

Research Article

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Multiple Herbicide-Resistant Italian Ryegrass [*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot] in California Perennial Crops: Characterization, Mechanism of Resistance, and Chemical Management

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Abstract

Italian ryegrass [*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot] is a troublesome weedy species in many regions of California. Its control has been chiefly dependent on herbicides due to their effectiveness and practicality and, as result, herbicide-resistant populations have been selected. Poor control of a population of *L. multiflorum* with paraquat was recently reported in a prune orchard in Hamilton City, CA. A series of experiments were carried out to characterize the response of this population to several POST herbicides, study the mechanisms of resistance, and investigate alternative chemical management options in tree crops. A known susceptible (S) and the suspected resistant population (PRHC) were subjected to greenhouse dose-response experiments with clethodim, fluazifop-P-butyl, glufosinate, glyphosate, paraquat, pyroxsulam, rimsulfuron, and sethoxydim. A 310-bp fragment of the *EPSPS* gene containing position 106 was sequenced from PRHC and S. Field experiments were carried out in a prune orchard with PRE herbicides commonly used by perennial crop growers in California. Greenhouse dose-response experiments confirmed that PRHC is resistant to paraquat, as well as multiply resistant to clethodim and glyphosate. The *EPSPS* gene of PRHC is heterozygous for glyphosate resistance at position 106, where one allele exhibited proline substituted by serine and the other by alanine. Field experiments with PRE herbicides indicated that tank mixes containing indaziflam and flumioxazin can provide adequate *L. multiflorum* control up to 150 d after treatment. Poor weed management practices, such as overreliance on a single site of action, have frequently been associated with the selection of herbicide-resistant *L. multiflorum* populations around the world, and adequate herbicide-resistance management programs are necessary for growers to maintain economic sustainability even after evolution of herbicide-resistant weeds in their fields.

Introduction

Italian ryegrass [*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot] is a winter annual or biennial (DiTomaso and Healy, 2007), and it is commonly cultivated as pasture forage, turf, and cover crop in many parts of the world. *L. multiflorum* is also a weedy species of worldwide occurrence, infesting roadsides, agronomic crops, orchards, and vineyards and competing efficiently for environmental resources (Hashem et al. 2000).

In the United States, California leads the nation in the production of almonds [*Prunus dulcis* (Mill.) D. A. Webb var. *dulcis*], clingstone peaches [*Prunus persica* (L.) Batsch.], dried plums [*Prunus domestica* L.], and walnuts (*Juglans regia* L.), among other nuts and fruits (USDA 2017). In the Central Valley of California, fruit and nut growers routinely mow between tree rows to reduce weed competition, but rely heavily on herbicides for weed control within the tree line. A typical herbicide program in most of these crops consists of a PRE/POST tank mix applied in late fall before rainfall of one or a mixture of the herbicides listed in Table 1, followed by a burndown treatment in early spring (March to April) and a second burndown treatment (often glyphosate or paraquat) in summer or fall after harvesting operations are completed. Glyphosate is the most widely used herbicide in California (California Department of Food and Agriculture 2015), due to its efficacy, broad-spectrum weed control, and safety. However, populations of *L. multiflorum* exhibiting resistance to glyphosate have been reported in almond orchards and vineyards (Jasieniuk et al. 2008). Since the first report of glyphosate-resistant *L. multiflorum* in California, several other cases of herbicide resistance in this species have been identified (Heap 2018).

The overreliance on herbicides has selected for herbicide-resistant *L. multiflorum* populations around the world (Preston et al. 2009). This widespread resistance may be

associated with the obligate-outcrossing, self-incompatible breeding system of *L. multiflorum*. This breeding strategy facilitates the dispersal of herbicide-resistance genes within and among populations (Loureiro et al. 2016), resulting in the selection of multiple herbicide-resistant biotypes in response to continued selection pressure (Mahmood et al. 2016).

Herbicide-resistant weeds increase weed control costs (Mueller et al. 2005). Weed interference in orchard crops may result in reduced performance and mortality of young trees (Belding et al. 2004), as well as reduced fruit size, total yield, and fruit number in older trees (MacRae et al. 2007). Even if the direct effects of weed competition can be overcome with additional water or fertilizer inputs, efficiency of many field operations is reduced. Adequate herbicide-resistance management programs are necessary for growers to maintain economic sustainability even after evolution of herbicide-resistant weeds in their fields. Using multiple herbicides with different sites of action within a cropping season delays the evolution of resistance and mitigates the direct and indirect impacts of herbicide-resistant weeds (Diggle et al. 2003). Therefore, diversified management practices are crucial for both proactive and reactive weed management.

