Atrazine Metabolism and Herbicidal Selectivity
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Summary. Metabolism of the herbicide 2-chloro-4-ethylamino-6-isopropylamino-s-triazine (atrazine) was investigated in resistant corn (Zea mays L.) and sorghum (Sorghum vulgare Pers.), intermediate susceptible pea (Pisum sativum L.), and highly susceptible wheat (Triticum vulgare Vill.) and soybean (Glycine max Merril.). This study revealed that 2 possible pathways for atrazine metabolism exist in higher plants. All species studied were able to metabolize atrazine initially by N-dealkylation of either of the 2 substituted alkylamine groups. Corn and wheat, which contain benzoazinone, also metabolized atrazine initially by hydrolysis in the 2-position of the s-triazine ring to form hydroxyatrazine. Subsequent metabolism by both pathways resulted in the conversion of the parent atrazine to more polar compounds and eventually into methanol-insoluble plant residue. No evidence for s-triazine ring cleavage was obtained.

Both pathways for atrazine metabolism appear to detoxify atrazine. The hydroxylation pathway results in a direct conversion of a highly phytotoxic compound to a completely non-phytotoxic derivative. The dealkylation pathway leads to detoxification through one or more partially detoxified, stable intermediates. Therefore, the rate and pathways of atrazine metabolism are important in determining the tolerance of plants to the herbicide. Both quantitative and qualitative differences in atrazine metabolism were detected between resistant, intermediate susceptible, and susceptible species. The ability of plants to metabolize atrazine by N-dealkylation and the influence of this pathway in determining tolerance of plants to atrazine are discussed.

Two substituted s-chlorotriazines that are widely used today as selective herbicides for the control of annual weeds in fields of corn and sorghum are 2-chloro-4-ethylamino-6-isopropylamino-s-triazine (atrazine) and 2-chloro-4,6-bis(ethylamino)-s-triazine (simazine). These compounds are effective inhibitors of the Hill reaction in photosynthesis (17) and are known to reduce the rate of $^{14}$CO$_2$ fixation in plants (3, 24).

Corn seems to be resistant to atrazine and simazine largely because of its ability to convert the 2 herbicides rapidly to non-phytotoxic 2-hydroxy-4-ethylamino-6-isopropylamino-s-triazine (hydroxyatrazine) and 2-hydroxy-4,6-bis(ethylamino)-s-triazine (hydroxyimazine) (5, 9, 12, 21). Other species have also been reported to metabolize atrazine (18) and simazine (10) to their hydroxy derivatives. The conversion of 2-chlorotriazines to their 2-hydroxy derivatives is probably non-enzymatic (5, 12, 20) and appears to be correlated with the presence of a cyclic hydroxamate, 2,4-dihydroxy-3-keto-7-methoxy-1,4-benzoazine (benzoazinone), in plants (10).

Sorghum, a resistant species, does not contain benzoazinone and is not able to metabolize the chlorotriazine to the hydroxytriazine (10). In the intermediately susceptible pea, atrazine was readily metabolized to the dealkylated derivative, 2-chloro-4-ethylamino-6-isopropylamino-s-triazine (compound I) (23). Hydroxyatrazine was not formed and benzoazinone was not detected in pea (23). Wheat contains benzoazinone (10) and soybean does not (8), but both species are highly susceptible to atrazine.

Although corn and sorghum are both resistant to the chlorotriazine herbicides, metabolism studies indicate that their basis for resistance is quite different. N-Dealkylation of atrazine, as it occurs in pea, may be an important mechanism of resistance to the herbicide in higher plants (22). This investigation was undertaken to determine if dealkylation of atrazine occurred in species other than pea. The occurrence of this reaction in higher plants and its significance as a basis for herbicidal selectivity are discussed.

Materials and Methods

Plant Material. Seeds of wheat (Triticum vulgare Vill. var. Justin), corn (Zea mays L. North Dakota KE 47101), soybean (Glycine max Merril. var. Hawkeye) and pea (Pisum sativum L. var. Little Marvel) were germinated between moist paper towels and seedlings were grown in nutrient solution as previously described (22). Sorghum seeds (Sorghum vulgare Pers. var. North Dakota 104) were germinated in vermiculite for 11 days in a controlled environment with a 12/12-hour photoperiod, $27\pm2^\circ$.
day and night temperature, 40 ± 5 % relative humidity, and a light intensity of 1600 ft-c. The young sorghum seedlings were removed from vermiculite and transferred to stainless steel trays containing half strength Hoagland’s solution and grown in the greenhouse. The nutrient solution was continuously aerated.

