## CHAPTER FOUR

## DETACHED SHOOT AND SITE OF ACTION STUDIES


### ABSTRACT

Intact plants of large crabgrass and goosegrass were treated with 0.56 kg ha $^{-1}$ of quinclorac and 1% v/v of "Merge" spray adjuvant. Immediately after the spray had dried, plant shoots were excised at the soil surface and placed in vials containing nutrient solution. Plants were maintained under greenhouse conditions. Six days after treatment, shoot fresh weights and visual injury ratings were recorded. Injury response of large crabgrass was similar to that observed with treatment to intact plants (in previous studies) with 94% visual injury and a fresh weight reduction of 65%. Response of goosegrass was different than that observed with intact plants. Visual injury was 75% with a fresh weight reduction of 25%. Very little effects were noted at this evaluated rate (and higher) in previous work conducted with intact plants.

Additional work was conducted evaluating the response of both species to applied 1-aminocyclopropane -1-carboxylic acid (ACC). The stimulation of ACC synthase has been a proposed main mechanism of action of quinclorac.

The subsequent oxidation of ACC leads to the production of ethylene and hydrogen cyanide (HCN) in stoichimetrically equivalent amounts. The formation of HCN has been proposed to be the lethal agent resulting from applications of quinclorac in sensitive species. Exposure over a six day period to root applied ACC at 10mM to intact large crabgrass plants showed similar visual response to that of foliar applied quinclorac. No visual effects were noted to goosegrass. Results support the proposed model for the mode of action with quinclorac in the case of large crabgrass. Results of the detached shoot studies with goosegrass suggested that translocation may play a vital role in the detoxification of quinclorac. The lack of goosegrass response to applied ACC suggested that goosegrass may have a higher tolerance level for HCN, or may possess more efficient detoxifying mechanisms.

## INTRODUCTION

Large crabgrass and goosegrass differ in their tolerance to quinclorac herbicide. Large crabgrass has been found to be sensitive, while goosegrass has been found to be quite tolerant. Investigations into differences in spray retention, absorption, and metabolism failed to reveal the actual mode of differential tolerance (Chapter 3). $^{14}C$ translocation studies did show that goosegrass translocated more $^{14}C$ quinclorac out of the treated leaf than large crabgrass. The main deposition sites of transported $^{14}C$ - quinclorac were the tillers and the crown and new leaf tissue. Differences in translocation are possible mechanisms for observed differences in response of weed species to herbicides (3,8,10). Additionally, results from the previous studies of this project (Chapter3) suggested that the differential tolerance between large crabgrass and goosegrass may involve physiological differences at the site of action.

Several papers have dealt with the investigation of the mode of action of quinclorac (5,13,14,15,16,17,18,19,22). The leading theory today has been proposed by Grossmann *et al.*(13,14,15,16) which strongly suggested that the synthesis of ACC (1-aminocyclopropane-1-carboxylic acid) and its subsequent oxidation into ethylene and cyanide is the key mechanism for the response observed with applied quinclorac.

Based on this theory, one could postulate that along this chain of reactions, the effect of quinclorac in large crabgrass is different than in goosegrass. Fig. 1 outlines the entire range of the major reactions that involve ACC and its subsequent oxidation and fate of co-products. One could speculate that, in goosegrass, quinclorac does not induce ACC synthase, thereby not allowing for the accumulation of ACC and subsequent oxidation to HCN. Alternatively, the induction may occur and ACC is formed and oxidized to ethylene and HCN as in large crabgrass. However, it may be that the activity and/or endogenous concentration of ß-cyanoalanine synthase (the major detoxifying enzyme for HCN) is higher in goosegrass than large crabgrass.

