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Toxicity of tomatine and tomatidine on weeds, crops and phytopathogenetic fungi

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ABSTRACT

α-Tomatine was not highly phytotoxic and slightly inhibited the stem elongation (7 to 13%) when applied as a spray to etiolated 4-day-old seedlings of sesbania [*Sesbania exaltata* (Raf.) Rybd.], sicklepod (*Senna obtusifolia* L.), mungbean (*Vigna radiata* L.), wheat (*Triticum aestivum* L.) and sorghum (*Sorghum vulgare* L.). Tomatidine had less effect on stem elongation than tomatine and reduced the elongation (5 to 10%). Tomatine was more effective than tomatidine in reducing chlorophyll content in excised etiolated tissues of hemp sesbania, sicklepod, mungbean, wheat and sorghum. Inhibition of chlorophyll accumulation by tomatine ranged from 16 to 89% of control values, whereas inhibition by tomatidine ranged from 0 to 30% of control in these species. Both tomatine and tomatidine increased electrolyte leakage of corn (*Zea mays* L.), kudzu [*Pueraria lobata* (Willd.) Ohwi], palmleaf morningglory (*Ipomea wrightii* Gray) and wild senna (*Cassia marilandica* L.) leaf disks at 24 to 72 h after exposure to the compounds (0.5 mM) and light (100 $\mu E\ m^2\ s^{-1}$). Tomatidine caused greater electrolyte leakage than tomatine in tissues of these species. When incorporated into growth media (agar) at 0.3 mM, tomatine and tomatidine inhibited the growth of three fungal bioherbicidal phytopathogens. *Alternaria cassiae* (weed host = sicklepod), the most sensitive pathogen was inhibited 70% by both compounds. Tomatine inhibited *Colletotrichum truncatum* (weed host = hemp sesbania) and *Fusarium subglutinans* (produces fumonisin, phytotoxic to many plants) growth by 63% and 50%, respectively; while tomatidine inhibited the growth of these latter two pathogens by 50% and 15%, respectively. These natural plant products were shown to have a broad range phytotoxicity and fungitoxicity which may be important in plant defense mechanisms.

Key words: Allelochemicals, bioassay, bioherbicide, electrolyte leakage, natural product, phytoxicity, saponin, tomatine, tomatidine, weeds.

INTRODUCTION

Plants produce a multitude of diverse secondary compounds. These chemicals can exhibit positive and negative interactions with other plants, animals and microorganisms, including defense behavior against pests and phytopathogens (26,28,36,38). Saponins are secondary plant metabolites that comprise a structurally diverse family of glycosylated triterpenes and steroids, and are constitutively present in over 100 diverse plant species and marine organisms. Saponins possess a wide range of

pharmacological activities and some such compounds are the active principles in traditional herbal medicines (37). The main mechanism of saponin antifungal activity is believed to be their interaction with sterols in plant pathogen membranes, causing a loss of the membrane integrity and cell lysis. Fungi that are resistant to such saponins, either produce enzymes that degrade these compounds, or possess resistant membrane structures. Various saponins have also been shown to be phytotoxic and capable of inhibiting plant and bacterial growth (21,40). α-Tomatine is a saponin (steroidal glyco-alkaloid) produced by tomato (*Lycopersicon esculentus* L.) and some other *Solanum* species (29) (Figure 1). Reports of the first isolation of this compound occurred in 1948 (12). α-Tomatine consists of a branched tetrasaccharide {β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-D-galactose}, attached to O-3 of the steroidal aglycone, tomatidine (Figure 1). This tetrasaccharide, called lycotetraose, is a recurrent glycone motif of glycoalkaloids occurring in a very wide variety of plant and marine species, *Solanaceae*ous species such as tomatoes and potatoes (*Solanum tuberosum* L.), and other plants (13). Tomatine can accumulate in plant stems, leaves, and roots and was shown to have fungitoxic activity against some plant pathogens as early as 1947 (23). Hydrolytic cleavage of sugar moieties of the tomatine molecule by enzymes can yield tomatidine. Hydrolysis of tomatine by *Septoria lycopercici* L., a plant pathogen, has been reported as a detoxification mechanism (1). Similar detoxification mechanisms by fungi have been implicated for analogous saponins such as the avenacins in oat (*Avena sativa* L.) (39). Enzymatic detoxification of host plant saponins by fungal pathogens suggests that such detoxification can be a determinant in the host range of certain fungi (6).