Atrazine Metabolism in Plants. Uniformly ring-labeled atrazine-14C (specific activity 7.8 μC per mg) was used in studying the metabolism of atrazine in plants. Atrazine-14C used in treating plants was purified by thin-layer chromatographic separation on a 250 μ silica gel HF plate, using benzene:acetic acid (50:4 v/v) (solvent A) as the developing solvent. Assay of 14C activity in purified atrazine-14C showed less than 0.6 % of the radioactivity present as impurities.

Roots of plants were immersed in an aqueous solution of atrazine-14C added to 200 ml of half strength Hoagland’s solution. Plants used for the study of species differences in atrazine metabolism were treated for 48 hours in a laboratory hood under conditions previously reported (23). Sorghum plants used in studying the metabolism of atrazine with time were treated in a controlled-environment room under conditions given previously except for a night temperature of 20 ± 2°C.

Plant Extraction and Assay for 14C Activity. At the end of the exposure period to atrazine-14C, the root and shoot tissues were extracted separately with 95 % methanol and the extracts purified as reported (23). In the controls, 0.05 μC of atrazine-14C was added to the tissues just prior to homogenization and extraction. Methanol was evaporated under vacuum at 35°C; the remaining plant residue was removed by centrifugation; and the resulting aqueous solution was concentrated for further assay. With corn and wheat the aqueous solution was boiled directly over an open flame for 5 minutes before centrifugation. This was required to inactivate benzoazinone which otherwise would catalyze the conversion of atrazine to hydroxyatrazine in the extract. An aqueous solution of atrazine-14C treated in a similar manner resulted in a loss of about 2 to 3 %, possibly due to volatilization. The aqueous extract was washed with chloroform to remove atrazine, compound 1, and other chloroform-soluble metabolites. The 14C activity in chloroform-soluble and water-soluble compounds was determined by liquid scintillation counting (23).

Unchanged atrazine and its chloroform-soluble metabolites were separated by thin-layer chromatography on 250 μ silica gel HF plates developed in solvent A (23). Detection of radioactive spots was made by autoradiography of thin-layer plates on Kodak no-screen x-ray film. Quantitative assay of unchanged atrazine, dealkylated metabolites, and other unidentified chloroform-soluble metabolites was made by careful removal of silica gel from the thin-layer plate and counting by gel scintillation counting (22).

Each sample counted was corrected for quenching and results were expressed as dpm.

The water-soluble radioactive metabolites were further purified by cation exchange chromatography on AG 50W-X8(H+) resin (23) and eluted with 10 × NH4OH. Atrazine must be removed from the aqueous solution before cation exchange chromatography is attempted because the strongly acidic resin will convert atrazine to hydroxyatrazine. The purified water-soluble metabolites were separated on silica gel HF thin-layer plates by multiple chromatography. The plate was first developed 3 times in 1 direction in benzene:ethy1acetate:acetic acid:water (25:50:20:3, v/v/v/v) (solvent B) and once in the same direction in n-butanol:acetic acid:water (120:30:50, v/v/v).

In sorghum, relative concentrations of compound I, and 2-chloro-4-amino-6-ethylamino-s-triazine (compound II), were determined by gas-liquid chromatographic separation as previously described (23). With the use of a 9 to 1 stream splitter and gas chromatographic fraction collector, 14C-labeled chloroform-soluble effluents at relative retention times (τg) of 2.0 (compound 1) and 2.7 (compound II) were collected and counted by liquid scintillation counting (23) (atrazine τg 1.0).

The 14C activity remaining in methanol-insoluble plant residue was measured by dry combustion in oxygen in Schoniger flasks as previously described (22). The same reported procedure was followed except for the use of 10 ml of a 2 to 1 (v/v) mixture of ethylene glycol monomethyl ether (methyl cellulose) to monoethanolamine as the CO2 trapping liquid in place of hyamine hydroxide 10-X. Three ml of the CO2 trapping liquid was added to 15 ml of scintillation counting liquid [6g/l PPO in methyl cellulose:toluene (1:2, v/v)] and assayed for 14C activity.

