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Target-Site ACCase-Resistant Johnsongrass (*Sorghum halepense*) Selected in Summer Dicot Crops

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Johnsongrass is a troublesome weed infesting spring–summer crops. Poor control of johnsongrass after fluazifop-p-butyl treatments has been reported in central to northern Italy. Greenhouse and outdoor dose–response experiments revealed that four populations were highly resistant to fluazifop-p-butyl. All four were cross-resistant to other aryloxyphenoxypropionate (FOP) herbicides—propaquizafop, quizalofop, and haloxyfop. The resistance indexes ranged between 8 and 25 for propaquizafop and quizalofop, whereas a greater variability between populations was detected in response to haloxyfop. Conversely, cycloxydim and clethodim determined only a shift in the susceptibility with resistance index (RI) values of 2 to 3. Molecular analyses revealed that resistant plants possessed an insensitive acetyl coenzyme-A carboxylase (ACCase) target enzyme due to an Ile-to-Asn substitution at codon 2041. To our knowledge, this is the first report of such a mutation endowing ACCase resistance in johnsongrass. A molecular marker (CAPS assay) was developed for its rapid detection. Alternative mode of action herbicides *S*-metolachlor and nicosulfuron controlled all the FOP-resistant populations. Only a few chemical options are still available, and they have different efficacy on germinating seeds and sprouting rhizomes. To maintain efficacy over time, herbicides should be integrated with agronomic practices.
Nomenclature: Fluazifop-p-butyl; propaquizafop; quizalofop; haloxyfop; cycloxydim; clethodim; *S*-metolachlor; nicosulfuron; johnsongrass, *Sorghum halepense* (L.) Pers.
Key words: Cross-resistance, resistance management, Asn2041Ile mutation, molecular marker.

Sorghum halepense es una maleza problemática que infesta cultivos de primavera y verano. En el centro y norte de Italia se ha reportado un control pobre de *S. halepense* después de tratamientos con fluazifop-p-butyl. Experimentos de respuesta a dosis en invernadero y al aire libre revelaron que cuatro poblaciones fueron altamente resistentes a fluazifop-p-butyl. Las cuatro poblaciones tuvieron resistencia cruzada a otros herbicidas aryloxyphenoxypropionate (FOP), tales como propaquizafop, quizalofop, y haloxyfop. Los índices de resistencia variaron entre 8 y 25 para propaquizafop y quizalofop, mientras que se detectó una mayor variabilidad entre poblaciones en respuesta a haloxyfop. En cambio, cycloxydim y clethodim determinó solamente una cambio menor en la susceptibilidad con valores del índice de resistencia (RI) de 2 a 3. Análisis moleculares revelaron que las plantas resistentes poseían una enzima acetyl coenzyme-A carboxylase (ACCase) insensible debido a una sustitución de Ile por Asn en el codón 2041. Con base en nuestro conocimiento, este es el primer reporte de esta mutación que confiere resistencia a ACCase en *S. halepense*. Un marcador molecular (ensayo CAPS) fue desarrollado para la detección rápida de la mutación. Herbicidas con modos de acción alternativos, como *S*-metolachlor y nicosulfuron controlaron todas las poblaciones resistentes a FOP. Solamente unos pocas opciones químicas están todavía disponibles, y estas tienen diferente eficacia sobre semillas germinadas y rizomas rebrotados. Para mantener la eficacia a lo largo del tiempo, estos herbicidas deberían ser integrados con prácticas agronómicas.

Johnsongrass is a weedy grass, commonly found in spring–summer crops, such as maize (*Zea mays* L.), soybean [*Glycine max* (L.) Merr.], and other dicots. It is also considered one of the worst weed pests worldwide, attesting to its tremendous vigor and adaptation (Holm et al. 1977). Johnsongrass is a geophyte, predominantly self-pollinating species that reproduces asexually, via extensive production of rhizomes that make it difficult to control, and also sexually by producing seeds (Holm et al. 1977). Plants that emerge from rhizomes (ramets) are more competitive and troublesome than those originating from seeds because of their earlier emergence and faster early growth rate (Bridges and Chandler 1987). Ramet competition may therefore cause higher crop yield losses (Mitskas et al. 2003).

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The most common way to control this troublesome weed is through herbicide application because nonchemical controls are only partially successful (McWhorter 1989). Many PRE herbicides are able to control johnsongrass seedlings, but they are less effective against ramets (McWhorter 1989). In contrast, POST herbicides introduced since 1980, mainly acetyl coenzyme-A carboxylase (ACCase) and acetolactate synthase (ALS) –inhibiting herbicides, have high efficacy on both seedlings and ramets. Sulfonylureas, ALS inhibitors, are widely used in maize, whereas aryloxyphenoxypropionates (FOPs) and cyclohexanediones (DIMs), two classes of ACCase inhibitors, are widely applied in soybean, cotton (*Gossypium hirsutum* L.), and other broadleaved crops (Kaloumenos and Eleftherohorinos 2009; Smeda et al. 2000).

