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Mechanism of resistance to quinclorac in smooth crabgrass (*Digitaria ischaemum*)

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Abstract

The mechanism of resistance to quinclorac was investigated in a smooth crabgrass biotype [*Digitaria ischaemum* (Schreb. ex Schweig) Schreb. ex Muhl] from Tulare County, California. Quinclorac (8.96 kg a.i. ha^{-1}) had no effect ($P = 0.18$) on the resistant (R) biotype, but reduced fresh weight of a susceptible (S) biotype by 93%. After treatment with 4.48 kg a.i. quinclorac ha^{-1}, the S biotype produced about three times more ethylene than the R biotype and accumulated cyanide in tissues. Similar amounts of endogenous cyanide resulting from treatment with KCN reproduced quinclorac phytotoxicity. Pre-treatment with the ACC synthase inhibitor AVG reduced quinclorac phytotoxicity by 37% and ethylene production by 89%. These data suggest a target site-based mechanism of resistance involving stimulation of ACC synthesis and accumulation of cyanide. Also, the R biotype had four times more β-cyanoalanine synthase activity than the S biotype, suggesting a higher ability to detoxify cyanide.

Keywords: *Digitaria ischaemum*; Quinclorac; Resistance; Turfgrass; Aminoethoxyvinylglycine; Ethylene; Cyanide; β-Cyanoalanine synthase; Abscisic acid; 1-Aminocyclopropane-1-carboxylic acid

1. Introduction

The quinolinecarboxylic acid herbicide quinclorac (3,7-dichloro-8-quinolinecarboxylic acid) is a highly selective auxinic herbicide that is widely used in rice (*Oryza sativa* L.) primarily for the control of *Echinochloa* spp. and certain dicot weeds including *Aeschynomene* spp., *Sesbania* spp., and *Monochoria* spp. [1]. It has also been developed for selective use in turfgrass [2], spring wheat (*Triricum aestivum* L.), barley (*Hordeum vulgare* L.), and canola (*Brassica napus* L.) against important dicot and monocot weeds including *Digitaria*

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spp., *Setaria viridis* (L.) P. Beauv., *Brachiaria* spp., *Trifolium* spp., *Taraxacum officinale* Web., and *Galium* spp. [3]. In chemical fallow, quinclorac is used to suppress *Convolvulus arvensis* L. and *Lactuca serriola* L. [1]. Its activity against important grasses differentiates quinclorac from other auxinic herbicides. Symptoms in susceptible grasses are characterized by necrosis and reddening that progress from the growing areas of young blades followed by wilting and necrosis of the entire shoot. These effects are not observed in tolerant rice or resistant grasses, which include biotypes of *Echinochloa crus-galli* (L.) Beauv., *E. hispidula* L., *E. colona* (L.) Link, *E. oryzoides* (Ard.) Fritsch, *E. oryzicola* Vasinger, and *Digitaria ischaemum* (Schreb. ex Schweig) Schreb. ex Muhl [3–7]. Lack of differences in quinclorac uptake, translocation, or metabolism between resistant and susceptible grasses [2,8] suggested a target site-based mechanism of selectivity and resistance [3].

By mimicking an auxinic effect, quinclorac stimulates the production of ethylene in plant tissues [1]. It has been proposed that this stimulation results from an induced de novo synthesis of the enzyme ACC synthase (EC 4.4.1.14) (reviewed by [3]), which catalyzes the biosynthesis of ACC (1-aminocyclopropane-1-carboxylic acid), which is later oxidized to ethylene. Cyanide is a by-product of the reaction that converts ACC to ethylene [9], and its accumulation in tissues can inhibit plant growth [10–13]. The accumulation of cyanide resulting from quinclorac-induced ethylene production has been proposed as the main mechanism of action of this herbicide in susceptible grasses. Tolerant species like *Oryza sativa* and resistant *Echinochloa* spp. do not respond to quinclorac treatment with large increases in ethylene and cyanide production, and it has been proposed that they are probably insensitive at the target site, namely the induction of ACC synthase [3,14,15].

A significant proportion of *D. ischaemum* could not be controlled when quinclorac was first tested in the mid eighties at a turfgrass site in Dinuba, California. This biotype persisted over the years and made up the majority of the population without having been subjected to a prolonged selection pressure from quinclorac applications. An earlier study [16], found that root cell wall biosynthesis in *D. ischaemum* from this location was significantly less inhibited by quinclorac than in a susceptible biotype. Quinclorac had similar inhibitory effect on the growth of roots and shoots of susceptible (S) *D. ischaemum*; but although roots of the resistant (R) biotype were fairly sensitive to quinclorac, the shoots were extremely tolerant [16]. Similar effects were observed in rice, a tolerant species. This suggests the presence of an additional mechanism of resistance and selectivity. Rather than a primary mechanism of action responsible for selective herbicide effects, the inhibition of cell wall formation could be a side effect of quinclorac associated with auxin-dependent regulation of hydrolytic enzymes, or the induction of ethylene, cyanide, or ABA production [1,3].