Recently, poor control of *L. multiflorum* with paraquat was observed in a prune orchard in Hamilton City, CA. Due to the presence of glyphosate-resistant *L. multiflorum* in the orchard, paraquat had been applied several times each year for at least 8 yr as the main weed management tool, primarily to reduce interference with irrigation practices. The objectives of this research were to characterize the response of a multiple herbicide-resistant population of *L. multiflorum* to POST herbicides, study the mechanisms of resistance involved in this field-selected population of *L. multiflorum*, and evaluate chemical control options with PRE herbicides currently registered in orchard cropping systems in California.

Material and Methods

Greenhouse Studies

Seeds of suspected multiply resistant *L. multiflorum* seeds (a population hereinafter referred to as “PRHC”) were collected in

April 2015 from plants that survived a burndown treatment with paraquat in a 28-ha prune orchard (39.752°N, 122.016°W) near Hamilton City, CA. The field collection was made by harvesting mature seeds from individual plants on grids of approximately 30 by 30 m (near every fifth tree in every fifth row) from across the orchard and bulking all samples. A previously characterized glyphosate-susceptible biotype of *L. multiflorum* (S) (Jasieniuk et al. 2008) was used for comparison purposes. Preliminary herbicide screening indicated that the S biotype was susceptible to clethodim, fluazifop-P-butyl, glufosinate, glyphosate, paraquat, pyroxsulam, rimsulfuron, and sethoxydim (unpublished data). Studies in this research focused on herbicides commonly used by perennial crop growers in California (Table 1).

PRHC and S seeds were germinated in petri dishes by alternating 5 C in darkness (14 h) with 25 C in light (10 h) until desired germination was achieved. Seedlings were then transplanted to 5 by 5 by 10 cm pots filled with commercial potting media, with each pot receiving 1 plant to minimize intraspecific competition and optimize spray coverage. When plants reached the BBCH-23 stage (3 visible tillers) (Hess et al. 1997), dose-response experiments were carried out with POST herbicides registered in orchard systems (Table 2). Each herbicide experiment included seven doses ranging from 0.125X to 8X the recommended use rate. A nontreated control treatment was included for comparison in each herbicide experiment. Treatments were applied with a spray cabinet equipped with an even flat-fan spray nozzle (8002E, Teejet®, Spraying Systems, Wheaton, IL), calibrated to deliver 187 L ha⁻¹. After treatment, plants were maintained in a greenhouse under natural light, supplemented with artificial light, with a total daylight period of 11 h. Pots were arranged in a completely randomized design with four replications. Visual assessments were performed at 7, 14, 21, and 28 d after treatment (DAT) using a scale that ranged from 0 to 100, where 0 represented absence of injury and 100 represented complete plant death. Following the final visual evaluation, aboveground biomass of each plant was collected and dried, and the dry weight was recorded. Several individual plants from the field population of PRHC were grown to maturity, and the seeds produced were used to repeat the dose-response experiments.

Table 1. List of herbicides (including trade names and manufacturers' details) used in greenhouse and field experiments.

Herbicide	Trade name	Manufacturer, city, and state	Manufacturer's website
Clethodim	Envoy Plus®	Valent U.S.A. Corporation, Walnut Creek, CA	www.valent.com
Fluazifop-P-butyl	Fusilade® DX	Syngenta Crop Protection, Greensboro, NC	www.syngenta-us.com
Flumioxazin	Chateau®	Valent U.S.A. Corporation, Walnut Creek, CA	www.valent.com
Glufosinate	Rely® 280	Bayer CropScience, Research Triangle Park, NC	www.cropscience.bayer.us
Glyphosate	Roundup PowerMax®	Monsanto Company, St Louis, MO	www.monsanto.com
Indaziflam	Alion®	Bayer CropScience, Research Triangle Park, NC	www.cropscience.bayer.us
Mesotrione	Broadworks®	Syngenta Crop Protection, Greensboro, NC	www.syngenta-us.com
Oxyfluorfen	GoalTender®	Dow Agrosciences LLC, Indianapolis, IN	www.dowagro.com
Oryzalin	Surflan A.S.®	United Phosphorus, Inc., King of Prussia, PA	www.upi-usa.com
Paraquat	Gramoxone® 2.0 SL	Syngenta Crop Protection, Greensboro, NC	www.syngenta-us.com
Pendimethalin	Prowl® H ₂ O	BASF Corporation, Florham Park, NJ	www.basf.com
Pyroxsulam	Simplicity™ CA	Dow Agrosciences LLC, Indianapolis, IN	www.dowagro.com
Rimsulfuron	Matrix® SG	DuPont, Wilmington, DE	www.dupont.com
Sethoxydim	Poast®	BASF Corporation, Florham Park, NJ	www.basf.com