The heavy reliance on ACCase inhibitors to control grass weeds in numerous cropping systems has led to the evolution of resistance in 42 grass weed species worldwide (Heap 2013), such as ryegrass (*Lolium* spp.), blackgrass (*Alopecurus myosuroides* Hudson), wild oat (*Avena* spp.), and green foxtail (*Setaria viridis* L. Beauv.). The first ACCase-resistant johnsongrass populations were detected in cotton and soybean fields in the United States (Mississippi) in 1991 (Smeda et al. 1997). These populations were resistant to FOP herbicides and cross-resistant to sethoxydim, but were controlled by the other DIM, clethodim. Since then, further ACCase-resistant cases have been reported in the United States (Bradley and Hagood 2001; Burke et al. 2006; Obermeier et al. 1997) and in Israel (Heap 2013). In Europe, only one resistant case has been reported in Greek cotton fields (Kaloumenos and Eleftherohorinos 2009), but no information was provided on the resistance mechanism. In northern Italy (central Po Plain), cropping systems based on dicot crops such as soybean, tomato (*Lycopersium esculentum* L.), and watermelon (*Citrullus lanatus* Thunb.), where grass control is exclusively or predominantly performed with the use of POST herbicides, are relatively common, and a few populations of johnsongrass poorly controlled by ACCase inhibitors have recently been reported by farmers.

Resistance to ACCase-inhibiting herbicides is related to two types of mechanisms: target-site–based resistance (TSR) and non-target–site based resistance that includes all mechanisms that act to minimize the amount of herbicide that reaches the target-site (Délye 2005; Powles and Yu 2010). Acetyl coenzyme-A carboxylase (ACCase, EC 6.4.1.2), a key enzyme in fatty acid biosynthesis (Harwood 1988), is the target site of the ACCase inhibitors. At least two ACCase isoforms are found in the cytosol and organelles, with the chloroplastic isoform accounting for more than 80% of total ACCase activity. The efficacy of ACCase-inhibiting herbicides, namely, FOPs, DIMs, and phenylpyraxoline (DEN), on Poaceae is based on their selective binding to the CT domain (one of the three catalytic domains involved in the carboxylation of acetyl-CoA) of the chloroplastic homomeric ACCase isoform, whereas other isoforms are insensitive and so are not affected (Nikolskaya et al. 1999; Zhang et al. 2004). Therefore, ACCase-inhibiting herbicides are selective graminicides with little or no activity on broadleaved species.

TSR is generally caused by point mutation(s) in the CT domain of the ACCase gene that decreases the affinity of this protein to herbicides. Different point mutations at codons 1781, 1999, 2027, 2041, 2078, 2088, and 2096 of the gene were identified in various grass species as being responsible for resistance to ACCase-inhibiting herbicides (Beckie and Tardif 2012; Powles and Yu 2010). Recently, a Trp to Cys change at codon 2027 of the ACCase gene was identified in four FOP-resistant johnsongrass populations (Kershner et al. 2012).

The aims of the study were (1) to test populations of johnsongrass collected in dicot crops in northern Italy for resistance to fluazifop and other ACCase inhibitors, (2) to assess the efficacy of other PRE and POST herbicides with different modes of action (MoA) on the same populations, (3) to collect field histories of agronomic practices, especially weed control treatments in the sampled fields, and (4) to investigate whether the resistance mechanism is related to an altered target enzyme and design a molecular marker for the quick detection of ACCase-resistant johnsongrass plants.

Materials and Methods

Seed Collection and Plant Material. Five seed samples of johnsongrass were collected in 2005, 2006, and 2007 in spring–summer dicot crops (soybean, watermelon/tomato rotation) located in different areas of the Lombardy region, central–

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northern Italy. Seeds (hereafter referred as 05-2, 05-3, 05-4, 05–6, 07–12) were collected following reports made to Syngenta Crop Protection regarding poor johnsongrass control. Each sample included seeds harvested from around 20 mature plants. In addition, johnsongrass seeds were collected in two different sites that had never been treated with herbicides for the last 10 yr and were used as susceptible reference (05-1 and 06–10). All seed samples were cleaned and dry stored at room temperature. Historical records of herbicide applications and other agronomic techniques used in the sampled fields were collected from the farmers.

Greenhouse Whole-Plant Bioassays. *PRE Treatment.* Two experiments were conducted to set up a protocol for the PRE treatments in a controlled environment. The first experiment was performed on two populations of johnsongrass, one reference (06–10) and one putative resistant (R) population (05-3), to evaluate the efficacy of different PRE application times of *S*-metolachlor.

