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1	The effects of water management, timing and the rate of several
2	herbicides on the growth of <i>Murdannia keisak</i> (Hassk.) Handel-Mazz
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10	Abstract
11	Murdannia keisak is a competitive rice weed found throughout the world. The aims of this
12	study were to investigate the influence of different water managements on the growth of M.
13	keisak under greenhouse conditions, and to assess the efficacy of common rice herbicides
14	against it under field conditions.
15	Three water management techniques were tested under greenhouse conditions:
16	saturation, intermittent irrigation, and continuous flooding. The efficacy against M. keisak
17	was evaluated in paddy fields during 2003-2005. In 2003, bispyribac-sodium was applied
18	alone or in combination with triclopyr or metosulam at 20, 27, and 32 days after seeding
19	(DAS). In 2004 and 2005, bispyribac-sodium was applied once (30 DAS), but at two
20	application rates.
21	Results for the three water treatments showed continuous flooding reduced M. keisak
22	biomass most as confirmed by aboveground fresh weights. In the paddy field experiments,
23	bispyribac-sodium also proved highly effective against <i>M. keisak</i> and was selective to rice
24	with more than 90% efficacy. The addition of tryclopyr or metosulam to bispyribac-sodium

failed to improve its herbicidal efficacy. Finally, the best *M. keisak* control at field condition
was obtained with continuous flooding combined with bispyribac-sodium.

27

28 Key words

29 Rice, bispyribac-sodium, marsh dayflower, water management.

30

31 **1. Introduction**

32 Murdannia genus belongs to the spiderwort (Commelinaceae) family and includes several 33 weed species of particular concern in rice fields. Murdannia species are native to eastern 34 Asia where they grow in both cultivated and natural areas (Baki, 1997) while *Murdannia* nudiflora (L.) Brenan (Bastidas López, 1996; Plaza, 19983; Rojas, 2002; Wilson, 1981) is a 35 36 common rice weed in Latin America. Murdannia keisak (Hassk.) Handel-Mazz., also 37 known as Aneilema keisak or by the common name Marsh dayflower, has been reported as a weed in South Carolina cultivated rice paddies (Swearingen, 2002), and has become 38 39 established and naturalised in other cultivated or natural areas (Dunn, 1990) of the USA. 40 In Italy *M. keisak* was first found in 1974 (Pignatti, 1982) in spontaneous vegetation; 41 significant rice field infestations have only been reported since the late '90s (Berti, 2006; 42 Tesio, 2004). 43 The ability of this plant to out-compete native vegetation is mainly caused by its fast 44 growth and its development of a dense canopy mat (King, 2000; Rundell, 1999). According 45 to Dunn and Sharitz (1991), the flexibility of *M. keisak* allows it to adapt readily to new

46 environments. *M. keisak* is considered invasive in marshes, swamps, ditches, creek and

- 47 riverbanks, and around ponds and lakes as it rapidly crowds out desired native
- 48 herbaceous vegetation by forming dense floating pads. Even in its native areas, this

species is a difficult paddy field weed as it grows rapidly in shade rather than in full sun(Dunn, 1990).

51 M. keisak presents alternating linear or lance-shaped leaves of about 6 cm long and 1 cm wide. Each leaf base has a closed tubular and hairy sheath. The stems are succulent, root 52 53 at the nodes and grow prostrate. Flowering generally occurs in September-October. The 54 flowers, solitary or in groups of 2-4 axillary racemes, usually develop at the stem ends or 55 from the leaf axil; they show 3 green sepals, ovate to oblong, and 3 pink petals that are a little longer than the sepals. The stamens are 6 (only 2 or 3 fertile) and the ovary is 56 57 superior. The fruits are 6-7 mm long smooth capsules, ellipsoid to oval in shape, pointed at 58 the tip, and contain about 48 grey and flat seeds (Pignatti, 1982). Reproduction occurs by 59 fragmentation and seed production. Temperatures of less than 8 °C, together with seed 60 burial depth and submersion, depress seedling emergence (Sago, 1996). 61 Even though *M. keisak* is of concern in several regions of the world, its eco-biology, 62 agronomic management, and herbicide sensitivity remain sketchy. Useful information on 63 the effects of different rice cropping and water management practices on its emergence, 64 growth, competitiveness, seed production and dispersal is absent in the literature. Some 65 reports indicate that species of the genus Murdannia are successfully controlled by several 66 herbicides: bispyribac-sodium (JinHao, 2000; HuiLi, 2002), bentazone, as well as mixtures