Another possible explanation of the tolerance exhibited by goosegrass may entail the alternate pathway of the metabolism of ACC to MACC as describe by Peiser *et al*. (26). If in goosegrass this mechanism is favored over the oxidation to ethylene and HCN, the accumulation of free HCN would be avoided along with its subsequent toxic effects. One also has to speculate on the role of the endogenous levels and synthesis formation of cysteine within the plant. Since this amino acid is the key substrate that is needed to trap the free HCN, its concentration within the plant would affect the efficacy of ß-cyanoalanine synthase and the capacity to trap the free cyanide.

The objectives of these studies were to investigate the response of detached shoots of large crabgrass and goosegrass to applied quinclorac and to evaluate the response of both species to applied ACC. A major assumption in this experiment was that some of the ACC would be converted to free HCN in the plant causing the phytotoxic effects.

## METHODS AND MATERIALS

### Detached Shoot Studies :

Both large crabgrass and goosegrass plants were cultured as previously described (Chapter 2). Quinclorac was applied at 0.56 kg ai $ha^{-1}$ when plants reached the one to two-tiller stage. "Merge" spray adjuvant was also added at a 1% (v/v) of the spray volume. Spray applications were made with an overhead track sprayer set to deliver 748 l $ha^{-1}$ at an operating pressure of 275 kPa using an 8004 even flat fan nozzle. Immediately after the spray dried, plants were excised at the soil surface and transferred into amber vials (100 ml) that contained 70 ml of a 0.1X Hoagland nutrient solution. Plants were supported in the vials by loosely fastening to plastic support stakes. Plants were maintained under incandescent lighting and temperature was maintained at 24 $^{0}C$ for the duration of the experiment. Visual injury ratings were taken at 2, 3 and 6 days after treatment. Plant fresh weights were also measured at 6 days after treatment.

### Data Analysis.

The experiment was conducted twice and consisted of 4 replications (one plant per replication) and was arranged as a Completely Randomized Design.

Data were subjected to analysis of variance and means separated using Fisher's Protected LSD at $\alpha$ =0.05. Data were combined across experiments since no experimental interactions were detected.

**Site of Action Studies :**

Large crabgrass and goosegrass plants were seeded and cultured as described in the translocation and absorption studies. A 10 mM stock solution of ACC was prepared using millipore water. The ACC rate selected was based on work conducted by Yip and Yang (35) with mungbean.
After removal of soil in the water bath, plants of both species were transferred into 15 ml centrifuge tubes containing either 10 mM ACC solution or millipore water. Plants were supported by means of a foam sleeve. No nutrient solution was introduced as to the unknown nature of possible interaction/ degradation that may occur with ACC. The experiment consisted of three treatments : untreated (millipore water), ACC, and foliar applied quinclorac at 0.56 kg $ha^{-1}$.

The quinclorac was only applied to plants immersed in millipore water. Quinclorac was applied with "Merge" adjuvant at 1% v/v using the spray chamber setup that was previously described in other sections (Chapter 2). Tubes were kept under greenhouse conditions as previously described (Chapter 2). Since no aeration was available, plants were supported with a portion of the root tissue above the solution level in the tubes. Tubes were checked each day and maintained at a constant volume with either millipore water or ACC.

To aid in aeration, as new solution was added, the entire volume of each tube was carefully removed momentarily by syringe. As the solution was reentered, air bubbles were introduced into the tubes via the syringe. At five days after treatment photographs of each treatment were taken.

## RESULTS AND DISCUSSION

### Detached Shoot Studies :

Results of the detached shoot studies are presented in Table 1 and Figures 1 & 2. The data showed that phytotoxicity was evident in both species at the 0.56 kg $ha^{-1}$ rate. Phytotoxicity was higher for large crabgrass than goosegrass evaluated either on a visual or fresh weight basis. These results concur with previous studies that showed that large crabgrass was more sensitive to quinclorac than goosegrass. However, the difference in these detached shoot studies was the degree of injury to goosegrass. Very little injury was ever observed in studies on intact goosegrass plants treated with quinclorac within prospective labeled rates (22). The detached shoots were considerably more sensitive than effects observed on intact plants. These results suggested that confinement of quinclorac to the treated goosegrass shoots may have an impact on the tolerance mechanism to quinclorac. The response observed with goosegrass may somehow be related to stress-induced ethylene production as described by Yang and Hoffman (32). Yang and Hoffman suggested that stress induced ethylene can be caused by factors such as wounding, cutting, chilling, etc.

