Plant Physiol. (1990) 92, 1220-1225
0032-0889/90/92/1220/06/$01.00/0

Received for publication September 1, 1989
and in revised form December 6, 1989

Selection and Characterization of Sethoxydim-Tolerant Maize Tissue Cultures[1]

William B. Parker[2], David A. Somers*, Donald L. Wyse, Robin A. Keith, James D. Burton, John W. Gronwald, and Burle G. Gengenbach

Department of Agronomy and Plant Genetics, University of Minnesota and U.S. Department of Agriculture-Agricultural Research Service, St. Paul, Minnesota 55108

ABSTRACT

'Black Mexican Sweet' (BMS) maize (*Zea mays* L.) tissue cultures were selected for tolerance to sethoxydim. Sethoxydim, a cyclohexanedione, and haloxyfop, an aryloxyphenoxypropionate, exert herbicidal activity on most monocots including maize by inhibiting acetyl-coenzyme A carboxylase (ACCase). Selected line B10S grew on medium containing 10 micromolar sethoxydim. Lines B50S and B100S were subsequent selections from B10S that grew on medium containing 50 and 100 micromolar sethoxydim, respectively. Growth rates of BMS, B10S, B50S, and B100S were similar in the absence of herbicide. Herbicide concentrations reducing growth by 50% were 0.6, 4.5, 35, and 26 micromolar sethoxydim and 0.06, 0.5, 5.4, and 1.8 micromolar haloxyfop for BMS, B10S, B50S, and B100S, respectively. Sethoxydim and haloxyfop concentrations that inhibited ACCase by 50% were similar for BMS, B10S, B50S, and B100S. However, ACCase activities were 6.1, 10.7, 16.1, and 11.4 nmol HCO_3^- incorporated per milligram of protein per minute in extracts of BMS, B10S, B50S, and B100S, respectively, suggesting that increased wild-type ACCase activity conferred herbicide tolerance. Incorporation of [^{14}C]acetate into the nonpolar lipid fraction was higher for B50S than for BMS in the absence of sethoxydim providing further evidence for an increase in ACCase activity in the selected line. In the presence of 5 micromolar sethoxydim, [^{14}C]acetate incorporation by B50S was similar to that for untreated BMS. The levels of a biotin-containing polypeptide (about 220,000 molecular weight), presumably the ACCase subunit, were increased in the tissue cultures that exhibited elevated ACCase activity indicating overproduction of the ACCase enzyme.

Sethoxydim[3] is a cyclohexanedione herbicide used in dicot crops to control grass weeds (2). Haloxyfop, an aryloxyphenoxypropionate, also is phytotoxic to most grasses (27). Cyclohexanedione and aryloxyphenoxypropionate herbicides inhibit ACCase[4] from susceptible monocot species (3–5, 10, 25–27, 31). ACCase catalyzes carboxylation of acetyl-CoA to form malonyl-CoA (12). Herbicide-induced depletion of malonyl-CoA would account for the inhibition of acetate incorporation into free fatty acids observed in herbicide-treated maize chloroplasts (5, 16) and root tips (18) indicating that herbicide-induced lethality is likely due to disruption of lipid biosynthesis in susceptible monocots. The role of ACCase in determining herbicide selectivity has been demonstrated with ACCase from maize (*Zea mays* L.) seedling chloroplasts, which is inhibited 50% by 2.9 and 0.5 μM sethoxydim and haloxyfop, respectively; whereas, ACCase from pea (*Pisum sativum* L.) chloroplasts is not inhibited by 10 μM sethoxydim or 1 μM haloxyfop (4). Differential sensitivity of ACCase also confers tolerance to some monocots. Red fescue (*Festuca rubrum* L.) is 2350- and 10-fold more tolerant to sethoxydim and haloxyfop, respectively, than tall fescue (*F. arundinacea* Schreb.) (31). Tall fescue ACCase is inhibited 50% by 6.9 and 5.8 μM sethoxydim and haloxyfop, respectively; however, red fescue ACCase is inhibited 50% by 118 μM haloxyfop and is not affected by 1 mM sethoxydim.