67 containing bentazone (Kitano, 1999), cloroacetamides (mefenacet, pretilachlor), molinate,

68 MCPA (Sago, 1996) and 2,4-D (Wilson, 1981). However, the effects of water management

69 on *M. keisak* must be better understood as they are fundamental to defining an integrated

strategy for control of a species so highly related to its aquatic environment.

The aims of this study were: (1) to evaluate the effects on *M. keisak* of different water management conditions in the greenhouse, and (2) to investigate the sensitivity of the

73 species to widely used rice herbicides under field conditions.

74 **2. Materials and methods**

The greenhouse experiments were performed during 2002 at the faculty of Agriculture of the University of Torino (Grugliasco, Italy). Field experiments were conducted during the 2003, 2004, and 2005 growing seasons in the Vercelli area (NW Italy), one of the most important areas for rice cultivation in Italy.

79 **2.1. Greenhouse experiments**

During the 2001 growing season, seeds of *Murdannia keisak* were collected from plants grown in naturally infested paddy fields of Northern Italy and multiplied in the greenhouse. Seeds harvested from these greenhouse-developed plants were air dried in open trays at room temperature for 30 days, and then cooled in deionized water at 4 °C for a minimum of two months.

The greenhouse was equipped with a shade tissue positioned outside of the structure to avoid excessive temperatures and to result in 47% light reduction. Natural light filtered through the shade tissue and was supplemented with metal-halide lamps, to obtain a total radiation of about 55 µmol s⁻¹ m⁻². The average maximum, minimum, and mean temperature values experienced were 30.3 °C, 19.3 °C, and 24.3 °C while the average maximum, minimum, and mean relative humidity values were 85.2%, 35.8%, and 62.3%, respectively.

Seeds of *M. keisak* and rice (*Oryza sativa* L.) cv. Selenio (medium grain *japonica*-type variety) were placed in germination trays and maintained at 25 °C until the root tip of both species had erupted and extended from the seed coat 2-3 mm. Germinated seedlings were transplanted into 100 cm² pots (9 seeds per pot) filled with water-saturated commercial potting media. After seeding, a 3x2 factorial design was applied to both *M. keisak* and rice. The first factor, represented by water management, had three levels: saturation (SA), intermittent irrigation (II), and continuous flooding (CF). SA treatment was performed by placing the pots in trays with a 5 cm water layer and letting the soil be saturated through the holes in the base of the pot. For treatment II, irrigation took place only when the rice began to show signs of drought stress (beginning of leaf folding) and was conducted by immerging the pots until soil saturation. In treatment CF, the soil was continuously flooded through maintenance of a 10 cm water layer above the pot.

The second factor, watering timing, referred to when the differing water management strategies (SA, II, and CF) occurred. Two different timeframes were used — immediately after seeding ("Early"), or ten days thereafter ("Late"). To avoid plant damage during the first ten days of treatment combination CF X Late, pots were maintained in trays containing a minimal water layer.

All the treatments were arranged according to a randomized complete block design structure with six replicates; each pot served as an experimental unit. Pots of both species were fertilized at about two weeks after seeding by replacing the water in the trays with solution containing 0.25 g L⁻¹ of N-P-K fertilizer (10-16-25). At approximately 50 days after seeding, the aboveground fresh weight of each pot of *M. keisak* and rice was measured. A two-factor analysis of variance was conducted on the fresh weight data in which *M. keisak* and rice responses were treated separately.

117

118 2.2. Field experiments

Field experiments were conducted during 2003-2005 in a paddy field highly infested by *M*. *keisak*. The paddy field was seeded with 180 kg ha⁻¹ of the cultivar Loto (long grain *japonica*-type variety) at the end of May. Control of *Heteranthera* spp., another weed in the
field, was achieved by applying oxadiazon at 270 g a.i. ha⁻¹. The field was drained at least

123 two days prior to oxadiazon treatment, submerged two days after being sprayed, and then 124 kept flooded for the duration of the study. Continuous submersion was the adopted water 125 management in the field experiments because this solution resulted to be the most 126 effective for the control of *M. keisak* in the greenhouse experiments.