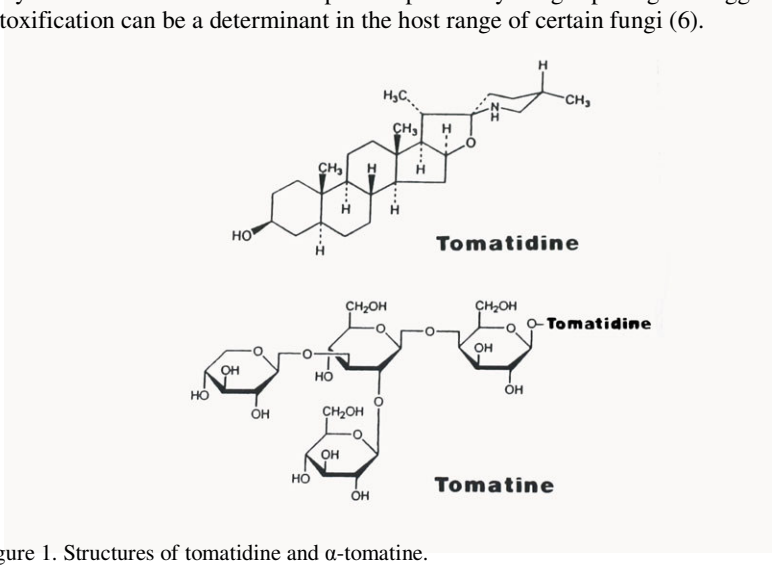

Figure 1. Structures of tomatidine and α-tomatine.

Plants defend against microorganisms and pathogens using diverse biochemical mechanisms. Most of the plant defense mechanisms that have been elucidated have dealt with pathogen: crop plant interactions. From the past to present, only a very few investigations and publications have been directed towards weed defense mechanisms

against plant pathogens. However, one example shows that the economically important weed, sicklepod (*Senna obtusifolia* L.) responds to infection by the pathogen, *Alternaria cassiae*, by elevating phenylalanine ammonia-lyase (PAL) activity (15) and the production of phenylpropanoid compounds (32,33). PAL has been implicated to play a key role in plant defenses against pathogens in many cases (19).

There is considerable interest to develop new compounds and/or methods to control weeds. This stems partly from the facts that many weed species have developed resistance to the currently used commercial synthetic herbicides, and some herbicides have been reported to adversely affect non-target organisms and the environment. Potential sources of new herbicides include microbial phytotoxins and pathogens that target weeds as hosts (11,16,18,19). Conceptually, knowledge of weed defenses could be used to design formulations (chemical and other) to weaken defense responses and thereby increase the efficacy of pathogens on weed hosts, or may even be useful to extend or to predict the weed host range of a given bioherbicidal pathogen.

Our laboratory is currently researching many aspects of weed control using plant pathogens or microbial compounds that possess phytotoxic activity. The objectives of the present studies were to examine the phytotoxic and antibiotic effects of tomatine and tomatidine in a variety of plant species (including weeds) and phytopathogenic fungi that have high potential to act as bioherbicides on several economically important weeds. Several bioassay systems were utilized on selected crop plants and weeds, and fungal spore/mycelial cultures of *Alternaria cassia* (host = the weed, sicklepod), *Colletotricum truncatum* (host = the weed, hemp sesbania), and *Fusarium subglutinans* [host = sunflower (*Helianthus annuus* L.) were used to test potential toxic effects by tomatidine and tomatine.

MATERIALS AND METHODS

α-Tomatine and tomatidine were high purity products obtained from Sigma chemical Co., St. Louis, MO, USA. Aqueous solutions (0.30 to 0.50 mM) of these chemicals were used in all of the bioassays used in these experiments.

Plant Species Sources and Growth Procedures. Crop seeds (corn, wheat, sorghum, and mungbean) were obtained from local seed companies. Weed seeds: palmleaf morningglory (*Ipomea wrightii* Gray), wild senna (*Cassia marilandica* L.), hemp sesbania [*Sesbania exaltata* (Raf.) Ex A.W. Hill], and sicklepod (*Senna obtusifolia* L.) were obtained from weed plots at the Southern Weed Science Research Unit, Stoneville, MS. Kudzu [*Pueraria lobata* (Willd.) Ohwi] seeds were obtained from Adams-Briscoe Seed Co., Jackson, GA 30233, USA.