Seeds were chemically scarified in concentrated sulfuric acid (97%) for 5 min, carefully rinsed with water, and placed on agar medium 0.6% (wt/v) and 0.2% (wt/v) KNO_3. Seeds were then subjected to a heat treatment (Harrington 1923) in a germination cabinet at the following conditions: 4 h at 45 C and 20 h at 24 C for 3 d, 12 h photoperiod with neon tubes providing a photosynthetic photon flux density (PPFD) of 15 to 30 μmol m^{-2} s^{-1}. The large diurnal fluctuations in temperature favor germination (Taylorson and McWorther 1969). Germinated seeds were then placed in plastic boxes (325 × 265 × 95 mm) containing soil and covered with a layer of soil. Water was applied both from above and by capillarity from the saucer beneath. This procedure favors retention of the herbicide at the proper depth for a good treatment efficacy. The experimental layout was a completely randomized design with two replicates containing 18 seeds each. Herbicide treatments were done at 1, 4, 7 and 10 d after sowing. For each population, a nontreated control was included. *S*-metolachlor (Dual Gold, 960 g ai L^{-1}, Syngenta Crop Protection, Milan, Italy) was applied at 1 L ha^{-1} with the use of a precision bench sprayer delivering 300 L ha^{-1}, at a pressure of 215 kPa, and a speed of 0.75 m s^{-1}, with a boom equipped with three flat-fan (extended range) hydraulic nozzles (TeeJet®, 11002). Three weeks after treatment (WAT), the number of seedlings in each box was counted and referred to the number of seedlings in the nontreated box. The visual estimate of biomass (VEB) was obtained through a visual comparison between treated and untreated boxes: a score ranging from 10 for plants not affected by the herbicide (compared with the nontreated control) to 0 when the plants were clearly dead was given to each treated box.

A second experiment was performed spraying *S*-metolachlor only 1 d after sowing on all populations.

POST Treatment. All five johnsongrass populations were tested for resistance to the FOP herbicide selecting agent fluazifop-p-butyl, for cross-resistance to the DIM herbicide cycloxydim and also for multi-resistance to the ALS inhibitor nicosulfuron. Two susceptible (S) reference populations were also tested (06–10 and 05-1).

Seeds were scarified and heat-treated as described above. Subsequently, the germinated seeds were maintained at 25/15 C (day/night) until the seedlings were transplanted into plastic trays (325 × 265 × 95 mm) containing a standard potting mix (60% silty loam soil, 15% sand, 15% perlite, and 10% peat), placed in the greenhouse where light was supplemented with the use of 400-W metal-halide lamps, which provided a PPFD of about 450 μmol m^{-2} s^{-1}, a 16-h photoperiod, and watered daily to maintain the substrate at or near field capacity. The experimental layout was a completely randomized design with two replicates (18 seedlings per replicate) for each herbicide and two doses per herbicide, recommended field dose in Italy (1 ×) and three times that (3 ×), with recommended surfactants. For each population, a nontreated control was included. The plants were sprayed when they reached the three- to four-leaf stage. Fluazifop-p-butyl (Fusilade Max, 125 g ai L^{-1}, Syngenta Crop Protection, Milan, Italy) was applied at 2 L ha^{-1}; cycloxydim (Stratos, 200 g ai L^{-1}, BASF Italia, Milan, Italy) was applied at 0.8 L ha^{-1} and nicosulfuron (Ghibli, 40 g ai L^{-1}, Syngenta Crop Protection, Milan, Italy) was applied at 1.2 L ha^{-1}. Plant survival and VEB in relation to the nontreated control were recorded three WAT for ACCase inhibitors and four WAT for nicosulfuron (see above). Plant survival was expressed as a percentage of surviving plants with respect to the number of treated plants. Plants were assessed as being dead if, regardless of color, they showed no

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Table 1. Herbicide details and recommended field dose (1 ×) used in dose–response experiments.

Common name	Trade name	Doses	Surfactant	Dose 1×	Manufacturer	Web site
		g ai L^{-1}		L ha^{-1}		
Fluazifop-p-butyl	Fusilade max	125		2	Syngenta Crop Protection	www.syngenta.com
Cycloxydim	Stratos	200	Mineral oil (1 L ha^{-1})	0.8	BASF Italia	www.basf.it
Clethodim	Select	240	Mineral oil 0.5%	0.6	Nufarm Italia	www.nufarm.com
Quizalofop-P-ethyl	Targa Flo	50		1.25	Bayer CropScience	www.bayercropscience.it
Propaquizafop	Agil	100		1	Dupont Crop Protection Italia	www.dupont.com
Haloxyfop-R-methyl	Gallant W30	30		3	Dow AgroSciences	www.dowagro.com

active growth. Standard error was calculated for each mean value. Populations were ascribed as being resistant when more than 20% of plants survived the recommended field dose.