In the present study, we elucidate a key mechanism of quinclorac toxicity and resistance in *D. ischaemum*. The first goal of this study was to characterize the level of resistance and compare it with the relative ability of quinclorac to induce ethylene and cyanide biosynthesis in R and S *D. ischaemum*. A causative role between stimulated ethylene production and growth inhibition was explored using an ethylene biosynthesis inhibitor. We then investigated whether quinclorac-induced accumulation of cyanide derived from stimulated ethylene formation was responsible for the observed phytotoxic effects, and whether those effects differed between shoots and roots. Finally, we assessed differences in the levels of endogenous cyanide in connection with the activity of β-cyanoalanine synthase in R and S plants.

2. Materials and methods

2.1. Plant material and growing conditions

Seeds of the resistant (R) *D. ischaemum* biotype were collected in 2000 from a site at the ARS-Research Station in Dinuba (Tulare County), California, where control failure was detected in the mid eighties. Herbicide experiments were conducted on that site in the following years with chemicals of diverse modes of action, including plots with quinclorac. Quinclorac had not been used for two years at the time seed samples were collected

for this study. Seeds of a susceptible (S) biotype that had never been exposed to herbicides before were acquired from a commercial source.[1] In experiments with foliar treatments, R and S *D. ischaemum* were grown from seed in plastic pots filled with 130 g of a peat-moss-based potting mixture[2] placed under a constant irradiance of 800 μmol m^{-2} s^{-1} photosynthetic photon-flux density in a growth chamber maintained at 25/18 °C day/night temperature, 16 h daylength, and 50% relative humidity. Emerged seedlings were thinned to four uniform and equidistantly spaced plants per pot. Plants were irrigated as needed with water and a fertilizer solution containing macro- and micronutrients.[3] For assays with plants in hydroponics, *D. ischaemum* seeds were placed in 250 ml flasks filled with water. The flasks had a 2.5 cm round opening at the top covered by a 2 mm plastic mesh, where the seeds were held in contact with the water for germination. At the one leaf stage of growth, plants were thinned to three or five plants per flask and the water replaced by 210 ml of 0.5 strength Hoagland solution [17]. Flasks were kept in a growth chamber under the same growing parameters as described above. All experiments were conducted twice.

2.2. Dose–response experiments

Dose–response experiments were conducted in pots to quantify the extent of resistance to quinclorac in the R biotype. Plants were sprayed at the three-leaf stage of growth with a commercial formulation (75% dry flowable) of quinclorac[4] applied at rates of 0, 0.56, 1.12, 2.24, 4.48, and 8.96 kg active ingredient ha^{-1} using a cabinet track sprayer equipped with a 6505E[5] (even-spray) flat fan nozzle that delivered a spray volume of 467.4 L ha^{-1} at 276 kPa. Plants were clipped at soil level two weeks after spraying and their fresh weight was determined. There were five replicates per herbicide rate, and fresh weight data were expressed as percentage of the mean of the untreated control plants.

2.3. Determination of ethylene and cyanide production

These experiments were conducted to assess differences in the amounts of endogenous ethylene and cyanide produced by R and S *D. ischaemum* plants in response to quinclorac treatment. Plants were sprayed with quinclorac as above at the three-leaf stage of growth with 0, 0.56, 1.12, 2.24, and 4.48 kg a.i. ha^{-1}. Treatments were replicated ten times. Five of the replicates were used for the ethylene bioassay and five for the cyanide production assay. Data were expressed as percentages of the mean of the untreated control R and S plants, and presented as means ± SE for common concentrations.

2.3.1. Ethylene assay

Three days after herbicide application, shoots of R and S plants were excised and placed in tared 10 ml glass syringes with the plunger set to 10 ml. The syringe was re-weighed to ascertain the tissue's fresh weight, capped with a rubber septum stopper, and incubated in the dark for 5 h at 25 °C. After incubation, ethylene was measured by withdrawing a 1 ml gas sample with a syringe and injecting it into a gas chromatograph[6] equipped with a 60/80 mesh aluminum column and a flame ionization detector for the quantitative determination of ethylene. The instrument's oven and injector/detector temperatures were at a constant 80 °C. Gas flow rates were 90 ml min^{-1} for N_2, and 75 ml min^{-1} for air and H_2.

2.3.2. Cyanide assay

Three days after quinclorac treatment, shoots from a second set of R and S plants were excised for cyanide determination using a modification of

[1] Herbiseed, New Farm, Mire Lane, West End, Twyford, England.
[2] ProMix BX; Premier Horticulture Inc., 326 Main St. Red Hill, PA 18076.
[3] Grow More, 15600 New Century Dr., Gardena, CA 90248.
[4] BASF Corp., 26 Davis Dr., Research Triangle Park, NC 27709.
[5] Spraying Systems Co. (Teejet), P.O. Box 7900, Wheaton, IL 60189-7900.
[6] Series 100 Carle Analytical Gas Chromatograph, Chandler Engineering, L.L.C.,Tulsa, OK 74147-0710.

Grossmann and Kwiatkowski's [14] method. A finely freeze-dried ground shoot tissue sample (1.5 g) was transferred to a glass vial 70 mm in height and 20 mm in diameter. One hundred microliters of NaOH (1.5 N) were applied to a 2 × 2.5 cm longitudinally folded filter paper[7] strip attached to the bottom of a rubber stopper cap sealing the vial. The filter paper strip remained suspended above the plant material inside the vial. Three milliliters H_2SO_4 (0.92 M) were added to the sample and the mixture was stirred at room temperature for 20 h to allow evolved HCN to be trapped at the NaOH-saturated filter paper. The filter was then eluted with 500 μl NaOH (0.1 N). A 200 μl aliquot of the eluent was analyzed colorimetrically according to [12], reading absorbance at 580 nm.