Table 2. Herbicide rates used in greenhouse POST dose–response experiments with multiple herbicide-resistant and susceptible *L. multiflorum*.

Herbicides ^a	Rates	
	—g ae ha ⁻¹ or g ai ha ⁻¹ —	
Clethodim	0; 34; 68; 136; 272; 544; 1,088; 2,176	
Fluazifop-P-butyl	0; 26; 52; 105; 210; 420; 840; 1,680	
Glufosinate	0; 143; 286; 572; 1,145; 2,290; 4,580; 9,160	
Glyphosate	0; 157; 315; 630; 1,260; 2,520; 5,040; 10,080	
Paraquat	0; 105; 210; 420; 840; 1,680; 3,360; 6,720	
Pyroxsulam	0; 2; 4; 7; 15; 30; 59; 118	
Rimsulfuron	0; 26; 52; 105; 210; 420; 840; 1,680	
Sethoxydim	0; 39; 79; 157; 315; 630; 1,260; 2,540	

^aAmmonium sulfate at 1% was added to all treatments. Nonionic surfactant at 0.25% was added to clethodim, fluazifop-P-butyl, glyphosate, paraquat, pyroxsulam, and rimsulfuron. Crop oil concentrate was added at 1% to sethoxydim.

Data were pooled between experiments in agreement with a Levene's ANOVA test for homoscedasticity of variance. Data were fit to log-logistic models, and the Akaike information criterion was used for comparison among several commonly used preselected models. The outliers were identified by comparing the semi-studentized residuals with a cutoff value based on a *t*-distribution with $\alpha=0.05$. Normality of residues and homoscedasticity of variance were assessed at a 5% level of significance. In cases in which the assumptions underlying nonlinear regression were not met (i.e., correct mean function, variance homogeneity, normally distributed errors, mutually independent

errors), the data were transformed with an optimal lambda obtained from a log-likelihood function (Box and Cox 1964; Kniss and Streibig 2015). Finally, data were fit to a three- or four-parameter log-logistic model (in agreement with the Akaike information criterion results), and the herbicide rate that reduced plant biomass by 50% (GR₅₀) was compared within each dose–response experiment and between PRHC and S using Student's *t*-test. The resistance index (RI) is reported as a means of comparison between the biotypes (the PRHC GR₅₀ was compared with the S GR₅₀). Statistical analysis was performed using the R software (R Core Team 2017).

Field Studies

An experiment was conducted twice to evaluate PRE herbicides commonly used by fruit and nut growers (Table 3) in the prune orchard near Hamilton City, CA, where failures to control PRHC with paraquat were observed. Experiments were initiated in November and December of 2015, spaced 3 wk apart in two different blocks in the same orchard with similar soil characteristics (Wyo silt loam with pH 5.9, 1.8% organic matter, 33% sand, 46% silt, and 21% clay). Approximately 3 wk and 1 wk before the start of each experiment, all plots were treated with a tank mix of glyphosate + glufosinate (1,260 + 1,160 g ha⁻¹), to ensure complete absence of weeds in the plots and, consequently, improve PRE herbicide application uniformity. A CO₂-pressurized backpack sprayer calibrated to deliver 187 L ha⁻¹ through three flat-fan extended-range 8002 nozzles (TeeJet®, Spraying Systems, Wheaton, IL) was used to apply the treatments. The experimental design was a randomized complete block design with four repli-

Table 3. Visual injury and biomass of multiple herbicide-resistant *L. multiflorum* after PRE herbicide treatments in a prune orchard in Hamilton City, CA, during 2015 to 2016.