Outdoor Dose–Response Experiment. To determine the level of resistance, the four johnsongrass populations that were found to be resistant to fluazifop in the greenhouse screenings were subsequently tested in an outdoor dose–response experiment. Seedling preparation and herbicide treatments were performed as described in the previous paragraph, with the following differences: seedlings were transplanted into 16-cm-diam pots (four seedlings per pot) and placed outside in a semicontrolled environment. In addition to fluazifop and cycloxydim used in previous screening tests, herbicide treatments included four other ACCase inhibitors, three FOPs and one DIM (Table 1), whereas no ALS inhibitors were included due to the fact that this mode of action still controlled all johnsongrass populations. Herbicide doses for resistant populations ranged from 1/2 × to 32 × and from 1/16 × to 4 × for FOP and DIM herbicides, respectively, whereas doses for the susceptible population (06–10) ranged from 1/32 × to 2 ×. The experimental design was a completely randomized block with three replicates and each replicate included three pots. Plant survival and shoot fresh weight per pot were recorded 3 WAT and expressed as a percentage of the nontreated control.

Statistical Analysis. The dose–response data were analyzed with the use of a log-logistic equation (Seefeldt et al. 1995): $Y = C + [(D - C)/[1 + (x/I_{50})^b]$ where Y is the fresh weight or survival; C and D are the lower and upper asymptotes at high and zero doses, respectively; I_{50} is the dose giving a 50% reduction in plant biomass or survival; b is the slope; and x is the herbicide rate. LD_{50} (based on plant survival data), GR_{50} (based on fresh-weight data), and relative standard errors, were obtained with the use of the macro BIOASSAY® developed by Onofri (2005) and running in Windows Excel® environment.

The data were first analyzed separately as a single curve and then all the curves for each herbicide were regressed together. Data were analyzed as described by Seefeldt et al. (1995), regressing together all curves for each herbicide with independent parameters. The complex model with independent parameters for each curve was then compared with progressively simplified models having common parameters (i.e., I_{50} and b) among curves. The lack-of-fit F test was performed at each step, and the simplification stopped when a significant lack of fit occurred. For biological reasons (Onofri 2005) and to improve the estimates of the parameters, the upper and lower asymptotes were forced to 100 and 0, respectively. Resistance indexes (RI) were calculated as ratios between the LD_{50} (or GR_{50}) of each resistant population and the LD_{50} (or GR_{50}) of the susceptible check (06–10).

Molecular Analyses. Genomic DNA from young leaves was extracted with the use of the CTAB method (Doyle and Doyle 1987). Specific primers were designed to amplify the coding sequence of the ACCase gene encompassing the CT domain. The primer combination SORG-2-For (5′-TTGGCCCAAGGGAAGATGCAT-3′)/SORG-2-Rev(5′-AACCCTTGAGGTTCGAGAAC-3′) produced a genomic fragment of 1,280 base pairs (bp). PCR amplifications were done in 50 µl with 100 ng of DNA template, 0.6 µM of each primer, 0.2 mM of each dNTP, and 1 µl of Advantage 2 Polymerase Mix containing a proofreading polymerase (Clontech Laboratories Inc., Palo Alto, CA). The

