1** consiste en una mezcla herbicida con unos componentes definidos (Rad. N°13-040351-00000-00000, Pág. 21)

¹URL:http://worldwide.espacenet.com/publicationDetails/biblio?CC=US&NR=2006183637A1&KC=A1&FT=D&ND=3&date=20060817&DB=EPODOC&locale=en_EP
²Anexo 1
³URL: http://www.koreascience.or.kr/article/ArticleFullRecord.jsp?cn=JCHHBQ_2006_v26n3_246

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1	D2	D3
mezcla que comprende:	Composición que comprende:	Composición que comprende:	Composición herbicida que comprende:
a) penoxsulam	penoxsulam	penoxsulam	penoxsulam
b) bentazón	Bentazón, entre otros	Bentazón, entre otros	Bentazón, entre otros

Los documentos D1, D2 y D3 son considerados el estado de la técnica cercano a la invención definida en la reivindicación 1 porque divulga:

D1 divulga una mezcla en la que el penoxsulam a menudo es mejor aplicarse en conjunto con uno o más herbicidas para obtener control de una amplia variedad de vegetación indeseable. El herbicida en conjunto es bentazón, dicha mezcla de herbicidas puede ir en formulación con adyuvantes aceptados en la agricultura y portadores, los portadores líquidos pueden ser acetona, etanol, etilenglicol, entre otros; los portadores sólidos sílice, carbonato de calcio, arcilla, entre otros (Párr. [0014], Párrs. [0017] – [0021])

D2 divulga que los experimentos fueron llevados a cabo para estudiar el efecto de la velocidad de aplicación, etapa de crecimiento y tanque de mezcla de bentazón o azimsulfuron sobre la actividad de penoxsulam, entre otros (abstract). Los tratamientos con herbicidas se aplicaron a las plantas de cada especie cuando se encontraban en la etapa de crecimiento de tres a cuatro o de 5 a 6 hojas. Penoxsulam fue aplicado a 20, 30 o 40 g/ha solo o en mezcla con bentazón a 960g/ha, la proporciones empleadas entre bentazón a penoxsulam son 48:1, 32:1 y 24:1 (Pág. 994, col 1, Párr. 1; Pág. 996, col. 2; Pág. 997, tabla 4)

D3 divulga que los experimentos fueron llevados a cabo para estudiar la problemática del control de malas hierbas efectivamente en la etapa post-emergencia cuando el tratamiento herbicida se aplicaba en el suelo y la eficacia no era buena para el arrozal. En el tratamiento de penoxsulam 3%SC mezclado con bentazon 40% SL a 45 días después del trasplante, crecimiento de arroz, altura de la planta y N° de macollos fueron similares a los de la parcela de control no tratada y estos herbicidas proveen control a *Echinocloa crus-galli* (barnyard grass), entre otras, eran de 10 a 30cm de altura en los tiempos de aplicación. Además se enseña que el tratamiento 4 es penoxsulam 30g/ha+bentazon 1600g/ha en la cual la proporción de penoxsulam a bentazon es de 1:53,3 (abstract y tabla 4)

Como se evidencia en la tabla comparativa anteriormente presentada, la mezcla de compuestos herbicidas descrita en la reivindicación 1 de la presente solicitud corresponde a una selección de compuestos conocidos presentes en mezclas herbicidas que ya habían sido reveladas en el documento D1 o D2 o D3.

La diferencia entre las mezclas herbicidas enseñadas en la solicitud y la de la anterioridad D1 radica en que no describe de manera específica una combinación que comprenda únicamente **penoxulam y bentazón** tal como si lo hace la solicitud bajo estudio.

La diferencia entre las mezclas herbicidas enseñadas en la solicitud y la de la anterioridad D2 radica en que no describe de manera específica una combinación que comprenda únicamente **penoxulam y bentazón** tal como si lo hace la solicitud bajo estudio.

La diferencia entre las mezclas herbicidas enseñadas en la solicitud y la de la anterioridad D3 radica en que no describe de manera específica una combinación que comprenda únicamente **penoxulam y bentazón** tal como si lo hace la solicitud bajo estudio.