2.4. Effect of ethylene production inhibitors

Susceptible *D. ischaemum* plants were treated with the ACC synthase inhibitor aminoethoxyvinylglycine (AVG) [18], alone and in combination with quinclorac to establish a causative relationship between quinclorac-induced ethylene production and shoot growth inhibition. Plants were grown in hydroponics and treated at the three-leaf stage of growth with AVG (5.0 μM) one day before exposure to 40 μM quinclorac. Both chemicals were applied in the nutrient solution. Three days after quinclorac treatment, shoots were harvested and ethylene production was measured as described before. Treatments were replicated three times, and data were expressed as percentages of the mean of the untreated control plants.

2.5. Relating tissue cyanide to shoot fresh weight reduction

This study was conducted to investigate if endogenous cyanide resulting from quinclorac-induced ethylene production can be related to the observed herbicide toxicity. Thus, we compared the plant injury caused by the accumulation of cyanide in tissues following either quinclorac treatment or an external KCN application. This experiment was conducted on the S biotype, since the R biotype shows almost no ethylene increase in response to quinclorac. Quinclorac was added to the hydroponic medium of a set of three-leaf *D. ischaemum* plants to establish a range of 12 final concentrations from 0.87 to 50.0 μM a.i. at 1.5× increments. Another set of identical plants was similarly treated with KCN to establish a range of 11 final concentrations between 0.029 and 0.8 mM CN at 1.5× increments. In all cases, each concentration was replicated twice, and two untreated controls were included in each series. Four days after addition of quinclorac and KCN to the corresponding media, shoots were harvested to measure fresh weight and cyanide concentration. The experiment was repeated with quinclorac concentrations ranging from 1.3 to 75 μM and cyanide concentrations ranging from 0.019 to 0.8 mM CN, using three replicates per concentration and three untreated controls per series. Fresh weight values were expressed as percentages of the mean untreated controls. The cyanide concentrations in shoots resulting from the KCN treatments and the cyanide concentrations resulting from quinclorac treatment were regressed against the corresponding percent shoot fresh weights.

2.6. Cyanide toxicity and detoxification in R and S plants

Two studies were conducted to investigate differences between R and S plants in their sensitivity to cyanide and ability to metabolize it.

2.6.1. Effect of exogenously applied KCN on cyanide accumulation and growth of R and S plants

KCN was added to the nutrient solution (0.75 strength Hoagland solution) of hydroponically grown four-leaf R and S *D. ischaemum* plants to achieve final concentrations of 0.0, 0.1, 0.2, 0.4, 0.8, and 1.6 mM CN. After three days, shoots were excised to measure fresh weight and quantify endogenous cyanide as in Section 2.3.2. There were three replicates per concentration. Three types of relationships were studied for each biotype: (a) tissue cyanide concentration vs. shoot fresh weight (expressed as percent of the mean untreated control), (b) concentration of externally

[7] Whatman No. 1 Qualitative Filter Paper; Whatman Inc., www.whatman.com.

applied cyanide vs. tissue cyanide concentration, and (c) concentration of externally applied cyanide vs. shoot fresh weight (%).

2.6.2. Assay of β-cyanoalanine synthase enzyme activity

The methodology is based on procedures employed by Miller and Conn [11], Yip and Yang [12], Grossmann and Kwiatkowski [14], Goudey et al. [19], and Blumenthal et al. [20]. Plants of the R and S biotype were grown in pots up to the three-leaf stage and sprayed with 3.36 kg a.i. quinclorac ha^{-1} or left untreated. Three days after treatment, samples of shoot tissue were homogenized with 2.5 ml of 100 μM Tris buffer (pH 8.5) per gram of tissue. After centrifugation at 10,000*g* for 10 min at 4 °C, the supernatant was employed for the enzyme assay with NaCN and L-cysteine as the substrates, which were dissolved in 100 mM Tris buffer (pH 8.5) to a final concentration of 50 and 10 mM, respectively. The enzyme extract and the substrate solutions were equilibrated separately at 30 °C for 10 min. The assay was started in a sealed test tube by adding 0.5 ml of the buffered NaCN and 0.5 ml of the buffered L-cysteine to 1 ml enzyme extract. After incubation at 30 °C for 30 min, the reaction was stopped and the color was developed by injecting into the tube 0.5 ml of 30 mM $FeCl_3$ in 1.2 N HCl followed by 0.5 ml of 20 mM *N,N*-dimethyl-*p*-phenylenediamine sulfate in 7.2 N HCl. After 20 min, the samples were centrifuged at 1020*g* for 5 min to remove precipitated protein. The enzyme activity was determined colorimetrically after conversion of the released H_2S (from cysteine) to methylene blue, reading absorbance at 650 nm, and using Na_2S as the standard reference. Enzyme activity was expressed in nmol H_2S g^{-1} fresh $weight^{-1}$ min^{-1}. Treatments were a factorial combination of biotypes × quinclorac rates and were replicated four times.

2.7. Relative sensitivity of root and shoot growth to quinclorac

Hydroponically grown plants of the susceptible biotype were treated at the three-leaf stage with quinclorac (0, 0.20, 0.40, and 0.80 ~~m~~μM a.i.) in the growing medium. Three days after, the fresh weight of roots and shoots of five plants per treatment was determined. Each treatment was replicated five times, and fresh weight data were expressed as percent of the mean untreated control.