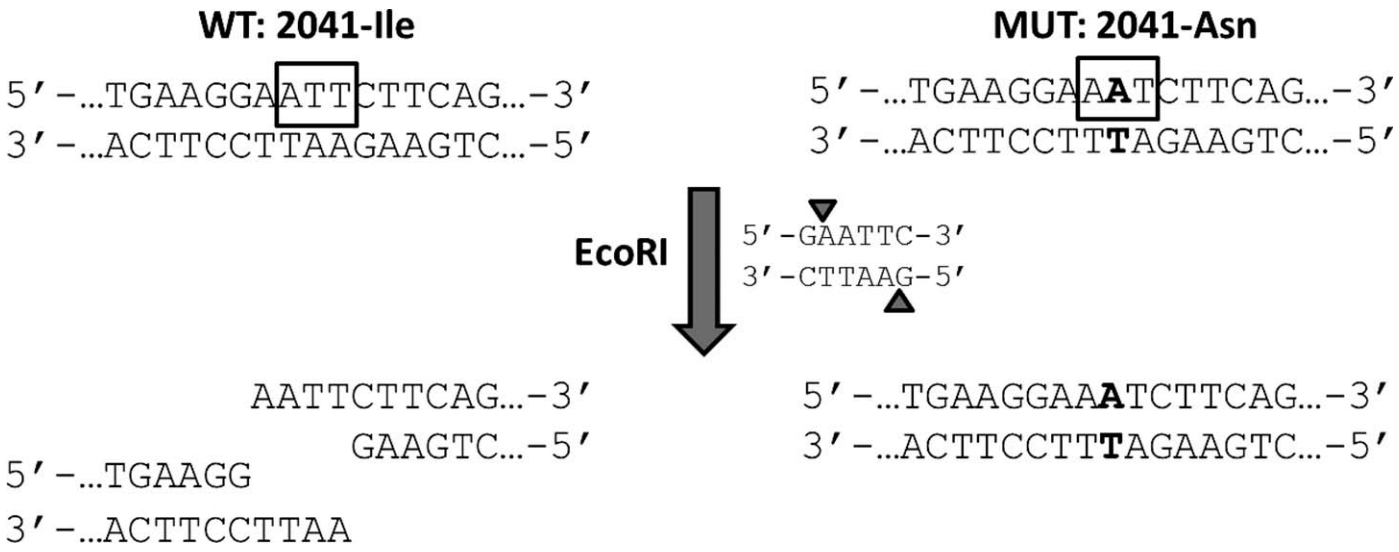

Figure 1. Scheme of the DNA-based molecular marker CAPS 2041. Above the sequence of the ACCase gene surrounding position 2041. The endonuclease EcoRI cuts the PCR product if the triplet ATT, encoding for the wild-type Ile, is present.

thermocycler (Biometra, Goettingen, Germany) program was as follows: 95 C for 1 min; 35 cycles of 95 C for 20 s, 60 C for 20 s and 68 C for 90 s; finally 70 C for 10 min. The amplicon was directly sequenced on both strands by extension of specific primers. Sequence analysis was conducted with the software BioEdit (Hall 1999).

A cleaved amplified polymorphic sequence (CAPS) marker was used to detect the presence or absence of the mutation at position 2041 of the ACCase gene in johnsongrass genomic DNA quickly (Figure 1). The assay was performed with the use of the method already described for ryegrass spp. (Scarabel et al. 2011; Zhang and Powles 2006) with some modifications for johnsongrass. Genomic DNA was extracted as described above and the combination of primers SORG-For-2027 (5′-CAGCTTGATTCCCATGAGCGATC-3′) and SORG-2-REV (see previous paragraph) were used to amplify a 360 bp DNA fragment. PCR was carried out with the use of Go Taq® Flexi DNA Polymerase (Promega). In a final volume of 25 µl, the following reagents were added: 5 µl 5 × colorless Go Taq® Flexi buffer, 1 µl $MgCl_2$ solution 25 mM, 0.5 µl PCR nucleotide mix 10 mM, each primer to a final concentration of 0.8 µM, 1 U of Go Taq® DNA polymerase, and 50 ng of genomic DNA. Amplification was performed using the following program: DNA denaturation for 2 min at 95 C, and 35 cycles of 30 s at 95 C, 30 s at 56 C, and 60 s at 72 C; finally a 5 min extension time at 72 C. The amplification was checked in 1% agarose gel and amplicon was purified with the use of the NucleoSpin Extract II (Macherey-Nagel). The PCR product (3 µl) was digested with 2 U of the endonuclease EcoRI (Promega) in a total volume of 20 µl, adding the specific buffer H and BSA 10 µg μl^{-1}, for 75 min at 37 C. The digestion was checked in 2% agarose gel. The homozygous resistant seedlings showed one noncut DNA band of 360 bp, whereas the homozygous susceptible plants showed two blurry DNA bands of 190 and 170 bp, respectively (Figure 1). Ninety seedlings were tested with this marker.

Results and Discussion

Field History of Sampled Fields. All putative resistant populations came from the southeastern part of the Lombardy region, where agriculture is very intensive and sometimes more than one crop is grown each year. The cropping system in the four "resistant" fields was based on dicot crops (i.e., mainly soybean, but also tomato and watermelon) sometimes rotated with maize every few years or alternated in the same year with winter monocot crops. Three out of the four FOP-resistant populations had been sampled in fields where minimum tillage had been adopted. In most cases, a PRE treatment was applied, but none of the active ingredients were effective against johnsongrass rhizomes. The most frequent ACCase POST selecting agent was fluazifop, with sporadic treatments with quizalofop, propaquizafop, and cycloxydim.