No obstante, el efecto que logra realizar la selección de **penoxulam y bentazon** en la mezcla herbicida ahora reivindicada no puede ser determinado porque no existe alguna evidencia que indique que dicha mezcla de compuestos tenga alguna ventaja técnica en su actividad herbicida sobre las mezclas de compuestos herbicidas ya conocidas en la anterioridad D1 o D2 o D3.

Por lo tanto el Problema Técnico Objetivo de la presente solicitud consiste en proporcionar una mezcla de compuestos con actividad herbicida seleccionados de compuestos ya comprendidos en mezclas con actividad herbicida enseñadas en el documento D1 o D2 o D3.

Como consecuencia de los hechos mencionados en los párrafos anteriores la persona medianamente versada en la materia habría derivado de manera obvia y sin mucho esfuerzo de su parte la mezcla de compuestos con actividad herbicida descrita en la reivindicación 1 de la presente solicitud a partir de las enseñanzas del documentos D1 o D2 o D3, toda vez que esta se limita a una simple selección de los compuestos con actividad herbicida presentes en las mezclas ya conocidas en la anterioridad D1 o o D2 o D3.

Puesto que las reivindicaciones dependientes 2 - 4 no mencionan características inventivas adicionales, tampoco pueden ser considerada inventivas.

Conclusión:

Las reivindicaciones 1 a 4, cumplen el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

La **reivindicación 5** consiste en una mezcla herbicida con unos componentes definidos (Rad. N°13-040351-00000-00000, Pág. 21)

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1
Composición que comprende	Composición que comprende:
mezcla de:	Mezcla de:
a)penoxsulam	penoxsulam
b) bentazón	Bentazón, entre otros
un adyuvante aceptado en la agricultura	un adyuvante aceptado en la agricultura y un portador

El documento D1 es considerado el estado de la técnica cercano a la invención definida en la reivindicación 1 porque divulga una mezcla en la que el penoxsulam a menudo es mejor aplicarse en conjunto con uno o más herbicidas para obtener control de una amplia variedad de vegetación indeseable. El herbicida en conjunto es bentazón, dicha mezcla de herbicidas puede ir en formulación con adyuvantes aceptados en la agricultura y portadores, los portadores líquidos pueden ser acetona, etanol, etilenglicol, entre otros; los portadores sólidos sílice, carbonato de calcio, arcilla, entre otros (Párr.

[0014], Párrs. [0017] – [0021])

Como se evidencia en la tabla comparativa anteriormente presentada, la mezcla de compuestos herbicidas descrita en la reivindicación 5 de la presente solicitud corresponde a una selección de compuestos conocidos presentes en mezclas herbicidas que ya habían sido reveladas en el documento D1.

La diferencia entre las mezclas herbicidas enseñadas en la solicitud y la de la anterioridad D1 radica en que no describe de manera específica una combinación que comprenda únicamente **penoxulam y bentazón** tal como si lo hace la solicitud bajo estudio.

No obstante, el efecto que logra realizar la selección de **penoxulam y bentazon** en la mezcla herbicida ahora reivindicada no puede ser determinado porque no existe alguna evidencia que indique que dicha mezcla de compuestos tenga alguna ventaja técnica en su actividad herbicida sobre las mezclas de compuestos herbicidas ya conocidas en la anterioridad D1.

Por lo tanto el Problema Técnico Objetivo de la presente solicitud consiste en proporcionar una composición de compuestos con actividad herbicida seleccionados de compuestos ya comprendidos en mezclas con actividad herbicida enseñadas en el documento D1.

Como consecuencia de los hechos mencionados en los párrafos anteriores la persona medianamente versada en la materia habría derivado de manera obvia y sin mucho esfuerzo de su parte la composición de compuestos con actividad herbicida descrita en la reivindicación 5 de la presente solicitud a partir de las enseñanzas del documento D1, toda vez que esta se limita a una simple selección de los compuestos con actividad herbicida presentes en las mezclas ya conocidas en la anterioridad D1.