The plant species (hemp sesbania, sicklepod, mungbean, wheat, and sorghum), used in some bioassays (spray tests and greening of excised tissue), were hydroponically grown in continuous darkness as described in detail elsewhere (17). Briefly, seeds of these species were planted near the top and between two paper towel sheets placed on top of one waxed paper sheet, then the papers were rolled into cylinders and the cylinders (seeds at top of cylinders) were placed in Erlenmeyer flasks (one cylinder per flask) containing 2.0 mM calcium sulfate solution. The flasks containing the cylinders were placed under

continuous darkness in an environmental chamber at 28° C for 4 days. Liquid from the beakers wicked upward to provide water to the seeds. Seeds in the top of the cylinders germinated and the seedlings roots grew downward. After this growth period, plants of uniform size were selected, transplanted into new unused paper towel cylinders (and beakers with calcium sulfate), and used in the spray application bioassays. Excised tissues of these species grown hydroponically were also used in the chlorophyll (greening) bioassays. Plant tissues for the electrolyte leakage bioassays were obtained by germinating and growing seeds of corn, kudzu, palmleaf morningglory, and wild senna in commercially available potting soil under controlled conditions in an environmental chamber (28° C, 16-h photoperiod) for 3 to 4 weeks. Leaf disks of these plants were prepared using hand-held cork borers.

Spray Application: After 96 h of dark growth, seedlings of uniform size of each species were selected, their shoot lengths measured (zero time), and then they were rolled into new, unused paper towel cylinders (4 to 6 seedlings per cylinder). The plants in the cylinders were then sprayed with tomatine (0.50 mM), tomatidine (0.50 mM), or water (control) using a hand-held sprayer. Then the seedlings were placed in the dark for an additional 72 h period and the shoot elongation (growth) was determined. All tests were performed in triplicate and the experiments were repeated. The data wer subjected to ANOVA and means were compared at the 5% level of significance using Fisher's LSD (35). Four to six seedlings per replication was used, and each treatment was triplicated.

Greening Protocol: Excised cotyledons or coleoptiles of 96 h-old dark-grown seedlings were placed in tomatine (0.5 mM), tomatidine (0.5 mM) solutions, or water (control) in well-plates (well diameter = 35 mm) for 2 h in the dark. After this short incubation period in the dark, the plates were transferred to continuous low light (~100 $\mu E\ m^{-2}s^{-1}$) for 72 h. Chlorophyll that was synthesized in these plant tissues during this additional 72 h photoperiod was extracted with dimethyl sulfoxide and quantified spectrophotometrically (4,14). Three to four sets of cotyledons or coleoptiles were used in each replication, and each treatment was performed in triplicate. The bioassay test was repeated.

Electrolyte Leakage: To broaden the scope and diversity of possible phytotoxicity effects of tomatine and tomatidine, a set of plants other than those used in the above bioassays were selected. Furthermore, previous tests using this set of plants had shown they were useful subjects in electrolyte leakage studies. Hand-held cork borers were used to cut 10 leaf/coleoptile disks (4 mm) from greenhouse-grown corn, wild senna (*Cassia marilandica* L.), kudzu, and palmleaf morningglory. Plant tissue disks were placed in petri dishes containing MES {(2-[*N*-morpholino]ethanesulfonic acid)} buffer (pH 6.8) alone, or in tomatine (0.5 mM) or tomatidine (0.5 mM) each prepared in MES buffer. Triplicate dishes for each treatment were incubated at 25°C in continuous light (150 $\mu E\ m^{-2}s^{-1}$). Leakage (conductivity) of electrolytes was determined via conductivity measurements of incubation solutions at, 0, 48, and 72 h after treatment. Ten to twelve cotyledon or coleoptile disks were used in each replication, and each treatment was performed in triplicate. The bioassay test was repeated.

Toxicity of Tomatidine and Tomatine to Fungal Phytopathogens: A predetermined amount of PDA was placed in conical flasks and sterilized in an autoclave (at 121°C at 15 psi) for 15 min. After autoclaving, a calculated amount of test chemical (tomatine or tomatidine) was added to the sterilized medium using a syringe filter (2 µ), and each flask was shaken thoroughly to mix each compound with the agar before pouring. The medium contained a final concentration of 0.3 mM of tomatine or tomatidine. An equivalent amount of sterile water (as used in the preparation of the chemicals) was added to agar flasks to be used as control treatments. Each chemical or water alone medium was then poured into sterilized plastic pertri dishes. After solidification of the agar, the dishes were inoculated with blocks (3 mm dia.) of spore/mycelial cultures of *Alternaria cassia* (weed host = sicklepod), *Colletotricum truncatum* (weed host = hemp sesbania), and *Fusarium subglutinans* (host = many species) in the center of each plate. All plates were incubated at 26 c in an incubator, and incubated at 28 °C. Three dishes of each treatment were utilized. After six days of incubation, the radial growth (colony diameters) of each fungus was measured by using two perpendicular determinations for each colony. The tests were repeated and data for each treatment were pooled and the radial growth of these phytopathogenic fungi in each treatment was compared to the growth in the control (water agar) plates.