To further investigate the role of ACCase in determining monocot susceptibility to sethoxydim and haloxyfop, we were interested in isolating and characterizing herbicide tolerant variants of maize. Because large differences in tolerance among maize genotypes to cyclohexanediones or aryloxyphenoxypropionates have not been previously reported, tissue culture selection was used to isolate variant cell lines exhibiting herbicide tolerance from BMS maize tissue cultures. BMS tissue cultures are easy to manipulate for selection experiments and would be expected to have accumulated a high frequency of tissue culture-induced mutations because the cultures were established more than 10 years ago (11). Although sethoxydim is phytotoxic to johnsongrass (*Sorghum halepense* [L.] Pers.) suspension cultures (32), its effect on maize tissue cultures has not been reported. Haloxyfop is toxic to BMS maize suspension cultures (7), but no attempt to select for sethoxydim or haloxyfop tolerance in monocot

[1] Cooperative investigation of the Minnesota Agricultural Experiment Station and the U.S. Department of Agriculture, Agricultural Research Service. Supported in part by a grant from BASF Corporation. Minnesota Agricultural Experiment Station publication No. 17,359.

[2] Present Address: Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167.

[3] Mention of a trademark, vendor, or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or the University of Minnesota and does not imply its approval to the exclusion of other products or vendors which may also be suitable.

[4] Abbreviations: ACCase, acetyl-coenzyme A carboxylase (EC 6.4.1.2); BMS, 'Black Mexican Sweet' maize; MS-2D, Murashige and Skoog medium (20) containing 2 mg L^{-1} 2,4-D; I_{50}, concentration of herbicide required to inhibit cell growth or ACCase activity by 50%; PPT, L-phosphinothricin; GS, glutamine synthetase; EPSP, 5-enolpyruvylshikimate 3-phosphate.

tissue cultures has been reported. The objectives of this study were to select sethoxydim-tolerant maize variants from BMS cultures and to determine whether herbicide tolerance was related to alterations in ACCase.

MATERIALS AND METHODS

Maize Cell Cultures

BMS maize (*Zea mays* L.) callus and suspension cultures (11) were maintained on Murashige and Skoog medium (20) supplemented with 150 mg L^{-1} L-asparagine and 2 mg L^{-1} 2,4-D at pH 5.8 (MS-2D). Liquid suspension cultures of BMS maize cells were diluted (1:20, v/v) with fresh MS-2D medium at 7-d intervals. After dilution, BMS cultures undergo a 3-d lag phase of growth followed by 6 to 7 d of logarithmic growth. BMS maize cultures also were maintained as callus on MS-2D medium containing 0.7% (w/v) agar and subcultured at 14-d intervals.

Selection for Sethoxydim Tolerance

Sethoxydim (BASF Corporation) was diluted in 95% ethanol and filter-sterilized, and 3 d after subculture was added to 80 mL of BMS suspension culture cells at a final concentration of 5 μM sethoxydim. After 4 d growth in the presence of sethoxydim, suspension cells were collected by filtration and spread onto solid MS-2D medium containing 5 μM sethoxydim. Sterile sethoxydim was added to solid medium after autoclaving. Growing cells were subcultured onto fresh agar-solidified medium containing 5 μM sethoxydim at 2-week intervals. Cell populations growing on 5 μM sethoxydim after 6 weeks were combined and transferred to solid medium containing 10 μM sethoxydim. Cell populations growing after 2 weeks on 10 μM sethoxydim were combined and designated B10S. The B10S cell line was reselected for 6 weeks for growth on MS-2D solid medium containing 50 μM sethoxydim, followed by a subsequent 6 week selection on 100 μM sethoxydim. Cell lines capable of growth on 50 and 100 μM sethoxydim were designated B50S and B100S, respectively. BMS, B10S, B50S, and B100S lines were maintained both as suspension and callus cultures in the presence and absence of sethoxydim.

Herbicide Tolerance

BMS, B10S, B50S, and B100S cells grown in the absence of herbicide for at least 14 d were collected from four to six Petri plates (approximately 20 g fresh weight for each line) and mixed to form uniform cell populations for determination of herbicide tolerance. Cells (0.5 g) were evenly spread onto sterile 7-cm Whatman No. 1 filter paper discs overlaying solid medium containing sethoxydim or haloxyfop (Dow Chemical Co). Technical grade haloxyfop was added to culture medium prior to autoclaving. After 2 weeks of growth, cells were collected and dry weights were determined after drying cells in an oven at 60°C for 48 h. Two experiments with three replicates of each herbicide concentration were conducted. Data were combined to determine mean dry weight increases and expressed as relative dry weight of the untreated tissue cultures for each herbicide concentration. The I_{50} values were determined from plots of relative dry weight and herbicide concentration for each cell line.