2.8. Statistical analysis

All experiments were conducted as completely randomized designs. Data from repeated experiments were pooled and statistical analysis involved either paired *t* tests to compare means, analysis of variance and mean separation using Fisher's protected LSD, or regression analysis. All statistical tests were conducted with $\alpha = 0.05$. When required, pairs of regressions were compared using a lack-of-fit *F* test [21]. For the dose–response experiments, a log-logistic model [22] was fitted to the data:

$$Y = c + \{(d - c/[1 + (X/g)^b]\}, \quad (1)$$

where *Y* is the fresh weight expressed as percentage of the untreated control, *c* and *d* are the coefficients corresponding to the upper and lower asymptotes, *b* is the slope of the curve around *g*, and the value of *g* corresponds to the dose that causes 50% response (GR_{50}). Regression analysis was conducted using the SigmaPlot version 8.0 (2002) statistical software.[8]

3. Results and discussion

Following failure to control *D. ischaemum* with quinclorac at a turf site in Tulare County, California, an accession of this biotype was evaluated for resistance in our laboratory. Studies were conducted to characterize the level of resistance, and to quantify ethylene and cyanide production by quinclorac-treated R and S plants. A causative relationship between quinclorac-stimulated ethylene and cyanide production was investigated by using a known ethylene biosynthesis inhibitor and by relating growth inhibition to cyanide accu-

[8] SPSS Inc. 233 S. Wacker Drive, Chicago, Illinois 60606.

mulation in shoot tissues. Finally, we also evaluated differences in the abilities of R and S plants to detoxify endogenous cyanide.

3.1. Characterization of resistance

The R biotype was highly resistant to quinclorac. While approximately 1.97 kg a.i. quinclorac ha^{-1} were required to reduce the growth of the S biotype by 50% (GR_{50}), a GR_{50} for the R biotype could not be calculated because no significant ($P = 0.12$) growth reduction in shoot fresh weight was observed at the doses tested (Fig. 1). Thus, the resistance ratio [GR_{50} (R)/GR_{50} (S)] was far larger than the maximum rate tested (8.96 kg ha^{-1}). Such high level of resistance is suggestive of insensitivity to the herbicide at its target site.

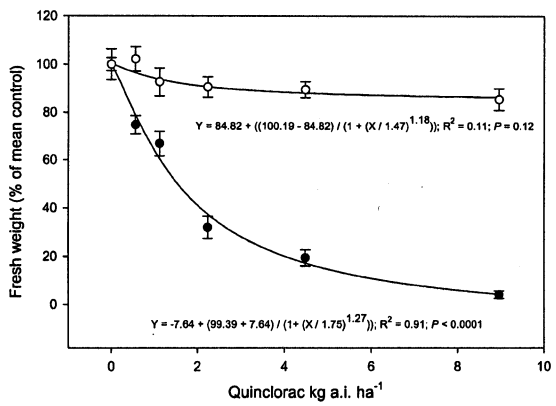

Fig. 1. Aboveground fresh weights of susceptible (●) and resistant (○) *D. ischaemum* two weeks after foliar treatment with quinclorac at the three-leaf stage of growth. Results from two separate experiments were combined, and data were expressed as percent of the mean fresh weight of untreated control plants. Vertical bars represent the standard error of the mean.

3.2. Physiological responses differ between biotypes

Our studies demonstrate a fundamental difference in the response of R and S *D. ischaemum* plants to quinclorac. After treatment with quinclorac, S plants drastically increased their production of ethylene with a concomitant accumulation of cyanide in their tissues (Figs. 2A and B). Contrary to this, R plants exhibit only a minor response. If ethylene, and ultimately cyanide, is responsible for the toxicity of quinclorac to *D. ischaemum*, then this differential response in ethylene production between both biotypes can be postulated as the mechanism of resistance.

3.3. Mechanisms of toxicity and resistance

The involvement of ethylene in quinclorac phytotoxicity to *D. ischaemum* was suggested by the effects of aminoethoxyvinylglycine (AVG) on plants treated with quinclorac. AVG is an inhibitor of ACC synthase and ethylene production [23,24].

