0971-4693/94 US $ 5.00
Allelopathy Journal 31 (2): 367-376 (2013) *International Allelopathy Foundation* 2013
Table: -, Figs : 4

Bioherbicidal effects of *Myrothecium verrucaria* on glyphosate-resistant and-susceptible Palmer amaranth biotypes

R.E. HOAGLAND*, N.D. TEASTER and C.D. BOYETTE[1]

USDA-ARS, Crop Production Systems Research Unit, Stoneville, MS 38776, USA
E. Mail; bob.hoagland@ars.usda.gov

(Received in revised form: March 4, 2013)

ABSTRACT

Bioherbicidal effects of the fungus *Myrothecium verrucaria* (MV) on glyphosate-resistant and -susceptible Palmer amaranth were examined on whole plants and in leaf bioassays of young and mature plants. Leaf bioassays using mycelia from the fermentation product of MV indicated that excised leaves of young greenhouse-grown (glyphosate resistant and -susceptible) and mature field-grown (glyphosate-resistant) plants were injured by the bioherbicide. Generally, injury was directly proportional to the MV mycelial concentration applied, and glyphosate-susceptible and -resistant plant leaves were equally sensitive to the MV phytotoxic effects as measured by reduction of chlorophyll content. Similar effects occurred on whole plants challenged by MV spray applications to foliage, as substantiated by plant growth reduction (fresh and dry weight accumulation) at termination of the time course. MV disease progression over a 7-d period in young (2-week-old) plants increased with time, and at 48 to 72 h after treatment, disease was severe with nearly 100% mortality occurring and there were no significant response differences in the glyphosate-susceptible and -resistant plants. As expected, disease progression in 4-week-old plants was slower, indicating more tolerance to the bioherbicide, but injury was moderately severe at the endpoint (168 h) after treatment. Results demonstrate that under greenhouse and laboratory conditions, MV can control both glyphosate-resistant and susceptible Palmer amaranth seedlings which could make this bioherbicide a possible candidate for use against this economically important weed.

Keywords: *Amaranthus palmeri*, biocontrol agent, bioherbicide, biological weed control, glyphosate-resistance, *Myrothecium verrucaria*, Palmer amaranth, pigweed.

INTRODUCTION

Palmer amaranth (*Amaranthus palmeri* S. Wats.), originally native to the North American southwest, is an invasive species that has spread rapidly to eastern North America and overseas to Europe, Asia and Australia (3). This major weed, distributed in the southeastern United States (42), has evolved resistance to several herbicides, including triazine, acetolactate-synthase inhibitors, and dinitroaniline herbicides (21,22,28,29,41, 44,47). This weed was originally controlled with the herbicide glyphosate in glyphosate-resistant crops, but now resistance to glyphosate has been documented in several states including Georgia (17) Tennessee, (42) Arkansas, (35) and North Carolina, New Mexico, Alabama, Mississippi, Missouri, and South Carolina (22) and has become widespread (17).

*Correspondence author, [1]USDA-ARS, Biological Control of Pests Research Unit, Stoneville, MS 38776, USA.

The molecular target site of glyphosate is inhibition of the enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Biotypes of glyphosate-resistant Palmer amaranth have high copy numbers of the EPSPS gene (5 to >160 copies), relative to glyphosate-susceptible biotypes (18). This resistance mechanism enables the plant to produces sufficient EPSPS to support amino acid production even after glyphosate treatment and shikimic acid does not accumulate.

The spread of resistance of this weed continues due to several factors. One Palmer amaranth plant can produce over 250,000 seeds during a season (39). The seeds can be moved by tillage, harvesting equipment, animals, or wind and water thus spreading resistance rapidly. Resistance traits can also be moved via pollen (genetic exchange). Palmer amaranth is a dioecious species, i.e., plants are either male or female, which means that plants are cross-pollinators, resulting in the exchange of genetic traits each year. Furthermore, resistance traits carried in pollen can travel up to 1000 feet from a known resistant male plant to susceptible female plants (40). Glyphosate-resistant Palmer amaranth has a very high copy number of the EPSPS gene (5 to >160 copies), relative to a glyphosate-susceptible population (18). A high EPSPS copy number ensures that the plant produces sufficient EPSPS to support required amino acid production even in the presence of glyphosate and the increased EPSPS gene copy number is a heritable trait when plants are cross-bred (18).