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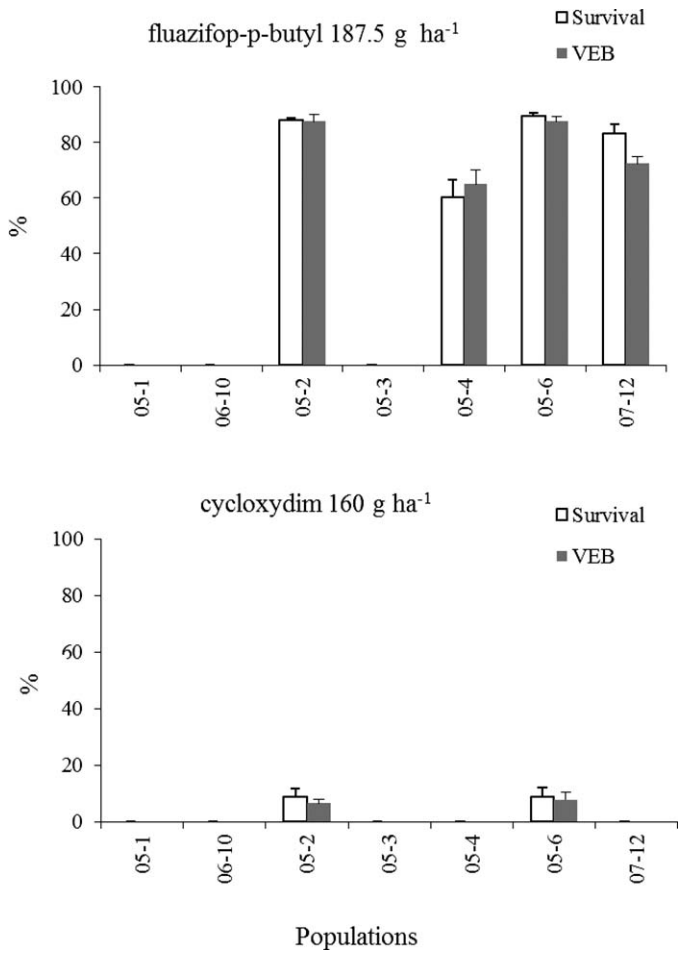

Figure 2. Percentage of plant survival and VEB of johnsongrass populations to fluazifop-p-butyl and cycloxydim herbicides applied at the four-leaf stage. Vertical bars represent standard errors. Populations 05-1 and 06–10 are susceptible checks.

Resistance Assessment. Fluazifop-p-butyl totally controlled both susceptible reference populations, 05-1 and 06–10 (Figure 2). Among the five putative resistant populations, four showed a high percentage of plant survival and VEB, ranging from 60 to 100% (Figure 2), even at three times the field dose (data not shown). The fluazifop-resistant populations treated with cycloxydim at the field dose showed survival as well as a VEB always below 10% (Figure 2).

In the dose–response experiments, the log-logistic equation fitted the data accurately without any data transformation ($\lambda = 1$), indicating that the range of doses was appropriate. Most standard errors of the parameters (LD_{50} and GR_{50}) were low, being one order of magnitude lower than the parameter values, with the exception of haloxyfop for which a greater variability was registered in all populations tested (Table 2). The lack-of-fit test on survival and fresh weight indicated that it was not possible to simplify the regressions to a model with common I_{50} and slope for all the populations, even if only the resistant populations were considered; a single curve approach was therefore preferred. The four populations proved to be highly cross resistant to all FOPs, in particular to the main selecting agent fluazifop-p-butyl, with RI > 392 and > 661 for LD_{50} and GR_{50}, respectively (Table 2). Based on LD_{50}, all resistant populations showed RIs ranging between 10 and 17 to propaquizafop and quizalofop, whereas considering GR_{50}, the RIs ranged between 8 and 25. Overall, plant survival and fresh weight gave similar results. The resistance indexes for cycloxydim and clethodim were always around 2 to 3, indicating only a variable shift in the

Table 2. Dose–response experiment: LD_{50}, GR_{50}, and resistance indexes (RI), calculated on the basis of the susceptible check 06–10 (S), of johnsongrass populations treated with POST ACCase inhibitors. LD_{50} and GR_{50} are the herbicide doses causing a 50% reduction in plant survival and fresh weight, respectively. Standard errors of LD_{50} and GR_{50} are given in brackets.

	Plant survival											
	Fluazifop		Propaquizafop		Quizalofop		Haloxyfop		Cycloxydim		Clethodim	
	LD_{50}	RI	LD_{50}	RI	LD_{50}	RI	LD_{50}	RI	LD_{50}	RI	LD_{50}	RI
	g ai ha^{-1}											
06–10 (S)	20.4 (1.18)		7.2 (0.47)		5.0 (0.21)		7.5 (0.19)		38 (0.9)		16 (0.6)	
05-2	> 8,000	> 392	110 (12.3)	15.3	80 (7.0)	15.9	736 (120.9)	98.3	56 (4.8)	1.5	30 (1.1)	1.9
05-4	> 8,000	> 392	120 (13.5)	16.7	65 (10.5)	13.0	479 (111.2)	63.9	67 (3.8)	1.8	52 (3.0)	3.3
05–6	> 8,000	> 392	111 (7.0)	15.5	76 (7.5)	15.2	750 (123.5)	100	78 (4.7)	2.1	47 (3.9)	3.0
07–12	> 8,000	> 392	79 (10.0)	10.9	47 (6.8)	9.4	696 (146.2)	92.8	50 (3.2)	1.3	–	–