Conclusión:

La reivindicación 5, cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

8. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	US20060183637	1 – 5	Nivel inventivo
D2	Damalas et Al, Weed Technology. Volume 20:992–998	1 – 4	Nivel inventivo
D3	Lee et Al, Korean Journal of Weed Science, Volume 26, Issue ,3, pp.246-253	1 – 4	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2014-08-12

Elaboró: Mayra Alejandra Quintero
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Control of Early Watergrass (*Echinochloa oryzoides*) and Late Watergrass (*Echinochloa phyllopogon*) with Cyhalofop, Clefoxydim, and Penoxsulam Applied Alone and in Mixture with Broadleaf Herbicides¹

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Abstract: Experiments were conducted to study the effect of application rate, growth stage, and tank-mixing azimsulfuron or bentazon on the activity of cyhalofop, clefoxydim, and penoxsulam against two morphologically distinct *Echinochloa* species from rice fields in Greece. Mixtures of penoxsulam with MCPA were also evaluated. Cyhalofop (300 to 600 g ai/ha) applied at the three- to four-leaf growth stage provided 62 to 85% control of early watergrass but 41 to 83% control of late watergrass averaged over mixture treatments. Control ranged from 37 to 80% for early watergrass and from 35 to 78% for late watergrass when cyhalofop was applied at the five- to six-leaf growth stage averaged over mixture treatments. Mixtures of cyhalofop with azimsulfuron or bentazon reduced efficacy on both species irrespective of growth stage or cyhalofop application rate compared with cyhalofop alone. Clefoxydim (100 to 250 g ai/ha) applied alone at the three- to four-leaf growth stage provided 98 to 100% control of early watergrass and 91 to 100% control of late watergrass; when clefoxydim was applied alone at the five- to six-leaf growth stage the control obtained was 91 to 100% for early watergrass and 79 to 100% for late watergrass. Mixtures of clefoxydim with azimsulfuron or bentazon reduced efficacy on late watergrass at the early growth stage and on both species at the late growth stage. Penoxsulam (20 to 40 g ai/ha) applied alone provided 94 to 100% control of both species at both growth stages. Mixtures of MCPA with penoxsulam reduced efficacy on late watergrass at the early growth stage and on both species at the late growth stage. Mixtures of penoxsulam with azimsulfuron or bentazon reduced efficacy only on late watergrass at the late growth stage.

Nomenclature: Azimsulfuron; bentazon; clefoxydim (BAS 625H); cyhalofop; penoxsulam (DE-638); early watergrass, *Echinochloa oryzoides* (Ard.) Fritsch. #³ ECHOR; late watergrass, *Echinochloa phyllopogon* (Stapf.) Koss. # ECHPH.

Additional index words: Antagonism, herbicide interactions, mixtures.

Abbreviations: DAT, days after treatment.

INTRODUCTION

Barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] is one of the most common and troublesome grass weeds in warm-season crops in many parts of the world (Holm et al. 1977). It is a major weed of rice (*Oryza sativa* L.) and considered one of the most competitive weeds in most rice-growing areas. In

particular, season-long competition from barnyardgrass has been reported to reduce rice yield by 28 to 65% (Smith 1988) and by 21 to 40% (Stauber et al. 1991), depending largely on rice cultivar as well as density and spatial distribution of barnyardgrass population.

Barnyardgrass control in rice is often inconsistent because of the high polymorphism of the species, which includes several subspecies and varieties (Asins et al. 1999; Barrett and Wilson 1981; Danquah et al. 2002; Norris 1996), and the development of resistant biotypes with varying levels of resistance to certain rice herbicides (Baltazar and Smith 1994; Carey et al. 1995; Fischer et al. 2000; Giannopolitis and Vassiliou 1989; Lopez-Martinez et al. 1997; Marambe and Amarasinghe 2002; Vasilakoglou et al. 2000).

¹ Received for publication September 28, 2005, and in revised form April 3, 2006.

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³ Letters following this symbol are a WSSA-approved computer code from *Composite List of Weeds*, Revised 1989. Available only on computer disk from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897.

Cyhalofop and clefoxydim (BAS 625H), members of the aryloxyphenoxypropionate and cyclohexanedione herbicide groups, respectively, provide post-emergence control of barnyardgrass and other grasses in rice (Guice et al. 2000; Mann et al. 2000). These herbicides control weeds by inhibiting acetyl co-enzyme-A carboxylase (ACCase; E.C. 6.4.1.2), a key enzyme responsible for the biosynthesis of fatty acids in selected grass species (Burton et al. 1989). In general, ACCase from most grasses is sensitive to aryloxyphenoxypropionate and cyclohexanedione herbicides, whereas ACCase from broadleaf species is tolerant (Burton et al. 1989).