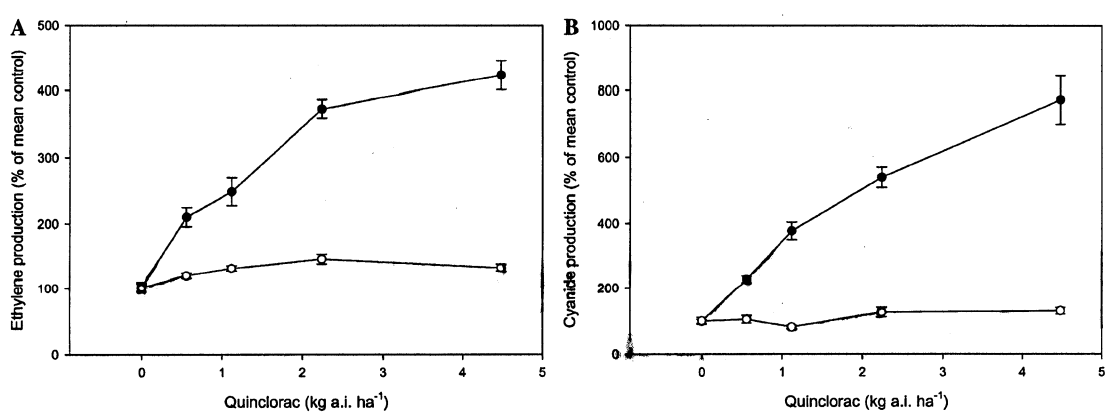

Fig. 2. Ethylene (A) and cyanide (B) production by susceptible (●) and resistant (○) *D. ischaemum* three days after foliar treatment with quinclorac at the three-leaf stage of growth. Results from two separate experiments were combined, and data were expressed as percent of the mean ethylene and cyanide content of untreated control plants. Vertical bars represent the standard error of the mean.

Table 1
Effect of AVG (aminoethoxyvinylglycine) on shoot fresh weight reduction caused by quinclorac applied to three-leaf-stage *Digitaria ischaemum* plants grown in hydroponics

Treatment	Herbicide rate (μM)	AVG concentration (μM)	Fresh weight[a] (%)	Ethylene[a] (%)
Untreated	—	—	100.00 ± 1.99	100.00 ± 11.59
Quinclorac	40	—	29.54 ± 1.39	305.65 ± 50.41
AVG		5.0	48.19 ± 2.04	14.23 ± 3.36
Quinclorac + AVG	40	5.0	40.57 ± 1.46	34.36 ± 6.23
LSD(0.05)[b]			5.15	77.01

[a] Values in these columns are means ± standard error of six observations expressed as percentage of the mean untreated control; data pooled from two separate experiments.
[b] Fisher's protected LSD ($P = 0.05$).

Shoot fresh weight of the S biotype was reduced by about 70% when quinclorac was added to the nutrient solution of plants growing in hydroponics; this was accompanied by a three-fold increase in ethylene production. The rate of ethylene production from plants pretreated with AVG was only 14% of the control (Table 1). Addition of quinclorac to these pre-treated plants increased production 2.4-fold, but even then, ethylene production was only 34% of the non-treated control, and 11% of the quinclorac treated plants. Plant growth (i.e., fresh weight) was reduced 70% and 50% by quinclorac and AVG, respectively. In AVG pre-treated plants, the 70% growth inhibition by quinclorac was reduced to 60%; or AVG pre-treated plants exposed to quinclorac were 37% heavier than plants exposed to quinclorac alone. In spite of the inhibitory effects of AVG per se, quinclorac phytotoxicity was partially overcome by the addition of AVG. A reversal of quinclorac phytotoxicity by inhibitors of ethylene biosynthesis was also observed with *Echinochloa* spp., which led [4,14] to conclude that ethylene plays a causative role in the toxicity of quinclorac. If this is true, the excess cyanide that accumulates in *D. ischaemum* as a result of the quinclorac-induced stimulation of ethylene biosynthesis should be capable of causing enough tissue toxicity to account for the observed growth reduction. The same level of growth inhibition caused by quinclorac was achieved with KCN once the same amount of endogenous cyanide was accumulated in each case (Fig. 3). That is, the range of toxicities (symptoms and fresh weight reduction) caused by the quinclorac rates used in this experiment were

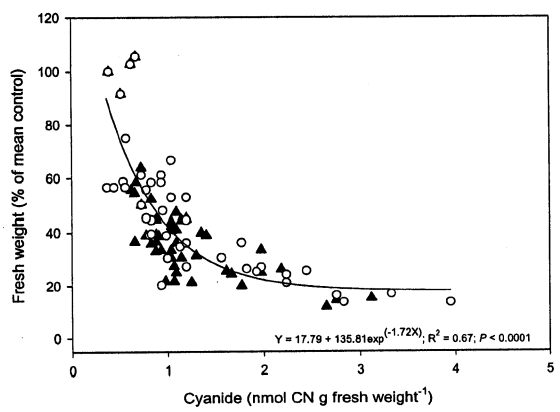

Fig. 3. *Digitaria ischaemum* (susceptible biotype) shoot fresh weight as affected by the cyanide concentration in shoots resulting from adding quinclorac (▲) or KCN (○) to the hydroponic medium of three-leaf plants followed by incubation for four days. Data from two separate experiments are presented; endogenous cyanide is expressed in terms of the CN anion and fresh weight as percent of the mean fresh weight of untreated control plants. A lack-of fit-test at $P = 0.05$ (Chow 21) suggested that data from quinclorac and KCN treatments can be described by a single regression line.

closely reproduced by accumulating, through an external application of KCN, equal amounts of cyanide in *D. ischaemum* tissues as those resulting from the quinclorac application. Although generated across different experiments, the data are extrapolatable. For example, three-leaf plants experienced about 75% reduction in shoot fresh weight when 1.75 nmol CN g^{-1} fresh weight^{-1} accumulated in shoots (Fig. 3). According to data used to construct Fig. 2B (not shown), the same cyanide concentration is found in three-leaf plants

after treatment with 3 kg quinclorac a.i. ha^{-1}, which in turn causes a shoot fresh weight reduction of about 62% when applied to similar plants (Fig. 1), a value not too far off from that observed in Fig. 3 if we consider that plants in Fig. 1 had two weeks to recover from treatment.