Palmer amaranth possesses several traits that help define its weedy character. Pigweed competition studies have been conducted in soybean with Palmer amaranth (31,33) and it has ranked first among three of the *Amaranthus* spp in affecting soybean yield loss (5,29). Weedy species of amaranth usually have prolonged seed dormancy and may persist for several years in the soil (36). Palmer amaranth can hybridize with several other *Amaranthus* species (19,38) thus herbicide resistance and other traits can be passed on within and among species. Since this weed is dioecious, the sex of the cloned plants from MS was compared for possible interaction with glyphosate resistance, but no correlation of plant sex and the presence or level of resistance was found (27,43). Although an elevated copy number of the EPSPS gene instills resistance to glyphosate, other factors may contribute to the overall glyphosate resistance of Palmer amaranth in the MS populations tested (43). The aggressiveness and rapid spread of Palmer amaranth may also be partially explained since it can exhibit allelopathic effects on plant growth (34). Several allelochemical compounds isolated from Palmer amaranth inhibit seed germination (13,14,16).

Biological alternatives to the use of synthetic herbicides for weed control have been proposed including the use of plant pathogens as bioherbicides (2,15,24,46). We have found that the bioherbicidal fungus, *Myrothecium verrucaria* (MV) can be used to control several weeds from various families (1,6,7,18,9,11,26). Host range studies of MV soon after its isolation showed that it was phytotoxic to *Amaranthus retroflexus* (45), but tests on Palmer amaranth have not been studied. Since Palmer amaranth is a very serious weed problem and has become resistant to glyphosate, our objectives were to: examine the effects of this bioherbicide on both glyphosate-susceptible and -resistant plants using whole plant and excised leaf bioassays, determine the vulnerability of this weed to MV, and ascertain if possible differences in bioherbicidal efficacy on glyphosate-resistant and -susceptible plants . To achieve this we used cloned Palmer amaranth plants that we have characterized for susceptibility/resistance to glyphosate (27,43) and included testing of young versus older plants to help determine MV efficacy differences with respect to plant age.

MATERIALS AND METHODS

I. MV Source and Production

MV spores [*M. verrucaria* (IMI 361690)] originally isolated from sicklepod (*Senna obtusifolia* L.) were cultured in petri dishes on potato dextrose agar (PDA) (Difco Laboratories, Inc., Detroit, MI, USA) incubated at 25°C, 5 d. Conidia (spores) were harvested by rinsing plates with sterile H_2O and conidial concentrations were determined using a hemacytometer. A fermenter (Model MF-214, New Brunswick Corp., Edison, NJ, USA). inoculated with MV spores and fungal growth (without spore production) proceeded on a proprietary liquid medium (12) for 48-72 h. The MV product produced via fermentation was harvested and stored at 4°C until use. Concentrations of the mycelia formulations used in these tests were based on percent (v/v basis) of the mycelia batch. Typically a fermentation batch produces MV mycelia at a density of 1.0×10^7 cfu mL^{-1}. The dry weight (mycelia and unspent nutrients) of a typical fermentation batch of MV was 0.05 – 0.06 g mL^{-1}.

II. Plant Propagation

Plants used in these tests were chosen from sets of cloned Palmer amaranth plants previously characterized as glyphosate-susceptible (labelled C3) or -resistant (C7 and R4) (27,43). After two weeks of growth, small cloned plants were transplanted into pots (9 x 7 cm) containing a potting soil mixture 50:50 mixture of 1:1 commercial potting mix/soil and grown in a greenhouse (20-24°C, and a 16 h photoperiod supplied with supplemental lighting). Plants were watered with de-ionized water and fertilizer [N:P:K (13:13:13)] was provided biweekly.