Experimental data on cyhalofop efficacy against barnyardgrass are relatively limited, whereas data on its efficacy against other *Echinochloa* species commonly found in rice fields, such as early watergrass or late watergrass, do not exist in the literature. Singh et al. (1996) reported increased barnyardgrass control with increasing dosages of cyhalofop, early application, and addition of adjuvants. Similar results have been reported by Sangwan et al. (1996) referring, however, to the species junglerice [*Echinochloa colona* (L.) Link]. Ntanos et al. (2000) observed better control of barnyardgrass with early application of cyhalofop or application in drained plots compared with late application or application in flooded plots.

Experimental data on clefoxydim efficacy are limited. Greenhouse research showed that clefoxydim applied at 30 g ai/ha was effective against barnyardgrass and broadleaf signal grass [*Brachiaria platyphylla* (Griseb.) Nash.], whereas the addition of bentazon or chlorimuron had an antagonistic effect on clefoxydim efficacy against the aforementioned species (Brommer et al. 2000). Furthermore, the addition of ethanol eliminated all antagonism with any of the herbicides used with either weed species.

Cyhalofop and clefoxydim do not control broadleaf weeds (Balyan et al. 1996). Thus, a mixture of cyhalofop or clefoxydim with other herbicides, such as azimsulfuron and bentazon, registered for effective control of broadleaf weeds and sedges in rice, could be a useful option. However, interactions between companion herbicides often affect herbicide activity in mixtures and may reduce weed control.

Penoxsulam (DE-638), a new triazolopyrimidine sulfonamide herbicide, is being developed for the control of annual grasses, sedges, and broadleaf weeds in rice culture (Jabusch and Tjeerdema 2005). It is applied postemergence and inhibits acetolactate

synthase (ALS; E.C. 2.2.1.6), a basic enzyme for branched-chain amino acid biosynthesis, in a similar mode like imidazolinone and sulfonylurea herbicides. Penoxsulam applied postemergence appears to provide excellent barnyardgrass control and poor to moderate broadleaf weed control (Ottis et al. 2003), which means that a companion herbicide is needed to broaden its efficacy against weeds found in rice fields.

The objective of this research was to study the effect of application rate, growth stage, and the addition of azimsulfuron or bentazon on the activity of cyhalofop, clefoxydim, and penoxsulam against two morphologically distinct *Echinochloa* species from rice fields in Greece. Mixtures of penoxsulam with MCPA were also evaluated.

MATERIALS AND METHODS

Seeds of *Echinochloa* species were collected by hand during September 2002 from mature plants grown in rice fields in Thessaloniki, northern Greece. Two morphologically distinct species (found exclusively in rice fields) were selected: early watergrass (having a typically drooping, closed, dense inflorescence, seeds with long awn, and showing early flowering) and late watergrass (having an erect, closed, dense inflorescence, awnless seeds, and showing late flowering). It must be noted here that there are serious taxonomic problems for species of the genus *Echinochloa* because of the existence of numerous intergrading polymorphic complexes, which are difficult to classify. Thus, the taxonomy of these *Echinochloa* species is controversial and different authors come up with different opinions. Identification keys and nomenclature for the species tested in this study are based on the classification proposed by Carretero (1981), which accurately describes the examined species. Seeds were dried in the greenhouse, air-cleaned to remove nonviable seeds and waste materials, and stored in plastic bags at 5 to 6 C until the initiation of the experiments.

Seeds were planted in late May in 2-L plastic pots filled with a soil mixture (soil and sand 2:1 by volume). The physicochemical characteristics of the soil used in the experiments were clay 32%, silt 56%, sand 12%, organic matter 1.6%, CaCO₃ 7.4%, pH (1:1 H₂O) 7.6, and CEC 27.7 meq/100 g. Pots were placed outdoors and watered once daily throughout the experiments by irrigating to soil saturation. One week after seedling emergence, plants were thinned to

30 per pot, where necessary, to obtain a uniform plant population in all pots. Plants grew normally throughout the experiments without experiencing any particular environmental stress conditions.