Quinclorac induces ACC synthase activity in the root of susceptible grasses like barnyardgrass (*Echinochloa crus-galli* (L) P. Beauv.) [8,25]. ACC is transported acropetally to the shoots where it acts as a signal stimulating ACC synthase leading to the release of ethylene and cyanide. The principal site of quinclorac action would be localized in the root, but endogenous levels of ACC, ethylene formation, and HCN accumulation increase predominantly in the shoot [1,8,25]. Consequently the shoots of root treated barnyardgrass were more susceptible to damage by quinclorac than the roots [14]. Our studies with root-treated *D. ischaemum* in hydroponics support this model. The ratio of GR_{40}(root)/GR_{40}(shoot) calculated[9] from the responses in Fig. 4 indicates that roots were 2.3 times more tolerant of quinclorac than shoots.

Our data strongly suggest that quinclorac toxicity to *D. ischaemum* is mainly caused by an accumulation of cyanide in shoot tissues. Cyanide builds up because of increased ethylene biosynthesis, which according to [1,8,14,15,25] in studies with grasses including *Digitaria sanguinalis*, occurs when quinclorac stimulates de novo synthesis of ACC synthase. Consequently, the mechanism of resistance in the R *D. ischaemum* biotype involves target site insensitivity to quinclorac stimulation of ethylene biosynthesis, which results in lower levels of endogenous cyanide produced in response to quinclorac compared to the S biotype. No significant differences in uptake, translocation or metabolism of quinclorac have been observed between resistant and sensitive grasses as reviewed by [3]. The strong relationships found in our work support the idea that other mechanisms proposed, such as cell wall biosynthesis inhibition in susceptible grasses [16], may not be the main site of herbicidal activity or resistance as discussed by [1,3,4].

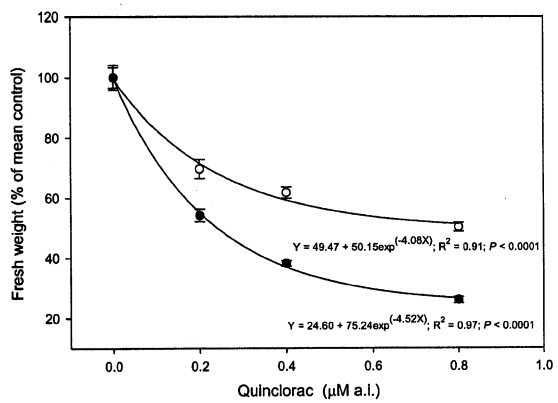

Fig. 4. Root (○) and shoot (●) fresh weight of susceptible *D. ischaemum* after adding quinclorac to the hydroponic medium of three-leaf plants and incubating for three days. Data from two separate experiments are presented and expressed as percent of the mean fresh weight of untreated control plants. Vertical bars represent the standard error of the mean.

3.4. Cyanide detoxification as an additional mechanism

Ethylene is a plant hormone that causes many physiological responses and its production is a normal response to stress in many plants for survival in unfavorable environments [3,26]. Besides the hydrolysis of cyanogenic glycosides, the oxidation of ACC by ACC oxidase (EC 1.14.17.4) to produce ethylene results in the formation of cyanide as a co-product [10,12,23]. This process is the main source of cyanide in tissues of many plants [19]. Cyanide can be a phytotoxic agent that inhibits enzymes involved in key metabolic processes [11–13], and plants have evolved a capacity to metabolize cyanide and prevent its potentially toxic accumulation [10,13]. The key enzyme in this process is β-cyanoalanine synthase (EC 4.4.1.9), which catalyzes the conjugation of HCN with cysteine to form hydrogen sulfide and β-cyanoalanine, which can be further metabolized to asparagine [11,12,14,19]. Following treatment with KCN in the hydroponic medium, both *D. ischaemum* biotypes were equally sensitive to the cyanide that accumulated in their shoots (Fig. 5A). But,

[9] A GR_{50} could not be calculated because the quinclorac rate for 50% root fresh weight inhibition fell beyond the range of rates tested.