14CO2 Evolution from Atrazine-14C Metabolism. Evolution of 14CO2 from sorghum and corn plants whose roots were exposed to uniformly ring-labeled atrazine-14C was determined by CO2 trapping experiments. The 14CO2 expired was trapped and assayed over a period of 168 hours. At the end of this period, the roots of plants were rinsed thoroughly, and the plants frozen immediately and freeze-dried for 24 hours. Two traps in dry ice-methanol baths were placed in the vacuum line to collect any volatile substances. No 14C activity was recovered from the condensate in the traps. The freeze-dried plant material was assayed for 14C activity by the dry combustion method.

Phytotoxicity of Atrazine and its Metabolites. A bioassay on phytotoxicity of atrazine and its metabolites was made on the extremely susceptible species, oats (Avena sativa L. var. Rodney). Oat seeds were germinated for 4 days and placed in nutrient solution in a controlled-environment room as reported for pea seedlings (22). Four days after transfer into nutrient solution, oat plants were placed in nutrient solutions containing 10-5 M concentrations of atrazine, hydroxyatrazine, compound I, and hydroxy-compound I.
Hydroxy-compound I was prepared from compound I in a manner similar to the conversion of simazine to hydroxysimazine (5). Compound I was hydrolyzed by adding 0.1 ml concentrated HCl per 15 mg compound I. The reaction mixture was allowed to stand at room temperature for 2 hours with frequent shaking. HCl was removed under vacuum and the cristallized product was dried. Aqueous solutions of hydroxy-compound I and compound I were analyzed for ultraviolet absorption. The Rp values of both compounds and hydroxytriazine were determined on silica gel HF, 250 μ thin-layer plate developed in solvent B.

Compound I showed a single absorption maximum at 204 to 206 μ and a new peak at 223 to 225 μ appeared for hydroxy-compound I. The Rp values indicated that hydroxy-compound I (Rp 0.08) was much more polar than compound I (Rp 0.80). However, hydroxy-compound I was very similar in polarity to hydroxytriazine (Rp 0.14).

Results

Metabolism of Atrazine in Different Species. The dealkylation products of atrazine were detected and identified by thin-layer and gas-liquid chromatographic separations. The Rp values and relative retention times of atrazine and its dealkylated metabolites were previously reported (23). Pea is the only species of higher plants in which dealkylation of atrazine has been reported to occur (22, 23). Thin-layer chromatographic analysis of chloroform-soluble compounds in plant extracts showed that dealkylation occurred in sorghum, pea, soybean, wheat, and corn. Both quantitative and qualitative differences in dealkylation of atrazine between the 5 species were detected.

Dealkylation of atrazine can result in 2 possible products. N-Dealkylation at the ethylamine side chain will result in the isopropyl derivative (compound I) and N-dealkylation at the isopropylamine side chain will result in the ethyl derivative (compound II). It was previously shown (23) that in shoots of mature pea plants, compound I (Rp 0.26) was the predominant metabolite of atrazine. However, a trace amount of compound II (Rp 0.22) was reported to be present in young pea seedlings (22); and it was also detected in plants used for this study. The predominance of compound I over compound II, as detected by autoradiography, was also true in soybean and wheat. In corn nearly equal amounts of compounds I and II were present as determined by autoradiography.

In sorghum high concentrations of both compounds I and II were detected (fig 1). The chloroform-soluble fraction from young plants exposed to atrazine-14C for 48 hours was diluted with authentic non-radioactive atrazine, and compounds I and II, and chromatographed by gas-liquid chromatography. The effluent up to tR 3.5 was collected and assayed for 14C activity. Of the total 14C activity chromatographed, 67.4% was in the hydroxy-compound I. A second set of each species served as a control at time of treatment, the ages of plants from date of germination were: sorghum, 35 days; wheat, corn, and

![Fig. 1. Chloroform-soluble metabolites of atrazine-14C in sorghum shoots at 5 different periods of time after an initial 48-hour exposure period. o-origin, a-compound I, b-compound II, c-atrazine, 1-48 hours, 2-96 hours, 3-144 hours, 4-authentic atrazine, 5-authentic hydroxytriazine, 6-240 hours, 7-336 hours. Chloroform-soluble compounds at origin are not hydroxytriazine. Thin-layer plate was developed in solvent A.](image-url)
Further metabolism may result in the incorporation of atrazine derivatives into methanol-insoluble plant residue. Assay of the different degradation products of atrazine present in the different species after a 48-hour exposure period to atrazine is given in figure 2.