Statistics: Data were subjected to ANOVA, and means within each treatment separated using Duncan's multiple range test ($P \geq 0.05$) (35). Percentage data were transformed using the arc sin-square root transformation. However, because no difference occurred between transformed or non-transformed data, the non-transformed data were used.

RESULTS AND DISCUSSION

Tomatine (0.50 mM) applied as sprays exhibited some phytotoxic effects, and reduced stem elongation of several species (Figure 2). Stem elongation reduction ranged from ~7 to 13 % as compared to untreated control seedlings. Sorghum was most affected and the weed, hemp sesbania was the least affected of the five species tested. Tomatidine applied as sprays had a somewhat less effect on these species, i.e., only a 5 to 10 % reduction of elongation, (data not shown).

Tests of these compounds each at 0.50 mM, to reduce chlorophyll accumulation of etiolated excised cotyledons or coleoptiles of several species when exposed to the chemicals and light, indicated that tomatine was more phytotoxic (growth reduction occurred in several species) than tomatidine when visually observed after 72 h treatment (data not shown). Extraction and quantification of chlorophyll accumulated in these exposed tissues revealed that both compounds had phytotoxicity effects that were substantial and significantly different from each other, and different from control treatment (Figure 3). Tomatine dramatically inhibited (16 to 89%) chlorophyll accumulation in excised tissues of these species, and the greatest effects were on tissues of hemp sesbania, wheat, and sorghum. Tomatidine was much less inhibitory (0-30%) on this parameter.

Both compounds at 0.50 mM, caused visual phytotoxic effects such as chlorosis, browning, and necrosis, 72 h after leaf disks were exposed to them and the electrolyte

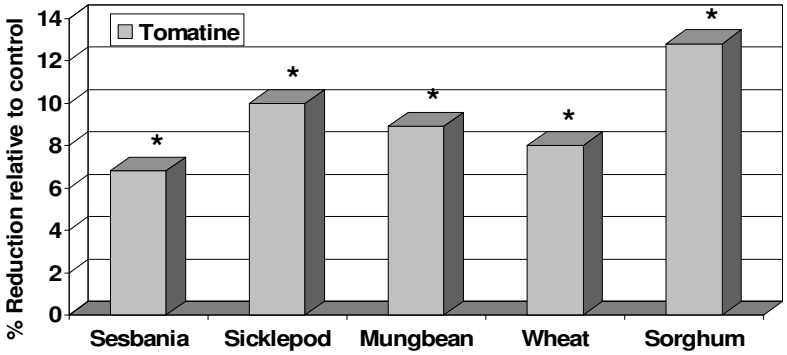

Figure 2. Effects of tomatine on stem elongation in 4-day old etiolated seedlings, 72 h after spray application. Results are presented as percent of control values. Histogram bars that differ significantly from control at $P \geq 0.05$ are marked with an asterisk.

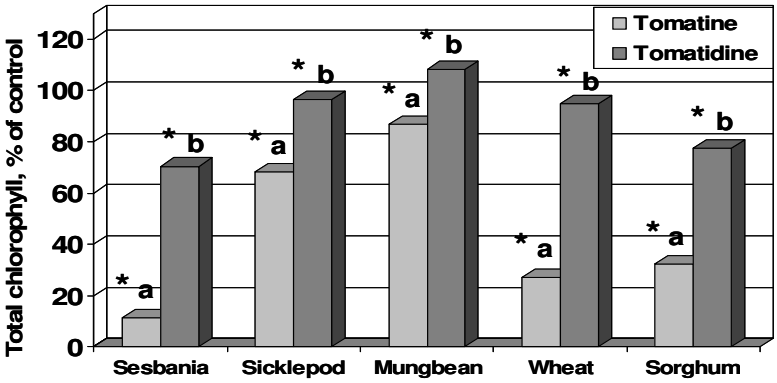

Figure 3. Comparison of total chlorophyll, 72 h after treatment of etiolated tissues with tomatine (0.50 mM) or tomatidine (0.50 mM) and light. Asterisks indicate significant difference from control treatments at the $P \geq 0.05$ confidence level. Different letters on histogram bars indicate significant differences ($P \geq 0.05$) between treatments.