Acetyl-CoA Carboxylase Activity

Callus (5 g fresh weight) was collected from two Petri plates of each cell line at 4, 7, and 11 d after an initial culture period of 14 d in the absence of sethoxydim. Cells were homogenized for 10 min at 4°C in a mortar and pestle containing 10 mL 0.1 M Tricine-KOH (pH 8.3), 0.3 M glycerol, and 5 mM DTT (4, 5). The extract was filtered through two layers of Miracloth and centrifuged at 10,000*g* for 30 min. A 2.5-mL portion of each supernatant was desalted on a Sephadex G-25 column equilibrated with 0.1 M Tricine-KOH (pH 8.3), 0.3 M glycerol, and 2 mM DTT. ACCase assays were conducted at 30°C in 7-mL scintillation vials in a fume hood. The reaction mixture contained 1 mM ATP, 2.5 mM $MgCl_2$, 50 mM KCl, 0.5 mM DTT, and 15 mM NaH $^{14}CO_3$ (2146 kBq/mmol) and 100 μL enzyme preparation in a final volume of 200 μL. Sethoxydim or haloxyfop were added as 10 μL aliquots 1 min prior to initiation of the ACCase reaction. The reaction was initiated by addition of 15 mM acetyl-CoA and terminated after 5 min by addition of 30 μL of 12 N HCl. A control lacking acetyl-CoA was included. A 2-cm Whatman glass microfiber disc was added to each vial as a support to facilitate $^{14}CO_2$ removal while drying in an air stream at 40°C. After drying, 50% ethanol (0.5 mL) was added to each sample followed by 7 mL of scintillation fluid. The amount of radioactivity in the acid stable fraction was determined using a liquid scintillation counter. Previous results (31) had confirmed that the acid stable [^{14}C] product from this reaction comigrated with malonyl-CoA. Protein concentrations were determined for each extract by the BCA protein assay (29). ACCase activity was expressed as nmol HCO_3^- incorporated·mg protein^{-1}·min^{-1}. Data were combined from the three sampling times with two replicates each to determine ACCase activity means and standard errors. I_{50} values were determined from plots of ACCase activity *versus* herbicide concentration.

[1-^{14}C]Acetate Labeling

To evaluate lipid biosynthesis potential of BMS and B50S cells, the incorporation of [1-^{14}C]acetate into the nonpolar fraction was determined. Following 5 d of growth in the absence of herbicide, BMS, and B50S suspension cultures were transferred to liquid medium containing 5 μM sethoxydim and 37 kBq [1-^{14}C]acetate (2072 kBq/mmol). Following a 60 min incubation, cells (40 mL containing about 0.8 to 1.0 g fresh weight cells) were collected by vacuum filtration onto Whatman No. 1 filter paper, washed twice with 10 and 5 mL cold distilled water containing 1.5 mM unlabeled sodium acetate, weighed, and homogenized in a polytron (40 s at setting 6) in 5 mL isopropanol:chloroform (3:1, v/v). The homogenate was centrifuged (10,000*g* for 10 min), the supernatant decanted, and the extraction repeated for 20 s homogenization. Supernatants were combined and evaporated to dryness under N_2 and the residue was taken up in 5 mL chloroform:methanol (2:1, v/v) and washed with 1.25 mL 0.12 M KCl. The upper aqueous layer was removed by aspi-

ration, and the lower nonpolar fraction was washed with 0.8 mL water:methanol (1:1, v/v). Radioactivity in a 0.2 mL sample of the nonpolar lipid fraction was determined using a liquid scintillation counter and expressed as dpm mg^{-1} fresh weight cells. Three flasks of each culture line and treatment served as replicates.

SDS-PAGE and Western Blots of Biotin-Containing Proteins

The extracts used for ACCase activity assays were diluted 1:1 (v/v) with 67 mM Tris (pH 6.7) buffer containing 20% (w/v) sucrose, 10% (v/v) β-mercaptoethanol, and 8% (w/v) SDS and boiled for 1 min. SDS-PAGE was carried out in 7.5% (w/v) acrylamide and 0.19% (w/v) bisacrylamide slab gels (0.15 × 16 × 18 cm) (19). Equal amounts of protein (about 10 μg per lane) from extracts of the four cell lines were applied to the gel and electrophoresed at 20 mA for 4 to 6 h. After separation, proteins were electrophoretically transferred from the gel to a PVDF Immobilon filter (Millipore) in 20 mM Tris (pH 8.3), 150 mM glycine, and 20% (v/v) methanol at 1.5 A overnight (33). Nonspecific protein binding was blocked by washing the filter for 1 h in 20 mM (pH 7.4), 0.9% w/v NaCl Tris 0.5% v/v Tween 20 (TBST) followed by 1 h in TBST containing 1% BSA. This blocking buffer was replaced with buffer containing a 1:1000 dilution of alkaline phosphatase-conjugated avidin (Bio-Rad) for 4 h at 24°C. Biotin-containing polypeptides were visualized by the alkaline phosphatase reaction.