Herbicide treatments were applied when plants of each species were at the three- to four- or five- to six-leaf growth stage. Cyhalofop was applied at 300, 450, or 600 g ai/ha alone or in mixture with azimsulfuron at 20 g ai/ha or bentazon at 960 g ai/ha. A nonionic surfactant⁴ at 0.2% (v/v) was added to all cyhalofop treatments and a nontreated control was included for comparison. Clefoxydim was applied at 100, 150, 200, or 250 g ai/ha alone or in mixture with azimsulfuron at 20 g/ha or bentazon applied at 960 g/ha. Crop oil concentrate⁵ at 0.25% (v/v) was added to all clefoxydim treatments and a nontreated control was included for comparison. Penoxsulam was applied at 20, 30, or 40 g ai/ha alone or in mixture with azimsulfuron at 20 g/ha, bentazon at 960 g/ha, or MCPA at 800 g ai/ha. A nontreated control was included for comparison.

There were four replications (pots) for each treatment in a completely randomized design. Herbicide treatments were applied with a propane-pressurized hand-held field plot sprayer at 250 kPa pressure using 300 L/ha of water. The study was repeated in time following exactly the same procedure. Environmental conditions during application were similar in both study periods.

Species control was evaluated by counting stems at 15, 30, and 45 d after herbicide treatments (DAT) and by determining fresh weight of all stems remaining at 45 DAT. Stem number and fresh weight data were expressed as a percentage reduction from the nontreated control. Furthermore, stem number data were square root-transformed and fresh weight data were log-transformed before the analysis to stabilize variance. Transformation did not affect data interpretation; thus original means are presented. The ANOVA for all data was conducted separately for each species using a $2 \times 3 \times 3$ factorial approach for cyhalofop (two growth stages by three cyhalofop rates by three cyhalofop mixture treatments), a $2 \times 4 \times 3$ factorial approach for clefoxydim (two growth stages by four clefoxydim rates by three clefoxydim mixture treatments), and a $2 \times 3 \times 4$ factorial approach for penoxsulam (two growth stages by three penoxsulam rates by four penoxsulam mixture treatments). The

Table 1. Cyhalofop efficacy (% reduction from nontreated control) on early watergrass (ECHOR) and late watergrass (ECPH) as affected by growth stage at application and application rate.

Treatments ^c	Rate	Stem number reduction ^{a,b}	
		ECHOR	ECHPH
		%	
Three- to four-leaf	g ai/ha		
Cyhalofop	300	62 d	41 e
Cyhalofop	450	71 c	68 c
Cyhalofop	600	85 a	83 a
Five- to six-leaf			
Cyhalofop	300	37 f	35 f
Cyhalofop	450	58 e	54 d
Cyhalofop	600	80 b	78 b

^a Means are pooled over two experiments and mixture treatments including bentazon at 960 g ai/ha and azimsulfuron at 20 g ai/ha.

^b Different letters within each column indicate statistically significant differences according to Fisher's protected LSD test at 5% level of significance.

^c Nonionic surfactant at 0.2% v/v added to all treatments.

statistical analysis indicated no significant three-factor interaction for all variables of cyhalofop, but significant main effect of growth stage, application rate, and mixture treatment, as well as growth stage by rate interaction for stem number reduction. Therefore, the main effect means of mixture treatment and the growth stage by rate interaction means are presented for stem number reduction, whereas the main effect means of all factors are presented for fresh weight. However, there was a significant three-factor interaction for all variables of clefoxydim and penoxsulam. Therefore, the three-factor interaction means are presented for clefoxydim and penoxsulam. Differences between treatment means were compared at 5% level of significance using Fisher's protected LSD test. No experiment interaction occurred for any parameters; therefore, stem count and fresh weight reduction means were averaged over time.

RESULTS AND DISCUSSION

Cyhalofop. Cyhalofop applied at 300 g/ha at the three- to four-leaf growth stage provided 62% control (percentage stem reduction from the nontreated control) of early watergrass but 41% control of late watergrass averaged over mixture treatments (Table 1). The same rate applied at the later growth stage resulted in only 37 and 35% control of each respective species. Control increased for both species at both growth stages with increasing application rate. Greatest control of early watergrass (85%) and late watergrass (83%) was observed with the highest rate

⁴ Extravon® 25EC, Syngenta Hellas, 153 49 Anthoussa Attiki, Greece.