Treatment ^a	Rate ^b	Visual injury		Biomass
		60 DAT	150 DAT	150 DAT
	g ai ha ⁻¹	----- % -----		g m ⁻²
Nontreated control	-	-	-	107.4 (5.3) a
Indaziflam 200 SC	51	100 (0) a	96 (3) ab	0.8 (0.3) d
Indaziflam 200 SC	73	100 (0) a	98 (1) a	1.0 (0.5) cd
Indaziflam 200 SC + flumioxazin	51 + 214	100 (0) a	100 (0) a	0.3 (0.1) d
Indaziflam 200 SC + oxyfluorfen	51 + 1,681	100 (0) a	100 (0) a	0.4 (0.1) d
Indaziflam 200 SC + rimsulfuron	51 + 35	100 (0) a	98 (1) a	0.2 (0.1) d
Rimsulfuron	70	100 (0) a	68 (7) b	5.8 (5.2) ab
Flumioxazin	428	100 (0) a	97 (2) ab	2.8 (2.5) d
Flumioxazin + pendimethalin	214 + 4,260	100 (0) a	98 (1) a	1.2 (1.0) d
Flumioxazin + oryzalin	214 + 2,242	100 (0) a	98 (1) a	1.7 (1.5) cd
Oryzalin	4,484	100 (0) a	90 (3) ab	7.1 (2.2) bcd
Oxyfluorfen	1,681	100 (0) a	98 (1) a	1.4 (0.8) cd
Pendimethalin	4,260	100 (0) a	91 (3) ab	5.8 (2.3) bcd
Mesotrione + pendimethalin	140 + 2,130	100 (0) a	76 (7) b	17.7 (5.7) abc
Mesotrione + oryzalin	140 + 2,242	100 (0) a	86 (4) ab	7.1 (2.2) bcd
Indaziflam 500 SC	51	100 (0) a	98 (1) a	1.0 (1.0) d
Indaziflam 500 SC + rimsulfuron	51 + 35	100 (0) a	97 (2) ab	1.3 (1.2) d

^aPlots were treated with a tank mix of glufosinate + glyphosate (1,680 + 1,260 g ha⁻¹) before treatment. Glufosinate at 1,680 g ha⁻¹, glyphosate at 1,260 g ha⁻¹, ammonium sulfate at 1%, and nonionic surfactant at 0.25% were added to all treatments.

^bMeans (± SE) followed by same letter within a column are not statistically different.

cations and 17 treatments, including a nontreated control. Visual injury assessments were performed at 30, 60, 90, 120, and 150 DAT using a scale that ranged from 0 to 100, where 0 represented absence of injury and 100 represented complete plant death. Aboveground biomass was collected at 150 DAT from 1-m⁻² quadrats randomly placed in each plot, then dried, and the weight was recorded. The original data did not meet the assumptions of ANOVA (i.e., normality of residues and homoscedasticity of variances); therefore, visual injury assessment and biomass data were subjected to Box-Cox transformations (Box and Cox 1964) based on a log-likelihood function built from the original data sets. Upon data transformation, the assumptions of ANOVA were met, and a multiple comparison test was performed with a Tukey's post hoc test, with the overall experiment-wise type I error rate corrected for 17 treatments using the Bonferroni correction and $\alpha = 0.05$.

Laboratory Studies

To determine whether polymorphism at position 106 of the *EPSPS* was involved in the resistance to glyphosate in PRHC, RNA was extracted (RNeasy Plant Mini Kit, Qiagen, Germantown, MD) from young leaves of untreated PRHC and S individuals from the field population; this was followed by cDNA synthesis (QuantiTect Reverse Transcription Kit, Qiagen).

Forward (AWF, 5'-AACAGTGAGGAYGTCTACTACATGCT-3') and reverse (AWR, 5'-CGAACAGGTGGGCAMTCAGTGCCAAG-3') degenerate primers were used (Adu-Yeboah et al. 2014) to amplify a 310-bp fragment of the *EPSPS* gene containing the encoded position 106, known to cause resistance to the herbicide glyphosate (Powles and Yu 2010). A polymerase chain reaction (PCR) composed of 1X PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTP mix, 0.2 μ M AWF, 0.2 μ M AWR, 50 ng of cDNA, and 2 U *Taq* DNA polymerase was performed with cDNA from four PRHC and S individuals. PCR cycles were as follows: an initial denaturation step at 94 C for 2 min, followed by 35 cycles of 94 C for 30 s, 60 C for 30 s, and 72 C for 1 min, with a final extension step at 72 C for 5 min.