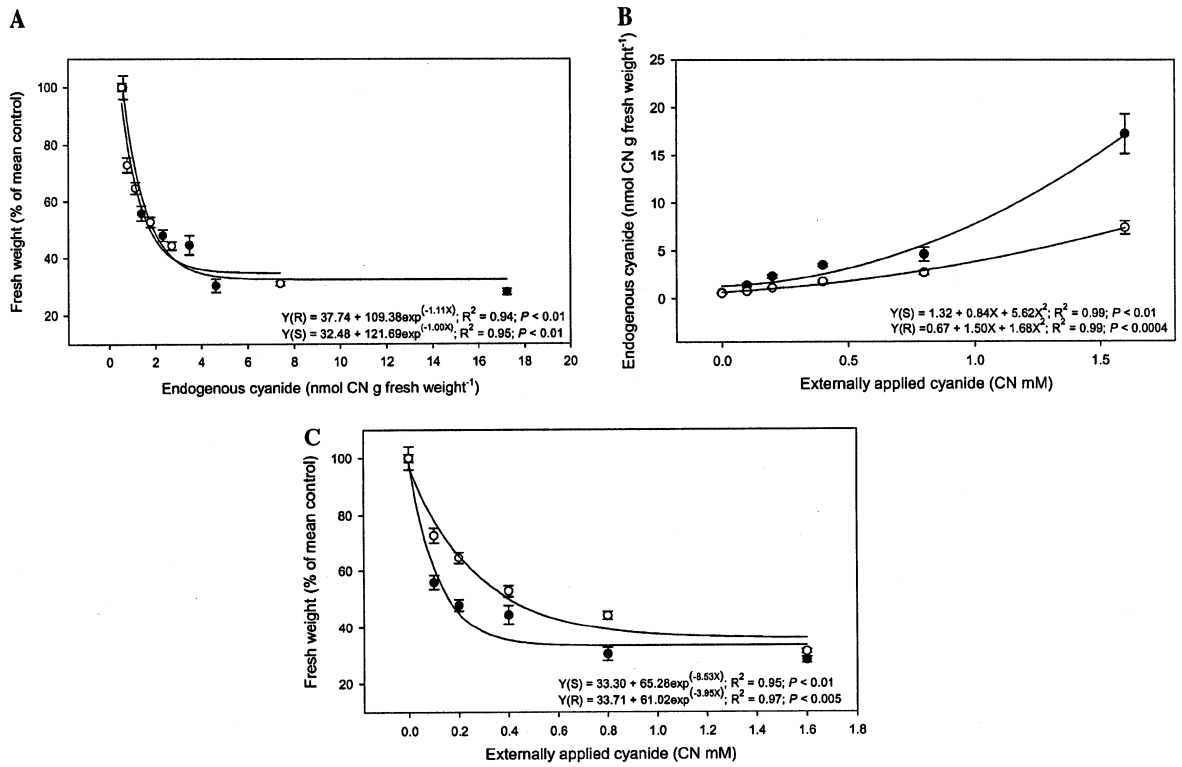

Fig. 5. Relationships between: (A) cyanide accumulation in shoots and shoot fresh weight, (B) externally applied cyanide and cyanide accumulation in shoots, and (C) externally applied cyanide and shoot fresh weight observed when KCN was added to the hydroponic medium of three-leaf R (○) and S (●) *D. ischaemum* followed by incubation for three days. Cyanide is expressed in terms of the CN anion and fresh weight as percent of the mean fresh weight of untreated control plants. A lack-of fit-test at $P = 0.05$ (Chow 21) suggested that data in (C) should be described by two different regression lines. Data are from two separate experiments and vertical bars represent the standard error of the mean.

the S biotype accumulated more cyanide (Fig. 5B), and was ultimately more suppressed than the R biotype (Fig. 5C). Differences in root absorption and translocation of cyanide between both biotypes were not studied, but basal β-cyanoalanine synthase activity was almost four times higher in R than in S shoots (Table 2). This difference became even greater when plants were pre-treated with quinclorac. Thus, the higher ability of young R *D. ischaemum* shoots to detoxify cyanide appears as an additional mechanism that can potentially contribute to its resistance to quinclorac. Previous research has shown [14] higher β-cyanoalanine synthase activity in shoots of the quinclorac-tolerant rice (*Oryza sativa*) than in those of susceptible *Echinochloa crus-galli* and *D. sanguinalis*; rice accumulated less cyanide and suffered less damage after treatment with KCN. These authors concluded that the higher β-cyanoalanine synthase

Table 2
Activity of β-cyanoalanine synthase in quinclorac-treated and untreated tissue of resistant and susceptible *Digitaria ischaemum* biotypes

Biotype	No quinclorac (nmol H_2S g^{-1} fresh weight^{-1} min^{-1})	Quinclorac 3.36 kg a.i. ha^{-1} (nmol H_2S g^{-1} fresh weight^{-1} min^{-1})
Susceptible	0.61 ± 0.06[a]	0.19 ± 0.03
Resistant	2.26 ± 0.05	2.17 ± 0.05
LSD (0.05)[b]	0.14	

[a] Values are means ± standard error of six observations from two separate experiments.
[b] Fisher's protected LSD ($P = 0.05$).

activity endowed *O. sativa* with another mechanism of selectivity towards quinclorac in addition to its lower target site sensitivity.

In conclusion, we propose that the mechanism of resistance to quinclorac in the R biotype is primarily a failure of the auxin pathway leading to the induction of ACC synthase and ethylene biosynthesis. Cyanide is a by-product of ethylene biosynthesis, and the toxic effects of quinclorac on susceptible *D. ischaemum* result from cyanide accumulation in plant tissues. In addition, we found that higher β-cyanoalanine synthase activity rates in the R biotype may contribute to resistance through increased cyanide detoxification. This biotype (R) may not have evolved through selection by continuous quinclorac use; this has also been observed with quinclorac-resistant *Echinochloa* spp. [3]. According to this author, plants in relative stress-free environments, like rice paddies, may not have evolved the need to produce ethylene via induction of ACC synthase activity for responding to stress. This hypothesis may relate to the ecology of the well groomed experiment station fields where the R biotype was found, or to favorable environments in turfgrass areas where this species is often a weed [27].

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References

[1] K. Grossmann, Quinclorac belongs to a new class of highly selective auxin herbicides, Weed Sci. 46 (1998) 707–716.

[2] W.J. Chism, S.W. Bingham, R.L. Shaver, Uptake, translocation, and metabolism of quinclorac in two grass species, Weed Technol. 5 (1991) 771–775.