Most of the $^{14}$C activity from atrazine-$^{14}$C was present in the shoots of each species except corn. The susceptible species, soybean and wheat, contained larger amounts of unchanged atrazine as compared to resistant sorghum and corn, andintermediately susceptible pea. A striking difference between the resistant species, sorghum and corn, and the other species was the higher percentage of water-soluble metabolites present in the resistant plants. Another obvious difference was the high percentage of dealkylated chlorotriazine metabolites present in intermediately susceptible pea as compared to other species. This agrees with previous reports (22, 23) showing the accumulation of dealkylated metabolites in pea. All species showed the presence of other chloroform-soluble metabolites. None of these have been isolated and identified, but they were definitely more polar compounds than the dealkylated chlorotriazines, compounds 1 and II. These unidentified metabolites remained at the origin when chromatographed in solvent A (fig 1).

 Autoradiograms of water-soluble compounds separated by thin-layer chromatography indicated that hydroxyatrazine ($R_p$ 0.62) was not present in sorghum, pea, and soybean. Figure 3 shows the presence of at least 3 predominant water-soluble metabolites in sorghum with $R_p$ values of 0.29, 0.24, and 0.20. Pea and soybean showed similar water-soluble metabolites except at much lower concentrations.

The predominant water-soluble metabolite in corn and wheat was hydroxyatrazine (fig 4). Detection
from sorghum plants (discussed in section on $^{14}$CO$_2$ evolution).

The time study showed almost total disappearance of unchanged atrazine in sorghum from 26.7% of total $^{14}$C activity after 48 hours to 1.0% after 336 hours. A marked increase occurred in $^{14}$C activity present as methanol-insoluble plant residue from 13.1% after 48 hours to 52.4% after 336 hours. $^{14}$C activity present as water-soluble compounds accounted for a large part of the total activity. It decreased slightly with time from a maximum of 48.0% after 96 hours to 30.8% at 336 hours. The amount of dealkylated chlorotriazines and unidentified chloroform soluble compounds remained fairly constant in the range of 10 to 12%, with slight reductions in the later periods.

Gas chromatographic separation and assay of $^{14}$C activity in compounds I and II in sorghum shoots from 48-, 96-, and 144-hour periods gave ratios of compound I to compound II of 1.8, 1.9, and 2.5 respectively. This indicated that in sorghum dealkylation of the ethyl side chain of atrazine may occur nearly twice as rapidly as the dealkylation of the isopropyl side chain.

Figure 1 shows relative changes in the amount of unchanged atrazine, compounds I and II, and unidentified chloroform-soluble metabolites (origin) in sorghum shoots over a period of 336 hours. Autoradiogram in figure 3 shows similar changes occurring in 3 predominant water-soluble metabolites in the shoots. It shows that with time further metabolism converts water-soluble derivatives to increasingly more polar metabolites. The predominant and least polar water-soluble metabolite at 48-hour period ($R_p$ 0.29) decreased from 43.2% to 6.6% of the total water-soluble $^{14}$C activity after 336 hours. Identification of these water-soluble metabolites has not been made.

$^{14}$CO$_2$ Evolution from Sorghum and Corn Exposed to Atrazine-$^{14}$C. No $^{14}$CO$_2$ from either sorghum or corn was detected over a 7-day period. Table 1 summarizes the data on recovery of $^{14}$C activity in the experiments. Previous reports (15, 19) have indicated triazine ring cleavage and evolution of $^{14}$CO$_2$. However, results from these experiments show that very little, if any, ring cleavage occurs with atrazine.