⁵ Dash® HC, BASF Agro Hellas, 151 25 Maroussi Attiki, Greece.

Table 2. Cyhalofop efficacy (% reduction from nontreated control) on early watergrass (ECHOR) and late watergrass (ECHPH) as affected growth stage at application, application rate, and tank mixture partner herbicide.

Treatments ^c	Stem number reduction ^{a,b}		Fresh wt reduction ^{a,b}	
	ECHOR	ECHPH	ECHOR	ECHPH
	%			
Growth stage				
Three- to four-leaf	N/A	N/A	65a	60a
Five- to six-leaf	N/A	N/A	61b	57b
Cyhalofop rate				
300 g ai/ha	N/A	N/A	50c	43c
450 g ai/ha	N/A	N/A	61b	55b
600 g ai/ha	N/A	N/A	79a	78a
Mixture treatment ^d				
Cyhalofop alone	82 a	76 a	81a	76a
Cyhalofop + bentazon	52 c	46 c	49c	46c
Cyhalofop + azimsulfuron	63 b	58 b	60b	55b

^a Means are pooled over two experiments.

^b Different letters within each factor of each column indicate statistically significant differences according to Fisher's protected LSD test at 5% level of significance.

^c Nonionic surfactant at 0.2% v/v added to all treatments.

^d Bentazon applied at 960 g ai/ha and azimsulfuron applied at 20 g ai/ha.

applied at the early growth stage. With each applied rate of cyhalofop, control of both species was greater with application at the earlier growth stage. Similar trends were observed for cyhalofop efficacy with respect to fresh weight reduction with application at the earlier growth stage and highest rate resulting in greatest control of both species (Table 2).

The increased control of *Echinochloa* species with increasing cyhalofop rates is in agreement with results reported by other researchers (Ntanos et al. 2000; Sangwan et al. 1996; Singh et al. 1996). The differences in control related to cyhalofop application rate indicate that high rates of this herbicide are required for adequate and consistent control of *Echinochloa* species in rice fields and particularly for the effective control of species with different susceptibility.

The reduced efficacy of cyhalofop with increasing growth stage of early watergrass and late watergrass indicates the necessity for early application of this herbicide for better control of *Echinochloa* species. These findings are in accordance with those reported in previous studies (Ntanos et al. 2000; Sangwan et al. 1996; Singh et al. 1996), where maximum control of barnyardgrass was achieved when cyhalofop was applied early, preferably at the two-leaf growth stage.

Inconsistent control of barnyardgrass in rice fields has been reported repeatedly by rice producers in

northern Greece (I. G. Eleftherohorinos, unpublished data). This reduced control was not due to failure in herbicide application or to herbicide resistance development, but in most cases it was due to the presence of multiple *Echinochloa* species or biotypes in the same field. Differences in herbicide efficacy against *Echinochloa* species could be related to genetic differences between species (Asíns et al. 1999; Danquah et al. 2002; Lopez-Martinez et al. 1999), which possibly imply different levels of species tolerance to certain herbicides. In particular, variability in tolerance to herbicides could be partially associated with growth rate differences between *Echinochloa* species, which may result in herbicide metabolism rate differences. Variability in herbicide efficacy has been observed also with propanil, molinate, and quinclorac against distinct *Echinochloa* species or biotypes from rice fields in Spain (Carretero 1989; Carretero et al. 1997; Lopez-Martinez et al. 1999).

The addition of bentazon or azimsulfuron in mixture with cyhalofop resulted in an 18 to 30 percentage point reduction, respectively, in control of both species, irrespective of growth stage or cyhalofop application rate, in comparison with cyhalofop applied alone (Table 2). Similar trends in cyhalofop efficacy in mixtures were also observed with stem fresh weight reduction. The reduced control with mixtures could be attributed to herbicide antagonism, which was evident irrespective of application timing for both weed species. Antagonism of cyhalofop activity by other rice herbicides when applied as a mixture has been reported on barnyardgrass (Ottis et al. 2005).