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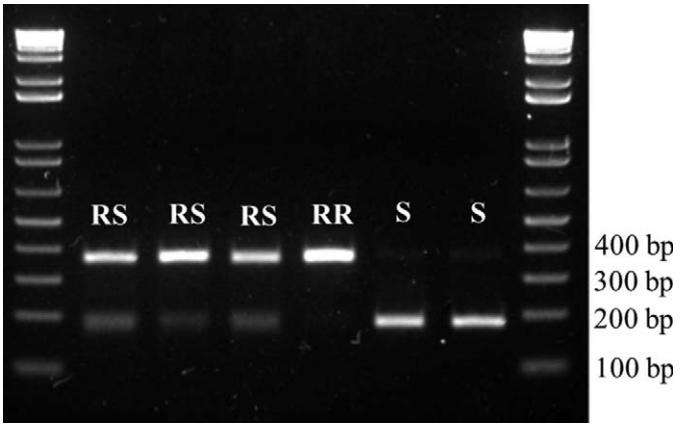

Figure 3. Genomic CAPS marker analysis for detecting 2041 ACCase mutation in johnsongrass plants. Homozygous susceptible (S), heterozygous resistant (RS), and homozygous resistant (R) plants.

susceptibility (Table 2). A similar resistance pattern was reported in two johnsongrass populations from Mississippi (Smeda et al. 1997). Bradley and Hagood (2001) documented a johnsongrass population from Virginia resistant to fluazifop, quizalofop, and sethoxydim but not to clethodim. Burke et al. (2006) reported that a clethodim-resistant johnsongrass population in Mississippi was also cross-resistant to fluazifop and sethoxydim, and Kaloumenos and Eleftherohorinos (2009) reported that a johnsongrass from northern Greece evolved cross-resistance to quizalofop and propaquizafop but did not evolve cross-resistance to fluazifop and cycloxydim.

Mechanism of Resistance. The comparison of the nucleotide sequences of the ACCase-amplified fragment of resistant and susceptible plants revealed a nucleotide substitution coding for an amino acid change at position 2041 (position is referred to the coding sequence of blackgrass chloroplastic ACCase sequence, EMBL/GenBank AJ310767). The susceptible plants had an ATT encoding isoleucine residue, whereas resistant plants had an AAT encoding asparagine (EMBL, accession numbers: KF885933, KF885934). All four resistant plants from each of the four ACCase-resistant populations analyzed presented the same point mutation at locus 2041. Other polymorphisms were observed either within individuals of a population or among the different populations, but they were not specific for the resistant or susceptible status of the plants. This is the first report of such a resistance-endowing mutation in johnsongrass, and it suggests that the Asn2041Ile change in the target enzyme is likely to be the major resistance mechanism in the Italian populations. Recently, a single point mutation that results in a cysteine replacing tryptophan at position 2027 of the ACCase gene has been discovered in four fluazifop-resistant johnsongrass populations (Kershner et al. 2012). Although data on the molecular basis of ACCase resistance in johnsongrass are limited, it can be hypothesized that the variability in the patterns of cross resistance could be ascribed to different point mutations at the ACCase binding site endowing target-site resistance or to different resistance mechanisms involved (Powles and Yu 2010).

When the susceptible plants were analyzed with the use of the CAPS marker, they displayed one band (190 bp) indicating only the presence of the 2041-Ile allele (Figure 3). Instead, all the fluazifop-resistant plants genotyped displayed both a resistant mutant (360 bp) and wild-type band (RS plant) or only one resistant mutant band (RR plant) (Figure

Table 2. Extended.

	Fresh weight											
	Fluazifop		Propaquizafop		Quizalofop		Haloxyfop		Cycloxydim		Clethodim	
	GR_{50}	RI	GR_{50}	RI	GR_{50}	RI	GR_{50}	RI	GR_{50}	RI	GR_{50}	RI
	g ai ha^{-1}											
06–10 (S)	12.1 (0.45)		4.7 (0.43)		4.2 (0.24)		3.4 (0.29)		24 (2.5)		7.1 (0.56)	
05-2	> 8,000	> 661	117 (9.4)	25.0	78 (7.2)	18.7	913 (139.1)	265.4	45 (5.0)	1.9	21 (1.8)	3.0
05-4	> 8,000	> 661	47 (7.9)	10.0	32 (5.3)	7.7	31 (20.9)	9.1	26 (2.8)	1.1	11 (1.3)	1.5
05–6	> 8,000	> 661	49 (8.7)	10.4	63 (8.2)	15.0	211 (56.5)	61.4	31 (4.7)	1.3	16 (1.9)	2.2
07–12	> 8,000	> 661	84 (18.4)	18.0	46 (4.7)	11.1	165 (31.2)	48.1	42 (2.6)	1.8	–	