The other observation was that the effect on large crabgrass we observed was in contrast with work conducted by Grossmann *et al.* (14) on another sensitive grass species, barnyardgrass. Detached shoots of barnyardgrass were found to be very tolerant to applied quinclorac. These observed differences may just be species specific or may have something to do with application techniques. In Grossmann's studies, the detached shoots were not treated with a conventional foliar spray, but rather exposed to solution concentrations of quinclorac in reagent tubes (14).

**Site of action studies :**

Results of applied ACC are presented in Figures 2 and 3. Exposure to ACC caused similar phytotoxic effects to large crabgrass as observed with quinclorac (Fig. 2). However, results showed that there was little observable effect of either ACC or the applied quinclorac to the goosegrass plants (Fig. 3). In the case of large crabgrass, these observations supported Grossmann's proposed model on the role of ACC and the mode of action of quinclorac (13). In the case of resistant grasses, Grossmann proposed that ACC synthase is not stimulated in the root and therefore, the subsequent effects of resultant HCN are not produced (13). One may speculate that since we observed little effect of the applied ACC to goosegrass, and one assumes that absorption occurred, there may be other mechanisms that resistant plants employ to avoid toxicity to quinclorac.

*Table 1*. Herbicidal effects of foliar applied quinclorac [1] to detached shoots of large crabgrass and goosegrass.

| Species | Rate | Visual Injury | Fresh Weight |
|---|---|---|---|
| | kg ha$^{-1}$ | % control | % of untreated |
| Lg. Crabgrass | 0 | 0 | 100 |
| | 0.56 | 94 | 35 |
| Goosegrass | 0 | 0 | 100 |
| | 0.56 | 75 | 70 |
| LSD (0.05) | | 6 | 13 |

[1] quinclorac applied @ 0.56 kg ha$^{-1}$ plus "Merge" @ 1% v/v

- Ultimate source of ethylene : derived from methionine (Adams and Yang [1]).

- Report by Adams and Yang (1) implicated SAM (*S*-adenosyl-l-methionine) as intermediate between methionine and ethylene . This lead to their discovery of a unique amino acid : 1-aminocyclopropane-1-carboxylic acid that was an immediate precursor to ethylene.

Methionine $\xrightarrow{\text{ACC synthase}}$ SAM $\longrightarrow$ ACC $\rightarrow$ Ethylene (from Apelbaum, *et al* [4]).

ACC $\xrightarrow{\text{ACC oxidase}}$ Ethylene + HCN

Carbon atoms 2 and 3 from ACC give rise to ethylene, carboxyl group and carbon 1 gives rise to carbon dioxide and cyanide (Peiser, *et al.* [26] & Yang and Hoffman [32])

Cysteine + HCN $\xrightarrow{\text{ßCAS (ß-cyanoalanine synthase)}}$ ß-cyanoalanine + $H_2S$ $\xrightarrow{\text{ß-cyanoalanine hydratase}}$ Asparagine

Blumenthal-Goldschmidt, *et al.* [6] Castric, *et al.* [9]

*Figure 1.* Schematic overview of the fate of cyanide derived from 1-aminocycclopropane -1-carboxylic acid (ACC).