[3] K. Grosmann, The mode of action of quinclorac: a case study of a new auxin-type herbicide, in: A.H. Cobb, R.C. Kirkwood (Eds.), Herbicides and their Mechanism of Action, CRC, Boca Raton, FL, 2000, pp. 181–214.

[4] N. López-Martínez, R.H. Shimabukuro, R. De Prado, Effect of quinclorac on auxin-induced growth, transmembrane proton gradient and ethylene biosynthesis in *Echinochloa* spp, Aust. J. Plant Physiol. 25 (1998) 851–857.

[5] O. Schmidt, O. Aurich, N. López-Martínez, R. De Prado, H. Walter, Botanical identification of Spanish *Echinochloa* biotypes with differential responses to quinclorac, Sixth EWRS Mediterraneum Symposium, Montpellier, France (1998), p. 232.

[6] S.J. Koo, J.C. Neal, J.M. Di Tomaso, Quiclorac-induced electrolyte leakage in seedling grasses, Weed Sci. 42 (1994) 1–7.

[7] I. Heap, The International Survey of Herbicide Resistant Weeds, Online, Internet, January 2, 2005, Available from <http://www.weedscience.org>.

[8] K. Grossmann, Highly selective, auxin herbicides of the quinolecarboxylic acid type. The mode of action of the rice herbicide quinclorac (Facet®), in: S.G. Pandalay (Ed.), Recent Research Development in Plant Physiology, 1, Research Singpost, Trivandrum (India), 1987, pp. 45–53.

[9] G.D. Peiser, T.T. Wang, N.E. Hoffman, S.F. Yang, H.W. Liu, C.T. Walsh, Formation of cyanide from carbon 1 of 1-aminocyclopropane-1-carboxylic acid during its conversion to ethylene, Proc. Natl. Acad. Sci. USA 81 (1984) 3059–3063.

[10] F.L. Tittle, J.S. Goudey, M.S. Spencer, Effect of 2,4-dichlorophenoxyacetic acid on endogenous cyanide, β-cyanoalanine synthase activity, and ethylene evolution in seedlings of soybean and barley, Plant Physiol. 94 (1990) 1143–1148.

[11] J.M. Miller, E.E. Conn, Metabolism of hydrogen cyanide by higher plants, Plant Physiol. 65 (1980) 1199–1202.

[12] W.K. Yip, S.F. Yang, Cyanide metabolism in relation to ethylene production in plant tissues, Plant Physiol. 88 (1988) 473–476.

[13] K. Grossmann, A role for cyanide, derived from ethylene biosynthesis, in the development of stress symptoms, Physiol. Plantarum 97 (1996) 772–775.

[14] K. Grossmann, J. Kwiatkowski, Selective induction of ethylene and cyanide biosynthesis appears to be involved in the selectivity of the herbicide quinclorac between rice and barnyardgrass, J. Plant Physiol. 142 (1993) 457–466.

[15] K. Grossmann, J. Kwiatkowski, The mechanism of quinclorac selectivity in grasses, Pestic. Biochem. Physiol. 66 (2000) 83–91.

[16] S.J. Koo, J.C. Neal, J. DiTomaso, Mechanism of action and selectivity of quinclorac in grass roots, Pestic. Biochem. Physiol. 57 (1997) 44–53.

[17] D.R. Hoagland, D.I. Arnon, The water-culture method for growing plants without soil, University of California Agric. Exp. Stn. Circ 347, University of California, Berkeley, California (1950).

[18] S.F. Yang, N.E. Hoffmann, Ethylene biosynthesis and its regulation in higher plants, Annu. Rev. Plant Physiol. 35 (1984) 155–189.

[19] J.S. Goudey, F.L. Tittle, M.S. Spencer, A role for ethylene in the metabolism of cyanide by higher plants, Plant Physiol. 89 (1989) 1306–1310.

[20] S.G. Blumenthal, H.R. Hendrickson, Y.P. Abrol, E.E. Conn, Cyanide metabolism in higher plants III. The biosynthesis of β-cyanoalanine, J. Biol. Chem. 243 (1968) 5302–5307.

[21] G.C. Chow, Tests of equality between sets of coefficients in two linear regressions, Econometrica 28 (1960) 591–605.

[22] J.C. Streibig, M. Rudemo, J.E. Jensen, Dose–response curves and statistical models, in: J.C. Streibig, P. Kudsk (Eds.), Herbicide Bioassays, CRC, Boca Raton, FL, 1993, pp. 29–55.

[23] F.B. Abeles, P.W. Morgan, M.E. Saltveit, Ethylene in Plant Biology, second ed., vol. 39, Academic Press, London, 1992, pp. 49–51.

[24] N. Amrhein, D. Wenker, Novel inhibitors of ethylene production in higher plants, Plant Cell Physiol. 20 (1979) 1635–1642.

[25] K. Grossmann, F. Scheltrup, Selective induction of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity is involved in the selectivity of the auxin herbicide quinclorac between barnyardgrass and rice, Pestic. Biochem. Physiol. 58 (1997) 145–153.

[26] L. Taiz, E. Zeiger, Plant Physiology, third ed., Sinauer Associates, Sunderland, MA, 2002, pp. 519–538.

[27] P. Dernoeden, C. Bigelow, J.E. Kaminski, J. Krouse, Smooth crabgrass control and creeping bentgrass tolerance to quinclorac, HortScience 38 (2003) 607–612.