leakage of the four species (three weed and one crop plant) tested were measured (visual data not shown). However, in these bioassays the effects of tomatidine were generally greater than those caused by tomatine. Conductivity test on leaf disks also indicate that leakage was greater in tomatidine-treated disks than in tomatine treated tissue except for corn, where tomatine caused more leakage than tomatidine (Figure 4). α-Tomatine has been shown to disrupt liposome membranes containing a 3-ß-hydroxy sterol, but liposome membranes containing sterols lacking 3-ß-hydroxy sterol were resistant (34). Furthermore, tests with plant tissues showed that α-tomatine caused electrolyte leakage of

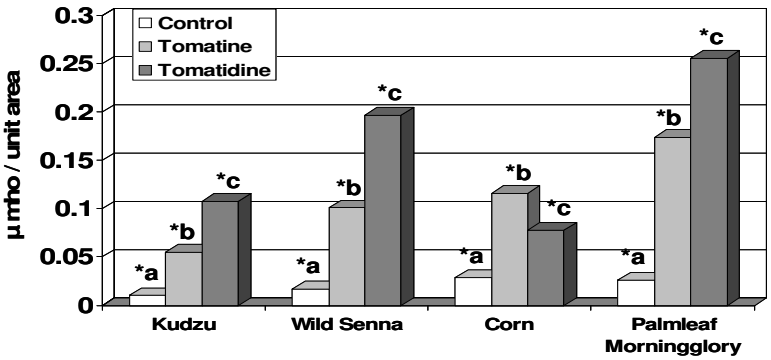

Figure 4. Effects of tomatine (0.50 mM) and tomatidine (0.50 mM) on electrolyte leakage (conductivity as µmho/unit area) of leaf disks of four plant species, 72 h after treatment. Different letters on histogram bars indicate significant differences ($P \geq 0.05$) between treatments.

several plant tissues [cucumber (*Cucumis sativus* L.), tobacco (*Nicotinia tabacum*), and apple-of-Peru (*Nicandra physaloides*)], but tomato and potato tissues were resistant (34). These results were directly correlated with the levels of sterols in these test plant tissues. However, in the present studies, the sterol content of the various species was not determined.

Fungal pathogens of tomato were more resistant to α-tomatine with respect to electrolyte leakage than non-pathogenic fungi (34). These authors also found that electrolyte leakage from *Phytophthora megasperma* (a phytopathogen that infests a wide variety of cultivated plants) was dependent upon on the level of sterol incorporation into the mycelium of this fungus, i.e. culturing on sterol-free medium resulted in more resistance to α-tomatine than when growth occurred on sterol-rich media. In the present studies, the sterol content in the membranes of the various fungal species was not determined.

Both tomatine and tomatidine had fungitoxic effects on the three fungal pathogens tested (Figure 5). *A cassiae,* a pathogen of the weed sicklepod, was the most sensitive pathogen tested. Generally, tomatine fungitoxicity was greater than that of tomatidine on these three weed phytopathogens. *Beauveria bassiana*, a pathogen of some insects, has also been found to be susceptible to tomatine (10). In this report, the authors suggested that conidial and hyphal growth of the pathogen might be inhibited if the host insect consumed sufficient levels of saponin containing plant tissues.

The overall results from these bioassays show that tomatine and tomatidine have phytotoxic effects as well as fungitoxic activity on several phytopathogens, including two that can control weeds. Generally, tomatine exhibited a higher level of phytotoxicity and fungicidal activity than its aglycone analog, tomatidine. In studies with several medicagenic acid alfalfa saponins, the aglycone medicagenic acid was generally as active as its corresponding saponin (medicagenic acid glucuronide) in its deleterious action on several species of rhizosphere bacteria (22). Similar saponin compounds may occur in