### Table 1. $^{14}$CO$_2$ Evolution from Sorghum and Corn Exposed to Atrazine-$^{14}$C for 7-day Period

<table>
<thead>
<tr>
<th>Species</th>
<th>$^{14}$CO$_2$ Nutrient solution (dpm)</th>
<th>Plants (dpm)</th>
<th>Recovered (%)</th>
<th>Absorbed by plants (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum</td>
<td>216,060</td>
<td>110,166</td>
<td>94.4</td>
<td>33.8</td>
</tr>
<tr>
<td>Corn</td>
<td>266,383</td>
<td>144,177</td>
<td>91.4</td>
<td>35.1</td>
</tr>
<tr>
<td>Control</td>
<td>162,460</td>
<td>. . .</td>
<td>94.1</td>
<td>. . .</td>
</tr>
</tbody>
</table>

* The following are the amounts of radioactivity from atrazine-$^{14}$C used to treat plants: sorghum, 345,456 dpm; corn, 449,093 dpm; control, 172,728 dpm per 35 ml of nutrient solution (sorghum plants were grown in the nutrient solution prior to the experiment."

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Phytotoxicity of Atrazine and Metabolites. Unchanged atrazine is the major phytotoxic form of the herbicide in higher plants. Hydrolysis of chlorine at the 2-position of atrazine to form hydroxyatrazine makes the original molecule completely non-phytotoxic (5, 9, 12, 21). Dealkylation partially inactivated the original molecule (fig 7), but it definitely retained some phytotoxic properties. This partial inactivation of atrazine by dealkylation was demonstrated in peas by comparing the inhibition of fresh weight increases by atrazine and compound I (22). Evidence indicates that metabolism of atrazine, leading to increasingly more polar derivatives, results in loss of phytotoxic properties inherent in original molecule. Hydroxy-compound I, a water-soluble derivative of compound I, was also completely non-phytotoxic (fig 7). The water-soluble metabolites in sorghum have not been identified, but they were found to be more polar than either hydroxyatrazine or hydroxy-compound I. These results indicate that the water-soluble metabolites of atrazine in sorghum are very likely non-phytotoxic derivatives of the original molecule.

Discussion and Conclusions

Resistance or susceptibility of different species to chlorotriazine herbicides does not seem to be corre-

Table II. Distribution of 14C Activity in Nutrient Solution After a 7-day Period

The nutrient solution and rinsings from washing of plant roots were combined, concentrated under vacuum at 35°, and assayed for atrazine-14C and its metabolites. The methods of analysis were the same as those for plant extracts.

<table>
<thead>
<tr>
<th>Species</th>
<th>Water soluble</th>
<th>Unchanged atrazine</th>
<th>Dealkylated chlorotriazines*</th>
<th>Unidentified chloroform soluble compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum</td>
<td>2.9</td>
<td>85.0</td>
<td>11.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Corn</td>
<td>27.7</td>
<td>46.9</td>
<td>13.6</td>
<td>11.8</td>
</tr>
<tr>
<td>Control</td>
<td>1.8</td>
<td>94.8</td>
<td>.</td>
<td>3.4</td>
</tr>
</tbody>
</table>

* Hydroxyatrazine was the predominant metabolite only in corn.
** This fraction included atrazine-4C and an unidentified compound.

Fig. 6. Chloroform-soluble compounds remaining in nutrient solutions of corn (1) and sorghum (2) after a 7-day exposure period. Authentic atrazine-14C added to nutrient solution in which sorghum plants had been grown was the control (3). a-origin, a-compound II, b-compound I, c-atrazine, 4-antibiotic atrazine and hydroxyatrazine. Chloroform-soluble compounds at origin are not hydroxyatrazine. Thin-layer plate was developed in solvent A.

Fig. 7. Photograph of out plants taken 7 days after exposure to 10⁻⁸ M concentrations of atrazine, hydroxyatrazine, compound I, and hydroxy-compound I. Control is on the left.
lated with the amount of herbicide absorbed by plants (6, 7, 9). Chloroplasts from resistant and susceptible species were equally sensitive to the inhibition of the Hill reaction by the herbicides (16). Evidence showing a possible interaction of atrazine with light to produce toxic substances which cause destruction of chloroplasts has been reported (1, 2). These observations indicate that the biochemical systems in resistant and susceptible plants may be equally sensitive to the action of triazine herbicides.