127 The field experiments included herbicides which in a previous work proved to be effective 128 against *M. keisak* under greenhouse conditions (Tesio et al., 2004).

129 The 2003 trial included four treatment combinations (Table 1): 1) bispyribac-sodium

130 combined with the anionic adjuvant Biopower (sodium salt of alchilether sulfate, Bayer

131 CropScience), 2) bispyribac-sodium combined with triclopyr or 3) metosulam and

132 Biopower, and 4) a mixture of propanil combined with triclopyr and Trend (ethoxylated

133 tridecyl alcohol, DuPont). Bispyribac-sodium was chosen because it performed best in the

134 greenhouse experiment. The bispyribac-sodium-based treatments were applied at 20, 27,

or 32 days after seeding (DAS) to coincide with the rice at BBCH stage 12-13, 13-21, or

136 15-23, and *M. keisak* at stage 11-13, 12-15, or 15-18, respectively. We applied the mixture

137 of propanil, triclopyr, and Trend twice, at 27 and 32 DAS.

The 2004 and 2005 experiments included treatments based on bispyribac-sodium applied at the rate tested in the previous experiments and at a 20% reduced rate when combined with wetting agents Biopower and Dash (methyl palmitate 172.5 g l⁻¹). In both years, the herbicides were applied only once—at 30 DAS—when the rice was at BBCH stage 13-21 and *M. keisak* was at 12-15.

All field experiments were laid out in a completely randomized design with four replicates and elementary plots of 24.75 m². The efficacy of the various herbicidal combinations on *M. keisak,* as well as their selectivity to rice, were evaluated according to EPPO/OEPP n. 62 guidelines by scoring the results against a range from 0 (no efficacy against weed; no phytotoxicity to crop) to 100 (complete control of weed; crop destroyed). We conducted
efficacy assessments throughout the growing seasons: at 10, 20, 40, and 80 days after
treatment (DAT) in 2003, and at 7, 14, and 30 DAT in 2004 and 2005.

The analysis of variance was performed on the efficacy and phytotoxicity data with the means separated using the LSD post-hoc test (P < 0.05).

- 152
- 153

154 **3. Results**

155 **3.1. Greenhouse experiments**

156 Water management significantly affected the growth of *M. keisak* and rice plants. Pots

157 maintained in saturated conditions (SA) resulted in the greatest aboveground fresh

weights, both in *M. keisak* and rice plants. On the other hand, the lowest fresh weights

159 were recorded for *M. keisak* plants kept continuously flooded (CF) and for rice plants

160 intermittently irrigated (II). CF values for the weed and rice were reduced when compared

161 to their counterpart plants under SA treatment (Figure 1).

162 A significant interaction between water management and application timing was also found

163 for both the weed and the rice plants. In fact, late application of the various water

164 strategies always yielded higher aboveground fresh weights except in one instance: *M*.

165 *keisak* grown in saturated conditions (Figure 1).

166

167 3.2. Field experiments

168 In the 2003 experiment, bispyribac-sodium showed effectiveness against *M. keisak* and

169 sufficient selectivity to rice plants in all water treatments (Table 2). Furthermore, all

170 treatments that included bispyribac-sodium combined with the wetting agent Biopower

171 scored an efficacy greater than 97% against *M. keisak* in assessments carried out from 20 172 DAT. Among the timing options, slightly better results, for both efficacy and selectivity, 173 were obtained when bispyribac-sodium + Biopower were applied at 27 DAS. In the case of propanil mixed with triclopyr and Trend, even when doubled in strength, the efficacy score 174 175 failed to exceed 94 (Table 2). Regardless of application time, the addition of metosulam or tryclopir to bispyribac-sodium did not significantly improve the control of *M. keisak*. 176 177 During 2004 – 2005 all bispyribac-sodium treatments were highly effective against M. keisak, showing a consistently high efficacy (> 92%) at 7 DAT (Table 3), and complete 178 179 control of the weed at 14 DAT and 30 DAT. Bispyribac-sodium applied in combination with 180 the wetting agent Biopower was less phytotoxic to rice than when combined with Dash at 181 the 7 DAT and 14 DAT assessments.