RESULTS AND DISCUSSION

Selection of Sethoxydim-Tolerant Maize Tissue Cultures

Growth of nonselected BMS tissue cultures was inhibited about 80% by ≥5 μM sethoxydim and about 70% by ≥0.1 μM haloxyfop as shown in Figure 1, A and B. BMS cells transferred from media containing these levels of herbicide to herbicide-free medium did not grow further, indicating these herbicide concentrations were lethal. Three sethoxydim-tolerant BMS culture lines were isolated by enrichment selection for growth on medium containing 10, 50, and 100 μM sethoxydim and were designated B10S, B50S, and B100S, respectively. B50S and B100S were subpopulations selected from B10S plated on medium containing the higher herbicide concentrations. The dry weight increases after 14 d were 107, 100, 114, and 114 mg for BMS, B10S, B50S, and B100S, respectively, in the absence of herbicide indicating that sethoxydim tolerance did not affect growth of the variant BMS cell cultures. Herbicide concentrations that reduced growth of BMS, B10S, B50S, and B100S by 50% were 0.6, 4.5, 35, and 26 μM sethoxydim and 0.06, 0.5, 5.4, and 1.8 μM haloxyfop, respectively (Fig. 1, A and B). The decreased tolerance of B100S relative to B50S indicated that there was no direct relationship between selection for growth on higher herbicide concentrations and higher tolerance.

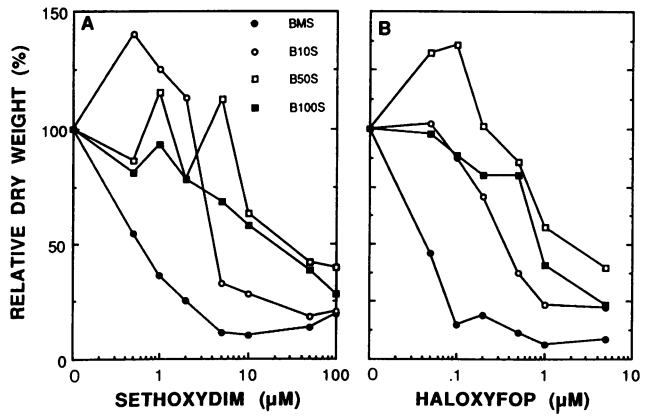

Figure 1. Effect of sethoxydim (A) and haloxyfop (B) on growth of BMS, B105, B50S, and B100S maize tissue cultures. Relative dry weight increases were determined after 14 d growth.

A subpopulation of B10S was maintained in the absence of herbicide for more than 1 year and continued to exhibit tolerance that was similar to B10S maintained on 10 μM sethoxydim during the same period (data not shown). Lines of B50S and B100S also remained tolerant to sethoxydim when maintained in the absence of herbicide. Because tolerance was retained by cells grown in the absence of herbicide, these results indicated that the variation causing herbicide tolerance was either genetic or epigenetic rather than an induced physiological response to herbicide exposure (6). However, we have subsequently observed a loss of herbicide tolerance in B10S maintained in the absence of herbicide for periods longer than 1 year, indicating that the phenotype was not completely stable.

Acetyl-Coenzyme A Carboxylase Activity

The parallel increases in tolerance of each variant cell line to sethoxydim and haloxyfop shown in Figure 1, even though selection was only for sethoxydim tolerance, indicated alterations in a site of action or a tolerance mechanism that was common to both herbicides. Sethoxydim and haloxyfop both specifically inhibit monocot ACCase (3–5, 10, 25–27, 31); therefore, the ACCase activity of the tolerant lines was compared to BMS to determine whether changes in ACCase were associated with herbicide tolerance. In the absence of herbicide, the mean ± SE specific activities of ACCase of BMS, B10S, B50S, and B100S were 6.1 ± 0.8, 10.7 ± 2.6, 16.1 ± 6.1, and 11.4 ± 3.0 nmol HCO_3^- incorporated · mg $protein^{-1}$ · min^{-1}, respectively, as shown in Figure 2, A and B; whereas, protein contents of the extracts were similar. The I_{50} estimates for sethoxydim inhibition of ACCase activity were 5.2, 7.0, 6.1 and 6.7 μM for BMS, B10S, B50S, and B100S, respectively. I_{50} estimates of haloxyfop inhibition of ACCase were 0.75, 0.94, 0.79, and 0.99 μM for BMS, B10S, B50S, and B100S. These data suggest that herbicide tolerance was likely conferred by an increase of the herbicide-sensitive, wild-type ACCase activity. For example, the concentrations of sethoxydim and haloxyfop inhibiting growth of line B50S by 50% were increased by 58-fold and 90-fold, respectively, compared to BMS. ACCase activity of B50S was elevated 260% compared to BMS ACCase; whereas, the concentrations of sethoxydim and haloxyfop that inhibited ACCase activity from B50S by 50% were increased only 17% and 5%, respectively, over the BMS I_{50} concentrations.