III. Leaf Bioassay and Chlorophyll Analysis

Leaves from 4 week-old cloned glyphosate-susceptible and -resistant plants, grown under greenhouse conditions (20-24°C, 16 h photoperiod and 45-65% relative humidity) were excised and used in excised leaf bioassays. A set of older mature plant leaves from a field-grown Palmer amaranth plant were also bioassayed to compare the effects of MV. This field population is resistant to glyphosate (27). Three to four leaves of each clone type or field plant were placed on an absorbent blotter paper in a petri dish containing 5.0 mL of deionized water. Five droplets of MV at various concentrations (100, 80, 50, 30 and 10% of the fermented product concentration) were pipetted in 10 µL applications onto the surface of each leaf. Control leaves were treated similarly with Silwet (0.15%, v/v) in water. Lids were placed on the dishes, followed by incubation at 20°C under continuous light (100 $\mu Em^{-2} s^{-1}$). Five days after inoculation, disks from each leaf were removed using a cork borer and placed in vials containing dimethyl sulfoxide. Chlorophyll was extracted without maceration into the dimethyl sulfoxide and quantitatively determined spectrophotometrically (4,23).

IV. Application of *Myrothecium verrucaria* Mycelial Formulation to Intact Plants

Five seedlings from each cloned biotype (6-week-old) were sprayed using fully-charged, hand-held compressed air spray canisters (Crown Spra-Tool, North American Professional Products, Woodstock, IL, USA) to run-off (ca. 300 L ha^{-1}), with each

treatment [Silwet at 0.15%, v/v (control)], or MV at 30 or 100% MV mycelia product plus Silwet. All treatments contained 0.15% (v/v) Silwet L-77 surfactant. Applications were administered in a bio-safety cabinet (NuAire, Model No. NU-425-400, Plymouth, MN, USA). After spray treatment, the seedlings were placed in a dew chamber (Percival Scientific, Model No. 1-35 DL, Boone, IA, USA) at 25°C for 16 to 18 h and then transferred to a greenhouse for further growth, observation, and measurements.

V. Determination of MV Effects on Plant Growth

After MV application as described above, the plants were visually examined for injury symptoms at various intervals after treatment. Plant shoot fresh and dry weights were determined 7 d after treatment on excised shoots at soil level. Excised shoot material used for dry weight determinations were placed in paper bags, labeled, and dried in a forced-air oven at 90 to 98°C for 48 h prior to weighing.

VI. Disease Progression Tests

Disease progression or severity on plants of these biotypes (6-week-old) after MV was applied as a spray [100% MV mycelial fermentation product (1.0×10^7 cfu mL^{-1}] prepared in 0.15% Silwet was monitored at several intervals over a 7- d period. A modified visual disease severity rating scale (30), where: 0 represented no infection, and 1.0, 2.0, 3.0 and 4.0 represented 20, 40, 60, and 80% leaf and stem lesion coverage/injury, respectively, and 5.0 = plant mortality was used to rate injury. Data were analyzed using standard mean errors and best-fit regression analysis.

VII. Experimental design and statistical treatments

A randomized complete block experimental design was used. Each treatment consisted of 2 to 4 plants and all treatments were triplicated and the experiments were repeated. The data were statistically compared using analysis of variance (ANOVA) at the 5% probability level. Values presented are means of replicated experiments. When significant differences were detected by the F-test, means were separated with Fisher's protected LSD test at the 0.05 level of probability. Error bars are ±1 SEM.

RESULTS AND DISCUSSION

Leaf bioassay tests

Generally older weeds are more difficult to control with herbicides or bioherbicides than young plants. However, when leaves from mature field-grown Palmer amaranth plants were bioassayed to compare MV effects, necrotic and chlorotic areas developed several days after treatment (Figure 1). The field-collected plants tested here were resistant to the herbicide. Generally, similar MV dose-dependent (applied at 100, 80, 50, 30 and 10%) symptomology was observed on the younger greenhouse-grown glyphosate-susceptible and -resistant plants tested in parallel studies (data not shown). Analysis of total chlorophyll content in the mature, field-collected treated leaves corroborated the visual appearance and injury caused by MV (Figure 2).