Clefoxydim. All rates of clefoxydim applied alone provided 98 to 100% control (percentage stem reduction from the nontreated control) of early watergrass at the early growth stage (Table 3). At the later growth stage, a clefoxydim rate of at least 200 g/ha was needed to provide similar control. The addition of bentazon or azimsulfuron did not have any significant effect on clefoxydim efficacy applied at the early growth stage of early watergrass. However, these mixtures applied at the late growth stage resulted in a 2 to 14 and 7 to 24 percentage point reduction, respectively, in control of early watergrass compared with clefoxydim applied alone. Similar trends were reflected in clefoxydim efficacy with respect to stem fresh weight reduction (Table 3).

Table 3. Clefoxydim efficacy (% reduction from nontreated control) applied at two growth stages of early watergrass (ECHOR) and late watergrass (ECHPH) as affected by application rate and tank mixture partner herbicide.

Treatments ^c	Rate g ai/ha	Stem number reduction ^{a,b}		Fresh wt reduction ^{a,b}	
		ECHOR	ECHPH	ECHOR	ECHPH
		%			
Three- to four-leaf					
Clefoxydim	100	98 ab	91 bc	98 b	89 d
(+) bentazon	(+) 960	97 b	73 f	98 b	76 h
(+) azimsulfuron	(+) 20	97 b	52 j	97 b	52 l
Clefoxydim	150	100 a	93 b	100 a	93 c
(+) bentazon	(+) 960	100 a	82 e	100 a	82 fg
(+) azimsulfuron	(+) 20	100 a	66 g	100 a	64 j
Clefoxydim	200	100 a	100 a	100 a	100 a
(+) bentazon	(+) 960	100 a	100 a	100 a	100 a
(+) azimsulfuron	(+) 20	100 a	86 d	100 a	84 f
Clefoxydim	250	100 a	100 a	100 a	100 a
(+) bentazon	(+) 960	100 a	100 a	100 a	100 a
(+) azimsulfuron	(+) 20	100 a	98 a	100 a	97 b
Five- to six-leaf					
Clefoxydim	100	91 d	79 e	91 e	81 g
(+) bentazon	(+) 960	77 g	57 i	81 h	55 k
(+) azimsulfuron	(+) 20	67 h	38 k	69 j	41 m
Clefoxydim	150	94 c	89 cd	95 c	87 e
(+) bentazon	(+) 960	84 e	70 f	85 f	73 i
(+) azimsulfuron	(+) 20	76 g	60 h	77 i	62 j
Clefoxydim	200	100 a	100 a	100 a	100 a
(+) bentazon	(+) 960	90 d	89 c	91 e	91 cd
(+) azimsulfuron	(+) 20	81 f	79 e	83 g	80 g
Clefoxydim	250	100 a	100 a	100 a	100 a
(+) bentazon	(+) 960	98 b	98 a	97 b	97 b
(+) azimsulfuron	(+) 20	93 c	88 cd	93 d	89 de

^a Means are pooled over two experiments.

^b Different letters within each column indicate statistically significant differences according to Fisher's protected LSD test at 5% level of significance.

^c Crop oil concentrate at 0.25% v/v added to all treatments.

Clefoxydim at 100 or 150 g/ha applied alone at the early growth stage provided 91 or 93% control of late watergrass but 79 or 89% control of the same species when applied at the late growth stage (Table 3). Increase of clefoxydim rate above 150 g/ha increased control of late watergrass to 100% at both growth stages. Mixtures of clefoxydim at 100 or 150 g/ha with bentazon or azimsulfuron resulted in considerably lower control of late watergrass after application at both growth stages. At rates greater than 150 g/ha, clefoxydim activity was reduced at the early growth stage at the 200 g/ha rate with addition of azimsulfuron (86 vs. 100%) and at the later growth stage with all combinations except clefoxydim at 250 g/ha applied with bentazon. Similar trends in clefoxydim efficacy were generally observed with stem fresh weight reductions. Reduced efficacy of clefoxydim at 30 g/ha after its simultaneous application with bentazon or chlorimuron has been reported previously on

barnyardgrass and broadleaf signal grass (Brommer et al. 2000).