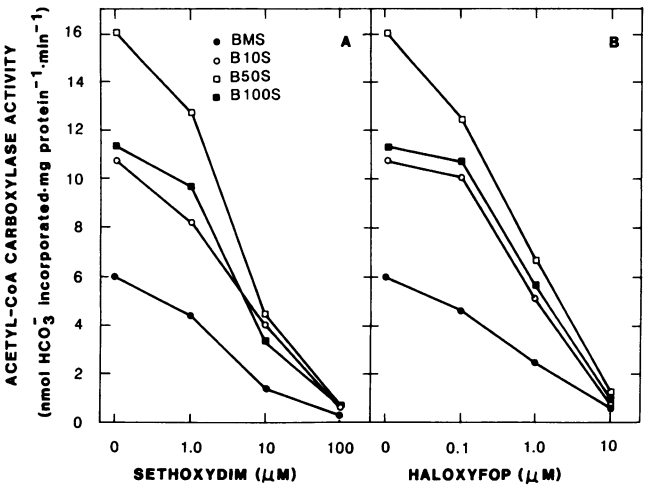

Figure 2. Effect of sethoxydim (A) and haloxyfop (B) on ACCase activity from BMS and herbicide-tolerant (B10S, B50S, B100S) maize cultures.

To provide further evidence for elevated ACCase in the selected cell lines, [1-^{14}C]acetate incorporation into the nonpolar fractions of BMS and B50S was investigated in the presence and absence of sethoxydim. The amount of [1-^{14}C] acetate incorporated during a 60 min labeling experiment was 2.6-fold greater in B50S in the absence of herbicide compared to BMS. Sethoxydim (5 μM) inhibited 87% of the [1-^{14}C] acetate incorporation of BMS cells. Acetate incorporation in herbicide-treated B50S was reduced 65% compared to untreated B50, which resulted in a level of incorporation similar to that of BMS in the absence of sethoxydim. Thus, the ability of B50S cells to maintain a normal rate of lipid biosynthesis in the presence of 5 μM sethoxydim, as indicated by [1-^{14}C] acetate incorporation, was associated with herbicide tolerance and elevated ACCase activity.

Biotin-Containing Polypeptides in Herbicide-Tolerant Cell Lines

ACCase is a biotin-containing protein in maize and other plants and therefore can be detected in Western blots using labeled or enzyme-linked avidin (22, 23). A biotin-containing protein that comigrated with highly purified ACCase (3) was detected in SDS-PAGE western blots of crude extracts from BMS and the tolerant lines as shown in Figure 3. The estimated subunit mol wt of about 220,000 differed from previous reports of subunit composition of maize leaf ACCase (22, 23), but was consistent with subunit mol wt of 210,000 and 240,000 determined for parsley (*Petroselinum hortens* Hoffm.) and wheat (*Triticum aestivum* L.) ACCase, respectively (9). Recently, it has been shown that inclusion of proteinase inhibitors and glycerol in extraction buffers used in conjunction with a rapid isolation procedure allows the resolution of a 220,000 mol wt subunit for maize leaf ACCase (14). Our ability to resolve the intact ACCase subunits from BMS cells extracted in the absence of proteinase inhibitors may have resulted from rapid extraction followed by storage of crude extracts in liquid N_2 before SDS PAGE, which would minimize proteolysis.

The amount of ACCase subunit, as indicated by intensity of alkaline phosphatase staining, was closely related to the level of ACCase activity in the various extracts, indicating that overproduction of ACCase resulted in elevated enzyme activity (Fig. 3). This experiment was conducted more than 1 year after initial isolation and characterization of the herbicide-tolerant cell lines. At this time, B10S grew more slowly than the other cell lines and was no longer sethoxydim-tolerant. ACCase activities of BMS, B10S, B50S, and B100S extracts used in Figure 3 were 3.7, 2.9, 10.1, and 7.7 nmol HCO_3^- incorporated·mg protein^{-1}·min^{-1}, respectively, indicating that the association between herbicide tolerance and levels of ACCase activity in the cell lines was maintained. Scanning reflectance quantitation of three replicate Western blots of these extracts indicated that the levels of ACCase protein of B10S, B50S, and B100S were 0.7-, 4.7-, and 2.9-fold that of BMS indicating close correspondence to the ACCase activities which were 0.8-, 2.7-, and 2.1-fold that of BMS, respectively.