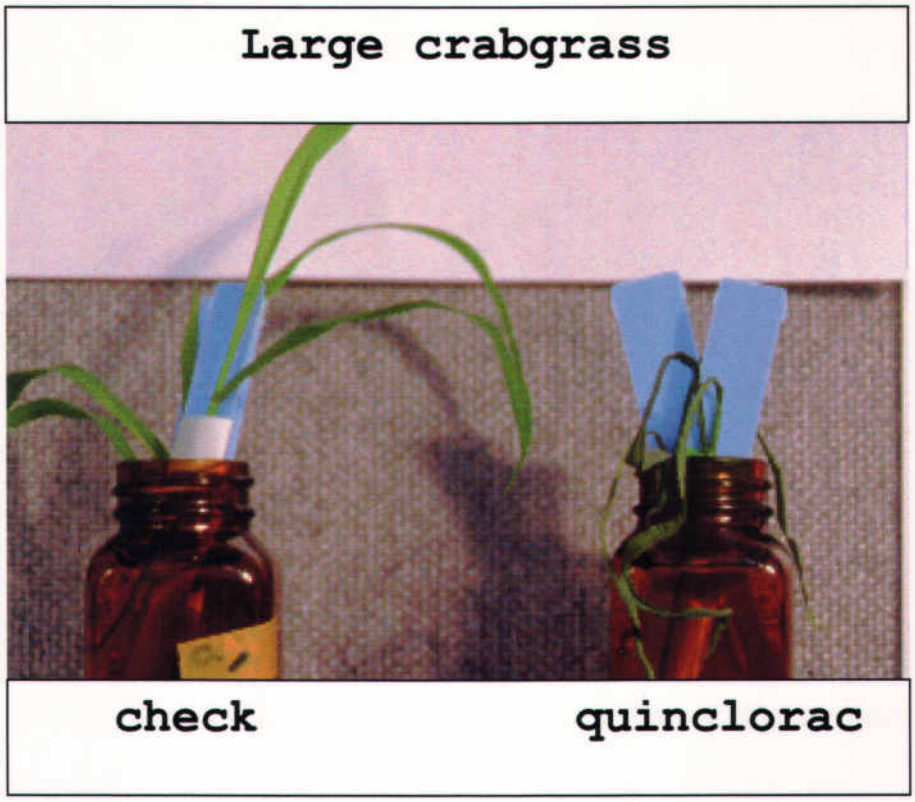


*Figure 2.* Influence of applied quinclorac at 0.56 kg $ha^{-1}$ to detached shoot tissue of large crabgrass.