Figure 1. Bioherbicidal effects of *Myrothecium verrucaria* in a bioassay of leaves from a mature, field-collected glyphosate-resistant Palmer amaranth, 7 d after inoculation. A = control; 0.15% (v/v) Silwet in water; B = 30% MV in 0.15% Silwet; C = 80% MV in 0.15% Silwet.

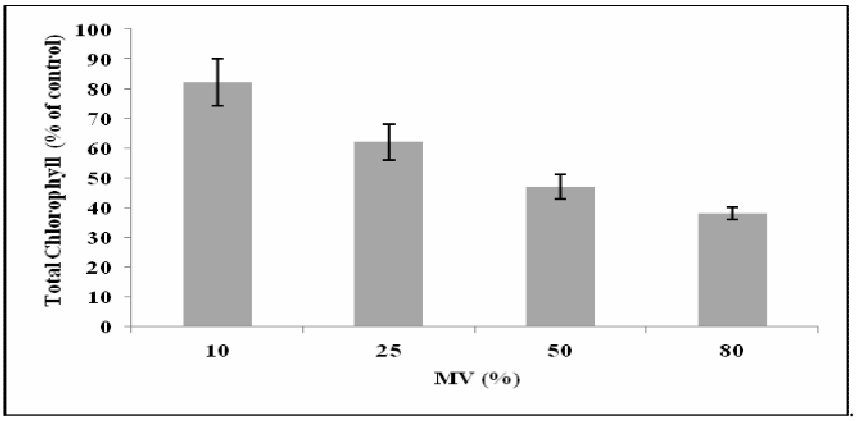

Figure 2. Plant injury caused by *Myrothecium verrucaria* at several concentrations on the reduction of total chlorophyll content in excised leaves from a mature, field-collected glyphosate-resistant Palmer amaranth, 7 d after inoculation.

Whole plant tests at two MV concentrations

Whole glyphosate-susceptible and -resistant Palmer amaranth plants (6-week-old) were sprayed with 30 and 100% (full strength) MV and grown under greenhouse conditions for 7 d. At the end of the time course, MV at both concentrations reduced the fresh weight and dry weight accumulation in both the susceptible and resistant plant biotypes. Since there were no significant differences in the fresh weight or dry weight values of either biotype, the weights from plants treated with each MV concentration were pooled following Bartlett's test for homogeneity of variance (20) (Figure 3). The high MV concentration caused greater growth reduction, but the 30% concentration did reduce both fresh and dry-weight accumulation. Because MV has a weed control response directly proportional to increased mycelial concentration in younger plants of other weed species (6), we suspect that this would also be the case if younger Palmer amaranth plants had been used.

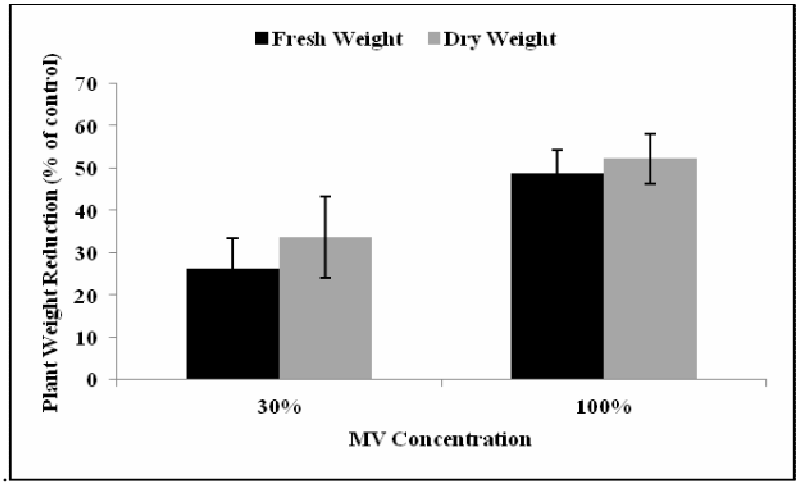

Figure 3. Bioherbicidal effects of spray applications of *Myrothecium verrucaria* at two concentrations (30% and 100%, v/v) on pooled fresh weight and dry weight accumulation values of glyphosate-resistant and –susceptible Palmer amaranth plants (6-week-old), 7 days after inoculation.