182

183 **4. Discussion and conclusions**

184 This study investigated the effects of different water management strategies on *Murdannia* 185 keisak as well as its sensitivity to common rice herbicides. Results indicated that the most common water management for European rice, continuous flooding, significantly affected 186 187 *M. keisak* growth. Rice biomass was highest in saturated conditions while rice growth was 188 mostly limited by intermittent irrigation. Our results were consistent with other studies 189 (Juraimi, 2009), in which the highest values of rice plant biomass were recorded in 190 saturated soil. Only a slight reduction in biomass was observed in continuously flooded 191 conditions relative to saturated conditions.

At the field scale, continuous flooding of the fields is the most adopted water management strategy, as a water layer maintained at the soil surface results in reduced thermal oscillations (Ikehashi, 2007). The results of the high growth we observed in saturated soil might be explained by the favourable environmental conditions of the greenhouse, and in particular the limited variation temperature, that is quite unusual in rice field cultivation.

197 An increased use of intermittent irrigation or saturation instead of continuous flooding has 198 been suggested (Chapagain, 2009; Yang, 2004) to reduce water utilization, but both 199 practices can adversely affect rice growth and favour *M. keisak* development. Similar behaviour has been observed in Fimbristylis littoralis Gaud., Ludwigia hyssopifolia (G. 200 201 Don) Exell, and Cyperus iria L. Yet, other rice weed species, such as Monochoria 202 vaginalis (Burm.F.) Presl and Cyperus difformis L. have shown no effect from continuous 203 flooding (Chauhan and Johnson, 2009). Generally, the effect of water on weeds depends 204 on species, water layer depth, and time submersion begins (Pons, 1982;Chauhan, 2009). 205 In field experiments bispyribac-sodium and triclopyr controlled *M. keisak* at lower rates 206 than those recommended on the herbicide product labels.

207 The results of this study can lead to a strategy for successful control of this weed through 208 optimal herbicide choice, rate, application timing and water management. In particular, our 209 results showed that continuous flooding, coupled with half-strength bispyribac-sodium and 210 the adjuvant Biopower applied once between the 3-leaf stage and first tiller of crop growth, 211 controlled *M. keisak* without any remarkable negative effects to rice. Similarly, *Echinochloa* 212 crus-galli in paddy rice is already controlled with this herbicide treatment at the same 213 growth stage as that required for *M. keisak* control (Koger et al. 2007; Vidotto et al. 2007), 214 which makes this strategy efficient to control both weeds in a single application.

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- 216

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282	Water Manage. 70:67-81.							
283								
284	Figure captions							
285								
286	Figure 1. Aboveground fresh weight (g plant ⁻¹) of <i>M. keisak</i> and rice. Water management strategies							
287	not sharing the same lower-case letter are significantly different at $P < 0.05$ (LSD test). Regular and							
288	italic letters refer to <i>M. keisak</i> and rice, respectively. Error bars indicate SE (n=6).							
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293 294

Figure 2. Aboveground fresh weight (g plant⁻¹) of *M. keisak* and rice. Water management strategies not sharing the same lower-case letter are significantly different at P < 0.05 (LSD test). Regular and italic letters refer to *M. keisak* and rice, respectively. Error bars indicate SE (n=6).

299

300 301

302303 Table 1. Herbicides included in the field experiments, rates, and application timings.

Voor	Trootmont	Rates	Application timing	
i eai	neathent	(g a.i. ha⁻¹)	(DAS) ¹	
	bispyribac-sodium + Biopower	31 + 265	20; 27, 32	
	bispyribac-sodium + triclopyr + Biopower	31 + 750 + 265	20; 27, 32	
2003	bispyribac-sodium + metosulam + Biopower 31 + 428 ·		20; 27, 32	
	propanil + triclopyr + Trend (repeated twice)	4000 + 750 +	27 + 32	
		400	21 . 02	
	bispyribac-sodium + Biopower	25.8 + 265	30	
2004 and 2005	bispyribac-sodium + Biopower	31 + 265	30	
2001 4114 2000	bispyribac-sodium + Dash	25.8 + 800	30	
	bispyribac-sodium + Dash	31 + 800	30	