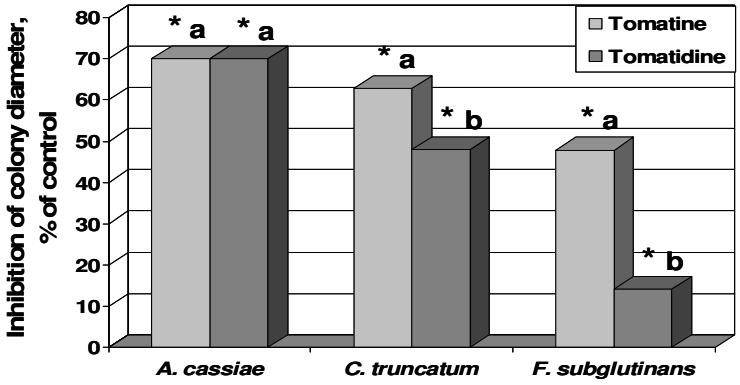

Figure 5. Antibiotic effects of tomatine (0.30 mM) and tomatidine (0.30 mM) of three fungal pathogens. Data presented as inhibition of colony diameter (mm), relative to control values. Asterisks indicate significant difference from control treatments at $P \geq 0.05$. Different letters on histogram bars indicate significant differences ($P \geq 0.05$) between treatments.

certain weeds, and the action of such chemicals could influence the host range of a given pathogen. More research will be required to isolate, identify, and characterize saponins and other antimicrobial compounds in weeds so that their mechanisms of action can be determined.

CONCLUSIONS

α-Tomatine-detoxifying enzymes, i.e. tomatinases can remove one or more sugar residues from α-tomatine leading to a modified saponin or aglycone (tomatidine) (26). These degradation products are generally substantially less toxic to fungi, but they can suppress other mechanisms of plant resistance to fungal attack (5,24). Twenty three fungal strains (phytopathogens, non-phytopathogens and saprophytes) were examined for their ability to degrade α-tomatine and for sensitivity to tomatine, $ß_2$-tomatine (lacks terminal glucose moiety), and tomatidine (31). Both saprophytes, and all five non-phytopathogens of tomato tested were sensitive to α-tomatine, while all but two tomato phytopathogens (*Stemphylium solani* and *Verticillium dahliae*) were tolerant (50% effective dose, ED_{50} > 300 μM) (31). Further, all tomato phytopathogens except *Phytophthora infestans* and *Pythium aphanidermatum* degraded α-tomatine, and there was a strong correlation between tolerance to α-tomatine, the ability to degrade this compound, and pathogenicity on tomato. A ß-1,2,- glucosidase from the tomato pathogen, *Septoria lycopersici* that hydrolyzes the ß -1,2, glucosyl bond on the tetrasaccharide moiety of tomatine was isolated and purified, and the gene encoding this enzyme was also isolated (30). Incubation of this pathogen with tomatine, caused tomatinase mRNA to accumulate, suggesting that the enzyme was substrate inducible. (30).

It is highly possible that a similar scenario based on these biochemical defense mechanisms may also exist in weed-pathogen interactions. Such resistance mechanisms could hamper lower the efficacy of bioherbicidal pathogens used for weed control. These defense processes may also help explain the host range of crop and bioherbicidal pathogens. Clearly, more research is needed to more fully understand these biochemical pathways in weeds in order to comprehend the complex interactions of bioherbicidal action on weeds and weed resistance mechanisms against such bioherbicides. If saponin compounds such as tomatine frequently occur in solanaceous species, it is possible that for example the important weed, jimsonweed (*Datura stamonium* L.) may produce these compounds. If so, these chemicals may help protect this weed species from attack by certain phytopathogens. However, one phytopathogen [*Alternaria crassa* (Sacc.) Sands], isolated from jimsonweed has high potential as a bioherbicide for this weed (7,8,9). Since this fungus is relatively specific for jimsonweed and some tomato varieties (7), either these susceptible plants do not accumulate such compounds at levels to ward-off attack, or the pathogen produces enzymes that degrade and detoxify these defense compounds. As reported earlier, phytopathogens must tolerate or detoxify the saponins of their hosts in order for pathogenesis to occur (27). Studies of defense mechanism of jimsonweed against *A. crassa* thus far have indicated that phenylpropanoid metabolism may be active in this weed-pathogen interaction (20), but the presence of saponin-like compounds was not examined. Since saponins do occur in jimsonweed (2,3,25), investigations of saponin-*A. crassa* interactions would be most interesting.