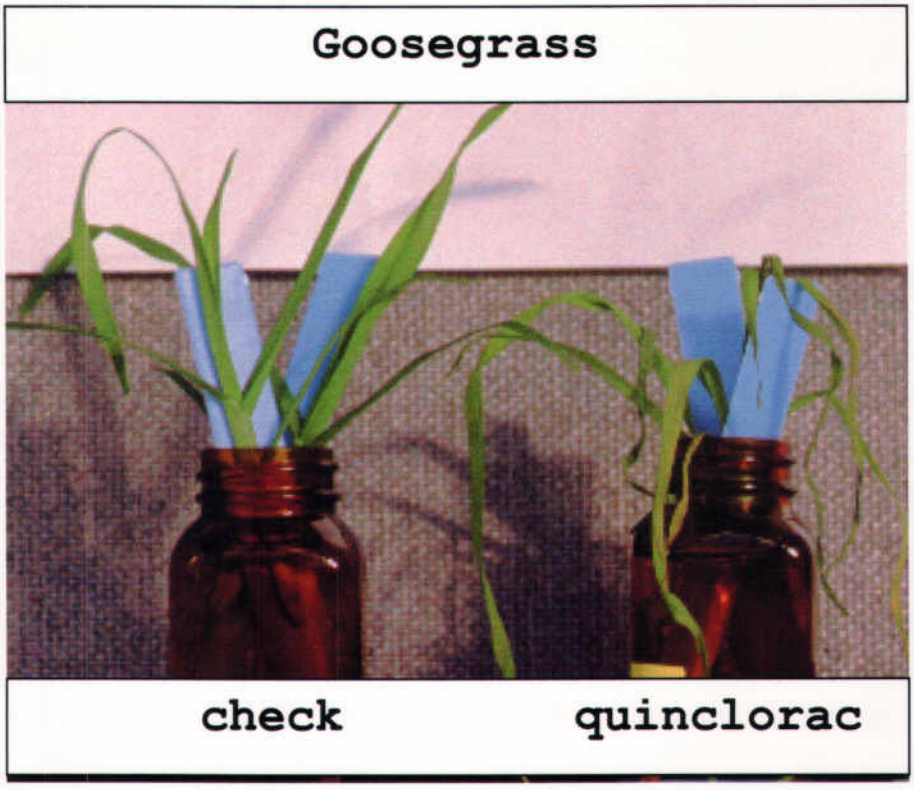


*Figure 3.* Influence of applied quinclorac at 0.56 kg $ha^{-1}$ to detached shoot tissue of goosegrass.

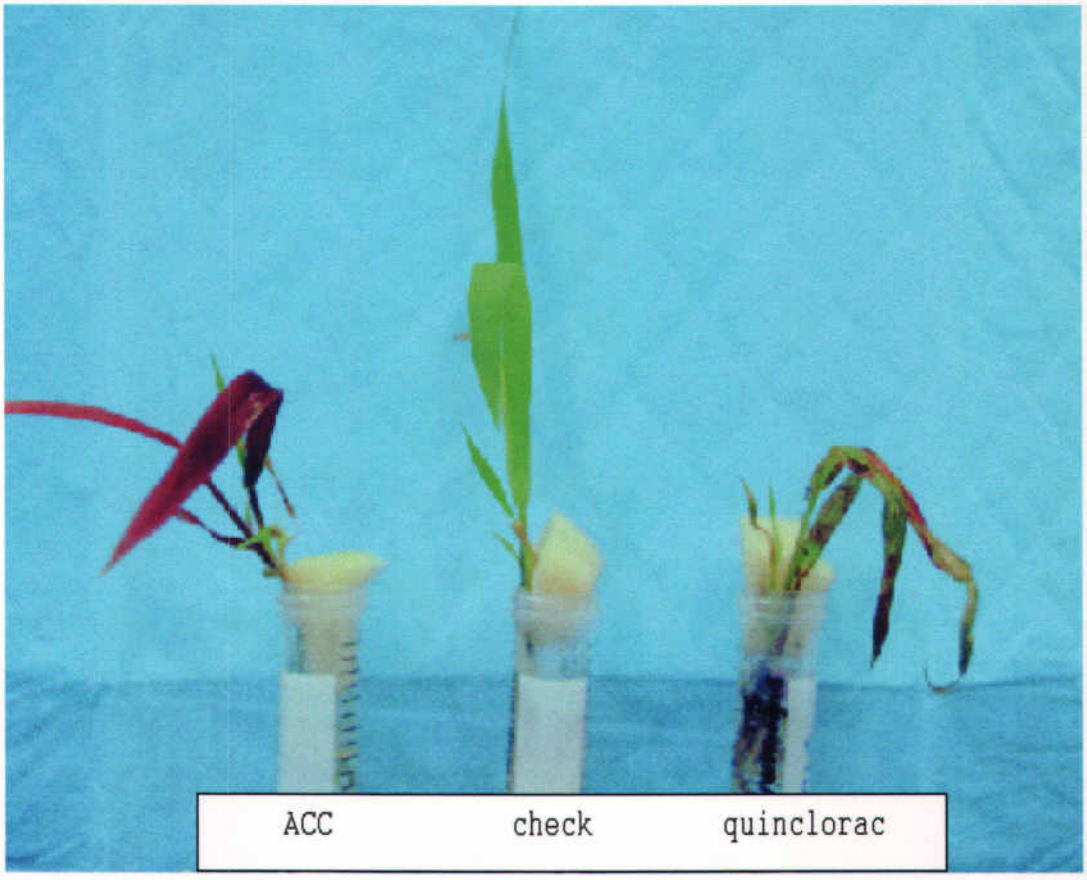


*Figure 4.* Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) at 10mM and quinclorac at 0.56 kg ha $^{-1}$ to large crabgrass.