Our results indicated that B50S exhibited 58-fold and 90-fold increases in tolerance to sethoxydim and haloxyfop, respectively, and that herbicide tolerance in maize BMS cultures may be conferred by a 2.6-fold increase in herbicide-sensitive ACCase activity. Similar ratios of enzyme activity increase and tolerance level have been reported in other examples of herbicide target site overproduction. An alfalfa (*Medicago sativa* L.) cell line that had a 60- to 100-fold increase in tolerance to PPT, an inhibitor of GS, only exhibited a 3- to 7-fold increase in herbicide-sensitive GS activity (8). A glyphosate-tolerant carrot (*Daucus carota*) cell line exhibited a 50-fold increase in tolerance and a 12-fold increase in EPSP synthase activity compared with the control (21). Alternatively, closer correspondence of EPSP synthase overproduction and glyphosate tolerance level were reported for a glyphosate-tolerant *Petunia hybrida* cell line (28, 30). Donn *et al.* (8) proposed that, in the case of GS, more than one molecule of herbicide binds the enzyme and that overexpressed but enzymatically inactive enzyme may also bind the

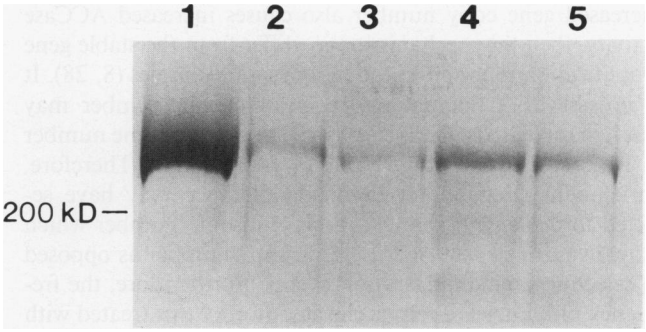

Figure 3. Biotin-containing, polypeptides detected in SDS-PAGE Western blots of total protein extracts of unselected and herbicide-tolerant maize tissue cultures using alkaline phosphatase-conjugated avidin. Highly purified BMS ACCase (lane 1) was presented for comparison of separation and detection of ACCase in extracts of BMS (lane 2), B10S (lane 3), B50S (lane 4), and B100S (lane 5). Extract protein contents were 0.53, 0.45, 0.51, and 0.49 mg/mL for BMS, B10S, B50S, and B100S, respectively. The same amounts of protein (11 μg) from the extracts were applied to each well.

herbicide. These explanations also may be applicable to our observations of relatively small increases in ACCase activity conferring herbicide tolerance. Cyclohexanedione and aryloxyphenoxypropionate herbicides are mutually exclusive noncompetitive inhibitors of monocot ACCases (3). Because these two classes of herbicides have different chemical structure, it would appear that restricted herbicide uptake or elevated herbicide detoxification may not account for cross-tolerance to sethoxydim and haloxyfop. However, a population of *Lolium rigidum* has been identified in Australia that is cross-tolerant to a number of different herbicide chemistries including aryloxyphenoxypropionates and cyclohexanediones (13). The tolerance mechanism in *L. rigidum* likely differs from that in the tolerant BMS maize tissue cultures because ACCase activity extracted from the susceptible and tolerant *L. rigidum* biotypes did not differ in activity level, affinity for acetyl-CoA or sensitivity to herbicide inhibition (15). It has been proposed that herbicide exclusion from the target site enzyme(s) or herbicide detoxification may confer cross-tolerance in the *L. rigidum* biotype. Thus, it may be possible that herbicide exclusion or detoxification may contribute to the tolerance to sethoxydim and haloxyfop conferred by elevated ACCase activity in the herbicide-tolerant BMS maize cell cultures.