REFERENCES

1. Arneson, P.A. and Durbin, R.D. (1967). Hydrolysis of tomatine by *Septoria lycopersici*: A detoxification mechanism. *Phytopathology* **57:** 1358-1360.
2. Aynehchi, Y.S. Sormaghi, M.A., Amin, G.H., Khaskhow, M. and Shabani, A. (1985). Survey of Iranian plants for saponins, alkaloids, flavanoids and tannins. *International Journal of Crude Drug Research* **23**: 33-41.
3. Banso, A. and Adeyemo, S. (2006). Phytochemical screening and antimicrobial assessment of *Abutilon mauritianum*, *Bacopa monnifera* and *Datura stramonium*. *Biokemistri* **18**: 39-44.
4. Barnes, J.D., Balaguer, L., Manriques, E. Elvira, S. and Davison, A.W. (1992). A reappraisal of the use of DMSO for the extraction and determination of chlorophylls *a* and in lichens and higher plants. *Environmental and Experimental Botany* **2**: 85-100.
5. Bouarab, K., Melton, R., Peart, J., Baulcombe, D. and Osbourn, A. (2002). A saponin-detoxifying enzyme mediates suppression of plant defences. *Nature* **418**: 889-92.
6. Bowyer, P., Clarke, B.R., Lunness, P., Daniels, M.J. and Osbourn, A.E. (1995). Host range of a plant pathogenic fungus determined by a saponin detoxifying enzyme. *Science* **267**: 371-373.
7. Boyette, C.D. (1986). Evaluation of *Alternaria crassa* for biological control of jimsonweed: host range and virulence. *Plant Science* **45**:23-228.
8. Boyette, C.D. and Turfitt, L.B. (1988). Factors influencing biocontrol of jimsonweed (*Datura stramonium*) with *Alternaria crassa. Plant Science* **56**: 61-264.
9. Boyette, C.D., Weidemann, G.J., TeBeest, D.O. and Quimby, P.C., Jr. (1991). Biological control of jimsonweed (*Datura stramonium*) with *Alternaria crassa. Weed Science* **39**: 678-681.
10. Costa, S.D. and Gaugler, R.R. (1989). Sensitivity of *Beauveria bassana* to solanine and tomatine: Plant defensive chemicals inhibit an insect pathogen. *Journal of Chemical Ecology* **15**: 697-706.
11. Duke, S.O. and Lydon, J. (1993). Natural phytotoxins as herbicides. In: *Pest Control with Enhanced Environmental Safety* (Eds., S.O. Duke, J.J. Mann, and J.R. Plimmer) ACS Symposium Series **524:** 110-124**.** American Chemical Society, Washington D.C**.**