Upon reaction completion, 5 μ l of individual PCR products were run in a 2% agarose gel, and the remaining products were sequenced with BigDye[®] Terminator v. 3.1 (Life Technologies, Burlington, Canada) and ABI Prism[®] 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Results and Discussion

Greenhouse results indicated that PRHC had a high RI when treated with clethodim (RI=10), fluzifop-P-butyl (RI=12), glyphosate (RI=10), paraquat (RI=19), and pyroxsulam (RI=20), and a moderate RI for rimsulfuron (RI=2) and sethoxydim (RI=3) (Table 4). However, statistically different GR₅₀ values between PRHC and S were only observed for clethodim, glyphosate, and paraquat. Conversely, both biotypes responded similarly to glufosinate (RI=1). Because dose-response error estimates for fluzifop-P-butyl were large, the RI was not significantly different between PRHC and S, even though field rates of this herbicide controlled S, whereas high rates (up to 8X) did not control PRHC (unpublished data). Similar observations were made for pyroxsulam, for which PRHC exhibited lower biomass reductions compared with S at field rates of this herbicide (unpublished data). Although the statistical criteria adopted in this research did not indicate differences between PRHC and S

when treated with fluzifop-P-butyl or pyroxsulam, it should be noted that field rates of these herbicides did not control PRHC, whereas S was controlled, and these responses were inheritable and reproducible across experiments. Because *L. multiflorum* is an outcrossing, self-incompatible weed species and PRHC is a field population, individuals within the population could have different mechanisms of resistance to fluzifop-P-butyl and pyroxsulam, increasing variability in the response to these herbicides. Although PRHC was less susceptible to pyroxsulam compared with S in the greenhouse study, this herbicide is not registered in prunes and was not tested in the field. Herbicides in the same chemical family of pyroxsulam (e.g., penoxsulam, flazasulfuron), however, are widely used in several other fruit and nut crops, and might have previously exerted selection pressure on *L. multiflorum* in this region.

EPSPS sequences from PRHC exhibited single-nucleotide polymorphisms at position 106. Surprisingly, all PRHC individuals sequenced presented heterozygous *EPSPS*, one allele exhibiting alanine at position 106 (Pro-106-Ala) and the other a serine (Pro-106-Ser), whereas S contained wild-type *EPSPS* alleles (proline at position 106) in both alleles. *EPSPS* duplication, which has been shown to be involved in glyphosate resistance in *L. multiflorum* populations from Arkansas (Salas et al. 2012), has not been assessed in this population. *L. multiflorum* is a diploid, obligate-outcrossing plant species (Loureiro et al. 2016), and the pollen-mediated movement of resistance alleles from glyphosate-resistant plants evolved independently in the field might explain the presence of a heterozygous *EPSPS* gene for resistance to glyphosate in PRHC.

Resistance to glyphosate can involve restricted herbicide movement (i.e., absorption and translocation) (Brunharo et al. 2016), enhanced herbicide metabolism (Carvalho et al. 2012), mutations in the target enzyme (Karn and Jasieniuk 2017), and increased expression and amplification of the *EPSPS* gene (Gaines et al. 2010). The mechanisms associated with paraquat resistance in PRHC have been characterized and involve the vacuolar sequestration of paraquat away from the chloroplast (Brunharo and Hanson 2017). The mechanisms involved in the resistance to clethodim have not been investigated in PRHC; however, several target-site mutations in the *ACC* gene are known to cause resistance to field rates of clethodim (Délye et al. 2008; Powles and Yu 2010). Non-target site mechanisms of resistance to acetyl-CoA carboxylase inhibitors (i.e., enhanced herbicide metabolism) have been identified in grass weed species (Hidayat and Preston 1997; Wang et al. 2017) and might also be involved in the resistance to clethodim in PRHC.