The major change in ACCase, the target site enzyme of sethoxydim and haloxyfop, observed in the herbicide-tolerant tissue cultures was an increase in the levels of activity due to overproduction of the enzyme. Determining the genetic mechanism(s) accounting for overproduction of ACCase in the sethoxydim-tolerant BMS variants is not currently possible because the maize ACCase gene(s) has not been isolated. Similar to both the PPT- and glyphosate-tolerant cell lines, our herbicide-tolerant maize culture lines were selected from nonregenerable tissue cultures that had been in culture for prolonged periods resulting in accumulation of genetic and chromosomal changes. Furthermore, stepwise increases in herbicide concentration were used to select the variants in each case. Elevation of GS and EPSP synthase in the respective PPT- and glyphosate-tolerant variants is due to gene amplification in both cases (8, 28). If a mechanism(s) causing increased gene copy number also causes increased ACCase activity, then the mechanism may differ from the stable gene amplification reported in the other plant examples (8, 28). It is possible that fluctuations in chromosome number may result in increased ACCase activity. The chromosome number in BMS suspension culture cells is variable (24). Therefore, enrichment selection for herbicide-tolerance may have selected for cells with changes in chromosome number which may, in turn, elevate ACCase gene copy number as opposed to selecting gene amplification events. Furthermore, the frequency of binucleate cells is elevated in root tips treated with sethoxydim, suggesting that the herbicide inhibits cell plate and wall formation (1, 17) which may result in increased chromosome numbers during herbicide selection. Further investigation of the relationships between herbicide tolerance, ACCase activity, and chromosome number will be required to elucidate a mechanism for increased ACCase levels in the variant cell lines.

Plant ACCase activity is modulated by adenine nucleotides, Mg^{2+}, and pH and may be rate limiting for *de novo* fatty acid synthesis (12). In the absence of herbicide, B50S cell line incorporated 2.6-fold more acetate into nonpolar lipids than BMS, presumably due to its 2.6-fold increase of ACCase activity. These results suggest that ACCase may be a rate limiting step in fatty acid biosynthesis in BMS cells. Therefore, it will be of interest to investigate the effect of elevated ACCase activity on cellular lipid biosynthesis in these herbicide-tolerant maize tissue cultures.

LITERATURE CITED

1. **Asare-Boamah NK, Fletcher RA** (1983) Physiological and cytological effects of BAS 9052 OH on corn (*Zea mays*) seedlings. Weed Sci **81:** 49–55
2. **Beste CE, Humburg NE, Kempen HM, Radke RO, Riggleman JD, Stritzke JF, Miller GR** (1983) Herbicide Handbook of the Weed Science Society of America, Ed 5. WSSA, Champaign, IL
3. **Burton JD, Gronwald JW, Keith R, Somers DA, Gengenbach BG, Wyse DL** (1989) Kinetics of inhibition of acetyl-CoA carboxylase by sethoxydim and haloxyfop (abstract No. 320). Plant Physiol **89:** S-54
4. **Burton JD, Gronwald JW, Somers DA, Connelly JA, Gengenbach BG, Wyse DL** (1987) Inhibition of plant acetyl-Coenzyme A carboxylase by the herbicides sethoxydim and haloxyfop. Biochem Biophys Res Commun **148:** 1039–1044
5. **Burton JD, Gronwald JW, Somers DA, Gengenbach BG, Wyse DL** (1989) Inhibition of corn acetyl-CoA carboxylase by cyclohexanedione and aryloxyphenoxypropionate herbicides. Pestic Biochem Physiol **34:** 76–85
6. **Chaleff RS, Carlson PS** (1975) In vitro selection for mutants of higher plants. *In* L Ledoux, ed, Genetic Manipulations with Plant Material. Plenum Press, New York, pp 351–363
7. **Cho H-Y, Widholm FM, Slife JW** (1986) Effects of haloxyfop on corn (*Zea mays*) and soybean (*Glycine max*) cell suspension cultures. Weed Sci **34:** 496–501
8. **Donn G, Tischer E, Smith JA, Goodman HM** (1984) Herbicide-resistant alfalfa cells: an example of gene amplification in plants. J Mol Appl Genet **2:** 621–635
9. **Egin-Buhler B, Loyal R, Ebel J** (1980) Comparison of acetyl-CoA carboxylase from parsley cell cultures and wheat germ. Arch Biochem Biophys **203:** 90–100
10. **Focke M, Lichtenthaler HK** (1987) Inhibition of the acetyl-CoA carboxylase of barley chloroplasts by cycloxydim and sethoxydim. Z Naturforsch **42:** 93–95
11. **Green CE** (1977) Prospects for crop improvement in the field of cell culture. Hortic Sci **12:** 7–10
12. **Harwood JL** (1988) Fatty acid metabolism. Annu Rev Plant Physiol Plant Mol Biol **39:** 101–138
13. **Heap I, Knight R** (1989) The occurrence of herbicide cross-resistance in a population of annual ryegrass, *Lolium rigidum*, resistant to diclofop-methyl. Aust J Agric Res **37:** 149–156
14. **Hellyer A, Bambridge HE, Slabas AR** (1986) Plant acetyl-CoA carboxylase. Biochem Soc Trans **14:** 565–568
15. **Holtum JAM, Matthews JM, Liljegren DR, Powles SR** (1989) On the mechanism of resistance to aryloxyphenoxypropionate, cyclohexanedione and sulfonylurea herbicides in annual ryegrass (*Lolium rigidum*). *In* Herbicide resistance in weeds and crops. Abstracts of the 11th Long Ashton International Symposium. University of Bristol, England, p 63
16. **Hoppe HH, Zacher H** (1985) Inhibition of fatty acid biosynthesis in isolated bean and maize chloroplasts by herbicidal phenoxylphenoxypropionic acid derivatives and structurally related compounds. Pestic Biochem Physiol **24:** 298–305
17. **Hosaka H, Inaba H, Satoh A, Ishikawa H** (1984) Morphological and histological effects of sethoxydim on corn (*Zea mays*) seedlings. Weed Sci **32:** 711–721
18. **Hosaka H, Takagi MK** (1987) Biochemical effects of sethoxydim in excised root tips of corn (*Zea mays*). Weed Sci **35:** 612–618
19. **Laemmli UK** (1970) Cleavage of structural proteins during the