12. Fontaine, T.D., Irving, G.W., Jr., Ma, R.M., Poole, J.B. and Doolittle, S.P. (1948). Isolation and partial characterization of crystalline tomatine, an antibiotic agent from the tomato plant. *Archives of Biochemistry* **18**: 467-475.
13. Freidman, M. and McDonald, G.M. (1997). Potato glycoalkaloids: Chemistry, analysis, safety and plant physiology. *Critical Reviews in Plant Science* **16**: 55-132.
14. Hiscox, J.D. and Israelstam, G.F. (1979). A method for the extraction of chlorophyll fromleaf tissue without maceration. *Canandian Journal of Botany* **57**: 1332-1334.
15. Hoagland, R.E. (1990). *Alternaria cassiae* alters phenylpropanoid metabolism in sicklepod (*Cassiae obtusifolia* L.). *Journal of Phytopathology* **130**: 177-187.
16. Hoagland, R.E. (Editor). (1990). *Microbes and Microbial Products as Herbicides*. ACS Symposium Series **439**, ACS Books, Washington, DC. 341 pp.
17. Hoagland, R.E. (1995). A hydroponic seedling bioassay for the bioherbicides *Colletotrichum truncatum* and *Alternaria cassiae*. *Biocontrol Science & Technology* **5**: 251-259.
18. Hoagland, R.E. (2000). Plant pathogens and microbial products as agents for biological weed control. In: *Advances in Microbial Biotechnology* (Eds., J.P.Tewari, T.N. Lakhanpal, J. Singh, R. Gupta, and B.P. Chamola) pp. 213-255. APH Publishing Corp., New Delhi, India.
19. Hoagland, R.E. (2001). Microbial allelochemicals and pathogens as bioherbicidal agents. *Weed Technology* **15**: 835-857.
20. Hoagland, R.E. and Boyette, C.D. (1994). Pathogenic interactions of *Alternaria crassa* and phenolic metabolism in jimsonweed (*Datura stramonium* L.) varieties. *Weed Science* **42**:44-49.
21. Hoagland, R.E., Zablotowicz, R.M. and Reddy, K.N. (1996). Studies of the phytotoxicity of saponins on weed and crop plants. In: *Saponins Used in Food and Agriculture* (Eds., G.R.Waller and K. Yamasaki) pp. 57-73. Plenum Press, New York.
22. Hoagland, R.E., Zablotowicz, R. M. and Oleszek, W. (2001). Effects of alfalfa saponins on *in vitro* physiological activity of soil and rhizosphere bacteria. *Journal of Crop Production* **4**: 349-361.
23. Irving, G.W. (1947). The significance of tomatine in plant and animal disease. *Journal of Washington Academy of Sciences* **37**:293-296.
24. Ito, S., Eto, T., Tanaka, S., Yamaguchi, N., Takahara, H. and Ikeda, T. (2004). Tomatidine and ycotetraose, hydrolysis products of α-tomatine by *Fusarium oxysporum* tomatinase, suppress induced defense responses in tomato cells. *Federation of European Biochemical Societies Letters* **571**:31-34.
25. Modawi, B.M., Iskander, G.M. and Karim, M.A. (1985). Steroidal components in plants of Sudan flora and seed germination: effects of withaobtusilonalide and withaphysanolide n *Eruka sativum*. *Fitoterapia* **56**: 53-55.
26. Morrissey, J.P. and Osbourn, A.E. (1999). Fungal resistance to plant antibiotics as a mechanism of pathogenesis. *Microbiology and Molecular Biology Reviews* **63**:708-724.
27. Osbourn, A.E. (1996). Saponins and plant defense - A soap story. *Trends in Plant Science* **1:** 4-9.
28. Prell, H.H. and Day, P.R. (2001). Plant-fungal pathogen interaction - A classical and molecular view. In: *Plant-Fungal Pathogen Interaction: A Classical and Molecular View*. Springer-Verlag, Berlin, 214 pp.
29. Roddick, J.G. (1974). The steroidal glycoalkaloid α-tomatine. *Phytochemistry* **13**: 9-25.
30. Sandrock, R. W., DellaPenna, D. and VanEtten, H.D. (1995). Purification and characterization of $ß_2$-tomatinase, an enzyme involved in the degradation of α-tomatine and isolation of the gene encoding $ß_2$-tomatinase *Septoria lycopersici*. *Molecular Plant-Microbe Interactions* **8**: 960-970.
31. Sandrock, R.W. and VanEtten, H.D. (1998). Fungal sensitivity to and enzymatic degradation of the phytoanticipin α-tomatine. *Phytopathology* **88**: 137-143.
32. Sharon, A. Amsellem,Z. and Gressel, J. (1992). Glyphosate suppression of an elicited defense response. *Plant Physiology* **98**: 654–659.
33. Sharon, A. and Gressel J. (1991). Elicitation of flavonoid phytoalexin accumulation in *Cassia obtusifolia* by a myco- herbicide: Estimation by $AlCl_3$-spectrofluorimetry. *Pesticide Biochemistry and Physiology* **41**: 142–149.
34. Steel, C.C. and Drysdale, R.B. (1988). Electrolyte leakage from plant and fungal tissues and disruption of liposome membranes by α-tomatine. *Phytochemistry* **27**: 1025-1030.
35. Steel, R.G.D. , Torrie, J.DH. and Dickey, D.A. (1997). *Principles and Procedures of Statistics-A Biometrical Approach*, 3rd edition. McGraw-Hill, New York.
36. Swain, T. (1977). Secondary compounds as protective agents. *Annual Review of Plant Physiology* **28**: 479-501.

37. Waller, G.R. and Yamasaki, K. (Editors). (1996). *Saponins Used in Food and Agriculture*. Advances in Experimental Medicine and Biology Volume **405**. Plenum Press, New York, NY. pp. 441
38. Wink, M. (2003). Evolution of secondary metabolites from an ecological and molecular phylogenic perspective. *Phytochemistry* **64:** 3-19.
39. Wubben, J.P., Price, K.R., Daniels, M.J. and Osbourn, A.E. (1996). Detoxification of oat leaf saponins by *Septoria avenae*. *Phytopathology* **86**: 986-992.
40. Zablotowicz, R.M., Hoagland, R.E. and Wagner, S.C. (1996). Effects of saponins on thegrowth and activity of rhizosphere bacteria. In: *Saponins Used in Food and Agriculture* (Eds., G.R.Waller and K. Yamasaki) pp. 83-95. Plenum Press, New York.