The primary factor which seems to determine the tolerance of plants to triazine herbicides is its ability to degrade and detoxify the phytotoxic parent molecule. With atrazine the rate and pathways of metabolism are important in determining the tolerance of plants to the herbicide. The rate of metabolism must be rapid enough to prevent accumulation of a lethal concentration of atrazine in the plant, and the pathway of metabolism must result in a change to the parent molecule which reduces or eliminates its phytotoxic property.

This investigation revealed that 2 possible pathways for detoxication of atrazine exist in higher plants (fig 8). The first involves the well-known hydroxylation reaction of chlorotriazines to form hydroxy-atrazine which results in complete detoxication of atrazine (5, 9, 12, 21). The second pathway involves dealkylation of either of the 2 alkyl groups of atrazine to form compounds I or II. Subsequent metabolism of compounds I and II may or may not proceed through a common intermediate. In this discussion, the pathways are classified only according to the initial chemical reaction which occurs in the degradation of atrazine. The formation of dealkylated derivatives is only a partial detoxication reaction (fig 7) (22). However, evidence strongly suggests that subsequent metabolism of the dealkylated derivatives results in complete detoxication of the parent molecule. The results show that the dealkylation pathway may be operating to some degree in most higher plants while both dealkylation and hydroxylation pathways for detoxication may operate in species containing benzoxazine. In species where both pathways exist, the relative activities of the 2 pathways may differ considerably, as in corn and wheat.

Evidence indicates that activities of both pathways may be very low in wheat. This results in a large accumulation of atrazine in plants. Although activity of the dealkylation pathway may be relatively low in wheat, the hydroxylation pathway is extremely active in this resistant species. Corn shoot was reported to contain 11.1 times as much benzoxazine derivatives as compared to wheat (10). Very little atrazine remained in corn plants after 48 hours. In sorghum and pea, dealkylation of atrazine occurs quite rapidly. However, subsequent metabolism of dealkylated derivatives in resistant sorghum appears to occur readily while high concentrations of these derivatives accumulate in pea. The relative concentrations of highly phytotoxic atrazine and the less phytotoxic dealkylated products accumulating in plants may explain intermediate degrees of tolerance as in peas (22).

In sorghum very little atrazine remained in the plants after 336 hours. The results indicate that in sorghum N-dealkylation is the initial degradation reaction in the conversion of atrazine to non-phytotoxic derivatives. Sorghum, although resistant, was less tolerant of high concentrations of atrazine than corn (10). Inhibition of growth in sorghum occurred at concentrations which did not affect corn. Although sorghum metabolized atrazine much faster than the susceptible species, it was not as rapid as in corn. In considering the efficiency or effectiveness of detoxication between the 2 pathways (fig 8), it is reasonable to assume that the hydroxylation pathway may be more effective. This pathway results in a direct conversion of a highly phytotoxic compound to a completely non-phytotoxic derivative. The dealkylation pathway leads to detoxication through one or more partially detoxified, stable intermediates. The difference in effectiveness between these 2 pathways may explain the observations made in comparing tolerances of corn and sorghum toward atrazine.

![Dealkylation and hydroxylation pathways for atrazine detoxication in higher plants. Broken lines signify unknown portions of pathways. Several unknown intermediates are present before formation of insoluble residue.](image)

Dealkylation of atrazine and subsequent rapid metabolism of dealkylated products to non-phytotoxic water-soluble and methanol-insoluble plant residue seem to be responsible for resistance in sorghum. In corn hydroxylation is primarily responsible for its resistance. The extent to which dealkylation contributes to the resistance in corn is unknown.

Results of this investigation and observations reported in the literature suggest that ring cleavage and complete oxidation of atrazine and other triazine herbicides do not occur very readily in higher plants (6, 11), microorganisms (13), or animals (4). In sorghum, and very likely in corn, the residue from atrazine metabolism may still have the symmetrical triazine ring intact. If ring cleavage occurs, the carbon in the heterocyclic ring appears to be rapidly metabolized to give metabolites or products.

**Fig. 8.** Dealkylation and hydroxylation pathways for atrazine detoxication in higher plants. Broken lines signify unknown portions of pathways. Several unknown intermediates are present before formation of insoluble residue.
Acknowledgments

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Literature Cited