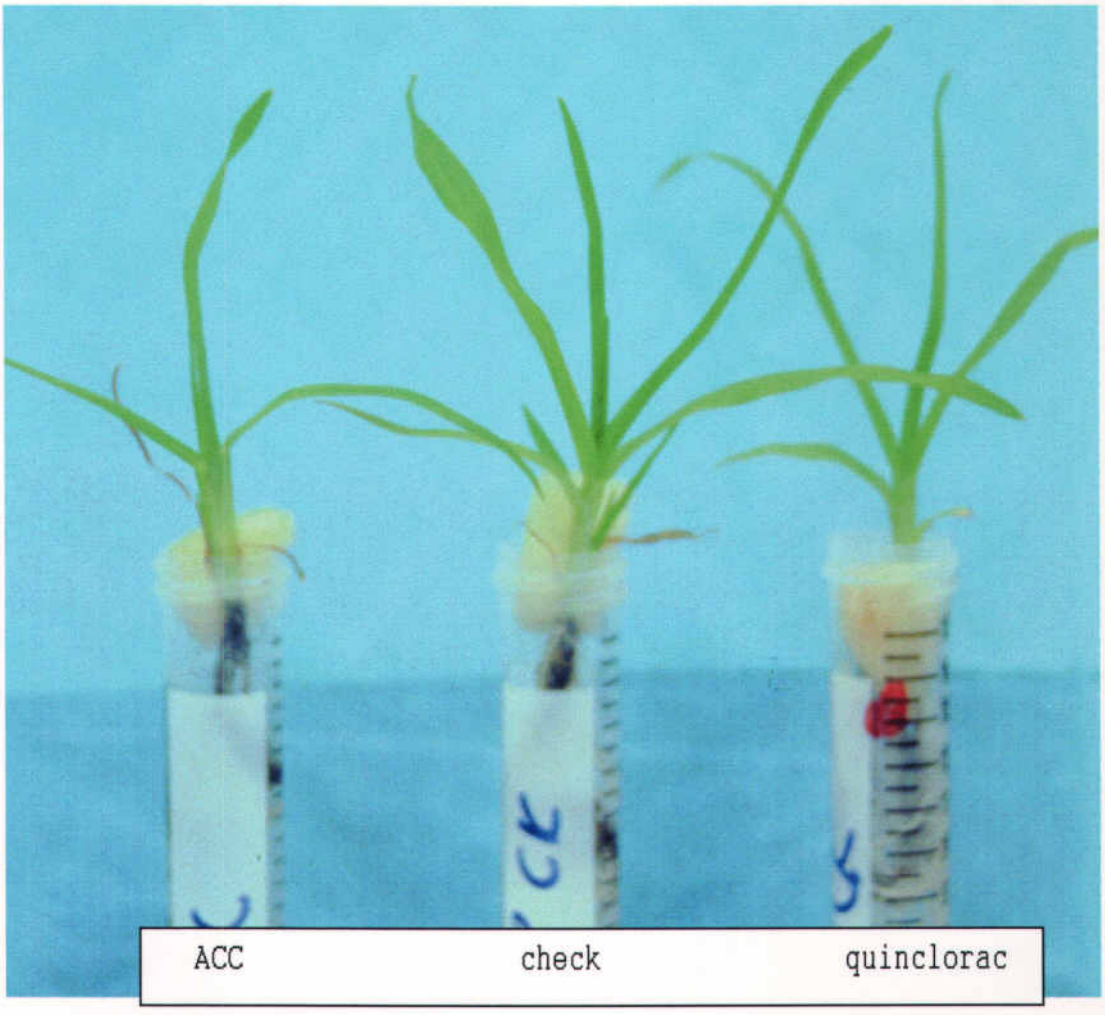


*Figure 5.* Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) at 10mM and quinclorac at 0.56 kg $ha^{-1}$ to goosegrass.

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