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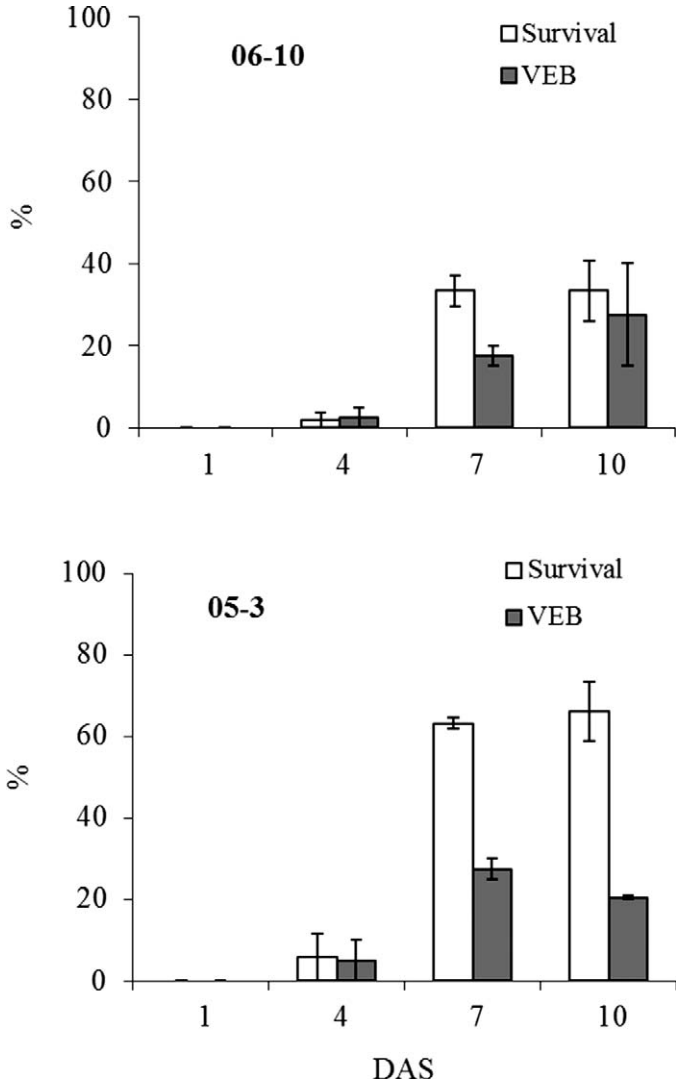

Figure 4. Effect of *S*-metolachlor applied at 1, 4, 7, and 10 d after sowing (DAS) pregerminated seeds of two populations of johnsongrass. Seedling emergence (i.e., survival) and visual estimation biomass (VEB) are expressed as a percentage of the nontreated pot units. Vertical bars represent standard errors.

3). In all the 91 fluazifop-resistant plants analyzed, at least one mutant band was detected, indicating the presence of the 2041-Asn mutation in the ACCase gene.

Alternative Herbicides and Resistance Management. The PRE treatments revealed that *S*-metolachlor adequately controlled johnsongrass when the treatment was done at 1 and 4 DAS (d after sowing). The few seedlings of populations 06–10 and 05-3 that were able to develop when treated at 4 DAS had a very low biomass, always below 10% of the nontreated check (Figure 4). Therefore, the treatment was only done at the best time, namely 1 DAS, in the second experiment where all populations were well controlled. Only a few seedlings with a very low biomass emerged in all populations. Also, the POST treatment with nicosulfuron had well controlled all the FOP-resistant populations (data not shown).

These findings indicate that the FOP-resistant populations were efficiently controlled by herbicides having different MoA; however, PRE herbicides (e.g., *S*-metolachlor, pendimenthalin, flufenacet, clomazone) commercially available for soybean and maize (or similar crops) are effective only on plants originating from seeds and not on those originating from rhizomes. The only POST active ingredient able to control plants from both seeds and rhizomes in dicot crops are ACCase inhibitors (i.e., fluazifop, propaquizafop, and quizalofop), whereas the only effective herbicides in monocot crops such as maize are sulfonylureas (i.e., ALS inhibitors). Therefore, in a cropping system based on dicot crops, once a resistant plant escapes a FOP POST treatment it will be able to produce rhizomes that in the following year(s) will not be controlled by any PRE or POST herbicide available for dicot crops. Therefore, the early diffusion process of FOP-resistant johnsongrass is driven by the spread of "resistant" rhizomes that will likely form patches of resistant plants, especially in minimum tillage conditions. The early recognition and destruction of these patches could significantly help to prevent or delay the spread of FOP-resistant individuals. Once present, resistance management options are very limited: e.g., stale seed bed preparation and nonselective presowing herbicides, rotation with maize and fallow, and use of POST sulfonylureas. However, the use of ALS inhibitors to control an ACCase-resistant population can lead to the relatively fast selection of a multiple resistant population, so should be considered as a short-term solution (Collavo et al. 2013), whereas a medium-long term strategy based on the integration of chemical and agronomic tools can significantly help to prevent or delay the spread of FOP-resistant johnsongrass.

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