Penoxsulam. All rates of penoxsulam applied either alone or in mixture controlled (percentage stem reduction from the nontreated control) both species 100% when treated at the three- to four-leaf stage with the exception of penoxsulam plus MCPA with 89 to 99% control of late watergrass (Table 4). Similarly, all rates of penoxsulam applied either alone or in a mixture controlled early watergrass 99 to 100% when treated at the five- to six-leaf stage with the exception of the two lower rates of penoxsulam plus MCPA, which provided 61 and 88% control, respectively (Table 4). In addition, penoxsulam applied alone provided 94 to 100% control of late watergrass at the five- to six-leaf stage. However, mixtures of penoxsulam at 20 or 30 g/ha with bentazon, azimsulfuron (except in combination with penoxsulam at 30 g/ha), or MCPA resulted in reduced control of late

Table 4. Penoxsulam efficacy (% reduction from nontreated control) applied at two growth stages of early watergrass (ECHOR) and late watergrass (ECHPH) as affected by application rate and tank mixture partner herbicide.

Treatments	Rate	Stem number reduction ^{a,b}		Fresh wt reduction ^{a,b}	
		ECHOR	ECHPH	ECHOR	ECHPH
g ai/ha					
%					
Three- to four-leaf					
Penoxsulam	20	100 a	100 a	100 a	100 a
(+) bentazon	(+) 960	100 a	100 a	100 a	100 a
(+) azimsulfuron	(+) 20	100 a	100 a	100 a	100 a
(+) MCPA	(+) 800	100 a	89 e	100 a	88 e
Penoxsulam	30	100 a	100 a	100 a	100 a
(+) bentazon	(+) 960	100 a	100 a	100 a	100 a
(+) azimsulfuron	(+) 20	100 a	100 a	100 a	100 a
(+) MCPA	(+) 800	100 a	94 d	100 a	92 d
Penoxsulam	40	100 a	100 a	100 a	100 a
(+) bentazon	(+) 960	100 a	100 a	100 a	100 a
(+) azimsulfuron	(+) 20	100 a	100 a	100 a	100 a
(+) MCPA	(+) 800	100 a	99 ab	100 a	99 ab
Five- to six-leaf					
Penoxsulam	20	100 a	94 cd	100 a	92 d
(+) bentazon	(+) 960	100 a	63 h	100 a	59 h
(+) azimsulfuron	(+) 20	100 a	76 f	100 a	75 f
(+) MCPA	(+) 800	61 d	51 i	60 d	48 i
Penoxsulam	30	100 a	98 ab	100 a	98 abc
(+) bentazon	(+) 960	100 a	89 e	100 a	88 e
(+) azimsulfuron	(+) 20	100 a	97 bc	100 a	96 c
(+) MCPA	(+) 800	88 c	70 g	86 c	62 g
Penoxsulam	40	100 a	100 a	100 a	100 a
(+) bentazon	(+) 960	100 a	100 a	100 a	100 a
(+) azimsulfuron	(+) 20	100 a	100 a	100 a	100 a
(+) MCPA	(+) 800	99 b	96 bcd	99 b	97 bc

^a Means are pooled over two experiments.

^b Different letters within each column indicate statistically significant differences according to Fisher's protected LSD test at 5% level of significance.

watergrass compared with a single application of penoxsulam. Similar trends in penoxsulam efficacy against the two species were also observed with stem fresh weight reductions (Table 4). The reduced control observed with penoxsulam mixtures compared with penoxsulam alone could be attributed to herbicide antagonism. Excellent control of barnyardgrass with penoxsulam applied at the three- to four-leaf growth stage has been previously reported by Ottis et al. (2003).

The results of this study indicated that cyhalofop at 600 g/ha, applied at the early growth stage evaluated, may be an option for effective postemergence control of certain *Echinochloa* species in rice. However, reduced activity may occur when cyhalofop is applied in mixture with azimsulfuron or bentazon. On the other hand, clefoxydim appears promising for effective postemergence control of troublesome *Echinochloa* species. However, its simultaneous application with azimsulfuron or bentazon at late growth stage of *Echinochloa* species may reduce control. Similarly, penoxsulam may be utilized for effective postemer-

gence control of diverse *Echinochloa* species even in late applications. However, although penoxsulam showed good compatibility with azimsulfuron or bentazon at the early growth stage of the *Echinochloa* species tested, its mixtures with these herbicides may reduce control of late watergrass in late applications. Similarly, mixtures of penoxsulam with MCPA were less effective than penoxsulam alone and these mixtures may be a problem for effective control of *Echinochloa* species, particularly in late applications.

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