assembly of the head of bacteriophage T4. Nature **227:** 680–685

20. **Murashige T, Skoog F** (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant **15:** 473–497
21. **Nafziger EM, Widholm JM, Steinrucken HC, Killmer JL** (1984) Selection and characterization of a carrot cell line tolerant to glyphosate. Plant Physiol **76:** 571–574
22. **Nikolau BJ, Hawke JC** (1984) Purification and characterization of maize leaf acetyl-coenzyme A carboxylase. Arch Biochem Biophys **228:** 86–96
23. **Nikolau BJ, Wurtele ES, Stumpf PK** (1984) Tissue distribution of acetyl-coenzyme A carboxylase in leaves. Plant Physiol **75:** 895–901
24. **Phillips RL, Somers DA, Hibberd KA** (1989) Tissue culture and *in vitro* genetic manipulations. *In* GF Sprague, JW Dudley, eds, Corn and Corn Improvement. American Society of Agronomy, Madison, WI, pp 345–387
25. **Rendina AR, Felts JM** (1988) Cyclohexanedione herbicides are selective and potent inhibitors of acetyl-CoA carboxylase from grasses. Plant Physiol **86:** 983–986
26. **Rendina AR, Felts JM, Beaudoin JD, Craig-Kennard AC, Look LL, Paraskos SL, Hagenah JA** (1988) Kinetic characterization, stereoselectivity and species selectivity of the inhibition of plant acetyl-CoA carboxylase by the aryloxyphenoxypropionic acid grass herbicides. Arch Biochem Biophys **265:** 219–225
27. **Secor J, Cseke C** (1988) Inhibition of acetyl-CoA carboxylase activity by haloxyfop and tralkoxydim. Plant Physiol **86:** 10–12
28. **Shah DM, Horsch RB, Klee HJ, Kishore GM, Winter JA, Tumer NE, Hironaka CM, Sanders PR, Gasser CS, Aykent S, Siegel NR, Rogers SG, Fraley RT** (1986) Engineering herbicide tolerance in transgenic plants. Science **233:** 478–481
29. **Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC** (1985) Measurement of protein using bicinchonicic acid. Anal Biochem **150:** 76–85
30. **Steinrucken HC, Schulz A, Amrhein N, Porter CA, Fraley RT** (1986) Overproduction of 5-enolpyruvylshikimate-3-phosphate synthase in a glyphosate-tolerant petunia hybrid cell line. Arch Biochem Biophys **244:** 169–178
31. **Stoltenberg DE, Gronwald JW, Wyse DL, Burton JD, Somers DA, Gengenbach BG** (1989) The effect of sethoxydim and haloxyfop on acetyl-coenzyme A carboxylase activity in tolerant and susceptible *Festuca* species. Weed Sci **37:** 512–516
32. **Swisher BA, Corbin FT** (1982) Behavior of BAS-9052 OH in soybean (*Glycine max*) and johnsongrass (*Sorghum halepense*) plant and cell cultures. Weed Sci **30:** 640–650
33. **Towbin H, Staehelin T, Gordon J** (1979) Electrophoretic transfer of protein and nucleic acids from slab gels to diazobenzylmethyl cellulose and nitrocellulose: procedure and some applications. Proc Natl Acad Sci USA **76:** 4350–4354