304 ¹ DAS= Days After Seeding

Table 2. Efficacy against *Murdannia keisak* and phytotoxicity on rice of herbicides applied
 in the 2003 field experiment. Letters refer to significance among treatments, according to
 the LSD test. Comparisons are made among values of a same column, thus within each
 assessment (DAT) for either efficacy or phytotoxicity.

	Efficacy ¹				Phytotoxicity ¹			
Treatment	10 DAT	20 DAT	40 DAT	80 DAT	10 DAT	20 DAT	40 DAT	80 DAT
bispyribac-sodium + Biopower	96.3ab	100.0a	99.5a	98.8ab	10.0bc	5.0abc	2.5bc	1.3b
(20 DAS)								
bispyribac-sodium + Biopower	97.3a	98.5a	99.0a	99.0ab	8.8bcd	3.8bcd	8.8a	1.3b
+ metosulam (20 DAS)								
bispyribac-sodium + Biopower	98.0a	99.5a	99.5a	99.5a	15.0a	8.8a	2.5bc	1.3b
+ triclopyr (20 DAS)								
bispyribac-sodium + Biopower	98.3a	100.0a	100.0a	100.0a	6.3cde	1.3cd	0.0c	0.0b
(27 DAS)								
bispyribac-sodium + Biopower	99.5a	100.0a	100.0a	100.0a	7.5bcde	1.3cd	1.3c	0.0b
+ metosulam (27 DAS)								
bispyribac-sodium + Biopower	98.3a	99.5a	99.5a	99.5a	8.8bcd	5.0abc	2.5bc	1.3b
+ triclopyr (27 DAS)								
bispyribac-sodium + Biopower	95.8ab	97.8ab	97.5a	98.8ab	3.8ef	1.3cd	0.0c	0.0b
(32 DAS)								
bispyribac-sodium + Biopower	97.5a	98.3a	100.0a	100.0a	6.3cde	2.5bcd	2.5bc	1.3b
+ metosulam (32 DAS)								
bispyribac-sodium + Biopower	97.0a	98.0ab	98.8a	98.8ab	5.0de	5.0abc	5.0b	5.0a
+ triclopyr (32 DAS)								
propanil + triclopyr + Biopower	87.5b	91.3b	93.8a	92.5b	7.5bcde	2.5bcd	2.5bc	2.5ab
(27 DAS and 32 DAS								

- 310¹: efficacy and phitotoxicity expressed as visual score. 0: no efficacy against the weed, no phytotoxicity
- 311 towards crop; 100: complete control of weed, crop destroyed.
- 312
- 313 **Table 3.** Efficacy against *Murdannia keisak* and phytotoxicity on rice of bispyribac-sodium
- 314 applied in 2004-2005 field experiments. Letters refer to significance among treatments,

315 according to the LSD test. Comparisons are made among values of a same column, thus

316 within each assessment (DAT) for either efficacy of phytotoxicity.

Treatment	Efficacy ¹			Phytotoxicity ¹		
	7 DAT	14 DAT	30 DAT	7 DAT	14 DAT	30 DAT
bispyribac-sodium 25.8 g a.i. ha ⁻¹ + Biopower	92.5a	100.0a	100.0a	0.5c	0.5c	0.0
bispyribac-sodium 31 g a.i. ha ⁻¹ + Biopower	93.8a	100.0a	100.0a	2.5b	2.5b	0.0
bispyribac-sodium 25.8 g a.i. ha ⁻¹ + Dash	92.5a	100.0a	100.0a	2.5b	2.5b	0.0
bispyribac-sodium 31 g a.i. ha ⁻¹ + Dash	95.0a	100.0a	100.0a	4.0a	4.0a	0.0

317 ¹: efficacy and phitotoxicity expressed as visual score. 0: no efficacy against the weed, no phytotoxicity

318 towards crop; 100: complete control of weed, crop destroyed.

319