Treatments with PRE herbicides provided high levels of weed control up to 150 DAT (Table 3). Tank mixes containing indaziflam and flumioxazin, as well as sole applications of indaziflam (51 and 73 g ai ha⁻¹), flumioxazin (428 g ai ha⁻¹), oryzalin (4,484 g ai ha⁻¹), oxyfluorfen (1,681 g ai ha⁻¹), and pendimethalin (4,260 g ai ha⁻¹) provided >90% weed control. Conversely, rimsulfuron alone (70 g ai ha⁻¹) and mesotrione + pendimethalin (140 + 2,130 g ai ha⁻¹) provided visual injury lower than 80% to *L. multiflorum*. Rimsulfuron has a half-life as low as 5.6 d under field conditions (Schneiders et al. 1993), and this short persistence may have contributed to the relatively poor control observed at 150 DAT. Rimsulfuron, however, may be an option when an herbicide with a short residual activity is practical, because it did control *L. multiflorum* at acceptable levels up to 60 DAT (Table 3). Mesotrione is an herbicide with primarily broadleaf activity (Mitchell et al. 2001), and its association with low rates of oryzalin and pendimethalin only provided marginal weed

Table 4. Dose–response experiments with a multiple herbicide-resistant (PRHC) and a susceptible (S) population of *L. multiflorum*.

Herbicides ^a	GR ₅₀ ^b			P-value	Log-logistic function ^d
	PRHC	S	RI (SE) ^c		
	–g ai ha ^{–1} or g ae ha ^{–1}				
Clethodim	416 (82)	40 (11)	10 (3)	<0.01	Four-parameter
Fluazifop-P-butyl	309 (1099)	25 (10)	12 (45)	>0.05	Four-parameter
Glufosinate	188 (17)	158 (12)	1 (0.1)	>0.05	Four-parameter
Glyphosate	1,647 (124)	170 (9)	10 (1)	<0.001	Four-parameter
Paraquat	1,089 (57)	57 (9)	19 (3)	<0.001	Three-parameter
Pyroxulam	12 (8)	1 (1.5)	20 (52)	>0.05	Four-parameter
Rimsulfuron	29 (3)	17 (3)	2 (0.3)	>0.05	Four-parameter
Sethoxydim	4 (2)	1 (1)	3 (2)	>0.05	Three-parameter

^aAmmonium sulfate at 1% was added to all treatments. Nonionic surfactant at 0.25% was added to clethodim, fluazifop-P-butyl, glyphosate, paraquat, pyroxulam, and rimsulfuron. Crop oil concentrate was added at 1% to sethoxydim.

^bHerbicide rate to reduce plant biomass by 50% (GR₅₀) compared with a nontreated control.


^cRI, resistance index: ratio between GR₅₀ of PRHC and S.

^d $Y = d / [1 + \exp(b(\log x - \log e))]$ or $Y = c + (d - c) \exp[-\exp(b(\log x - e))]$, where b is the relative slope around e , c and d are the lower and upper limits, and e is the dose required to reduce dry weight by 50% (GR₅₀).

control (<90%). Biomass data were comparable to visual control evaluations (Table 3).

In a seedling germination study conducted in Oregon (Ghersa et al. 1994), *L. multiflorum* seeds germinated from August through April, and in some instances germination was observed even with limited incidence of sunlight (10% of full sunlight), a condition commonly found in orchard and vineyard systems. This nonuniform germination pattern of *L. multiflorum* poses challenges to growers who rely solely on POST herbicides for weed management, as it requires multiple applications to provide acceptable control. In this context, tank mixes of PRE herbicides with residual activity, like those studied in the present research, can be extremely important for managing multiple cohorts of weeds with nonuniform germination and emergence patterns.

Glyphosate-resistant *L. multiflorum* populations are widespread in California, and the overreliance on POST herbicides from a variety of chemical classes has selected for a multiple herbicide-resistant population. Greenhouse data indicate that glufosinate, rimsulfuron, and sethoxydim may be adopted as POST chemical alternatives to control PRHC, although resistance to these herbicides has been reported in *L. multiflorum* and other weed species in other parts of the world (Heap 2018). Resistance to glyphosate in PRHC is due to double amino acid substitutions in the EPSPS, where one allele presented a proline-to-alanine substitution and the other a proline-to-serine substitution at position 106. Fruit and nut tree growers have a number of PRE herbicides registered in their crops that are effective options to control *L. multiflorum*, notably tank mixes that provide residual activity and high control levels, such as those containing indaziflam and flumioxazin.

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