Disease progression in whole plants

Analysis of MV disease progression over a 7-d period indicated that disease severity and the disease progression curves were not significantly different in the glyphosate-susceptible and the -resistant biotypes (Figure 4). In the younger (4-week-old) glyphosate-susceptible and -resistant biotypes, disease progressed over time and at 48 to 72 h after treatment with 100% MV, disease was severe and disease ratings of 4.8 to 5.0, respectively were exhibited. Furthermore, nearly 100% mortality occurred within 24 to 30 h after treatment when whole plants were inoculated with full strength MV mycelium. The older (6-week-old) Palmer amaranth plants were more resistant to MV than the younger plants used in these tests, with disease progressing at a slower rate (Figure 4). However, at the end of the testing period (168 h) disease was considered moderately severe (ca. 3.2 to 3.5). Although mortality did not occur during the testing period shown, the disease was sufficiently severe to cause growth cessation and stunting compared to control plants (data not presented). Under field conditions such injury would likely render the plants non-competitive with respect to crop plants and the harsher environmental conditions.

Although the pathogen *Phomopsis amaranthicola* occurs on *Amaranthus* species (34) and its bioherbicidal potential as a genus-specific agent for *Amaranthus* were evaluated (37), environmental factors can be problematic for its use as bioherbicide. The virulence of five isolates of *M. verrucaria* (ATCC 90310, isolated from leafy spurge) and isolates of several saprophytes (non plant pathogens) were evaluated on 28 species of *Amaranthus* including Palmer amaranth (50). Corn oil or Myvacet increased the injury caused by *M. verrucaria* in that study (51). It should be pointed out that the host range of MV isolate ATCC 90310 from leafy spurge (48,49) differs widely from the sicklepod isolate (IMI 361690) that we used as reported earlier (25,26,45).

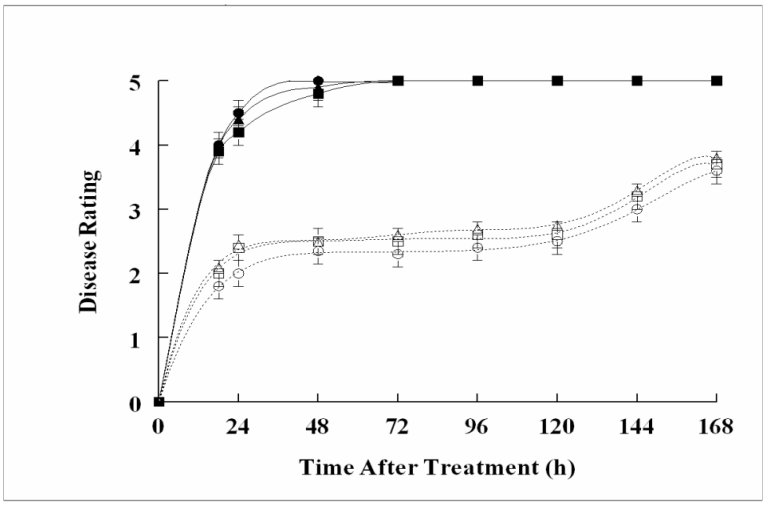

Figure 4. Disease progression of *Myrothecium verrucaria* on glyphosate-resistant and -susceptible Palmer amaranth biotypes over a 7-d period. Plants were tested at two different ages; 4 weeks and 6 weeks. A visual disease severity rating scale modified from Horsfall & Barrett (30) was used to estimate disease progression where: 0 represented unaffected, and 1.0, 2.0 3.0, and 4.0 = 20, 40, 60, and 80% leaf and stem lesion coverage/injury, respectively, and 5.0 = plant mortality. Solid symbols represent young (2-week old) plants [● = C7 (glyphosate-resistant), third degree polynomial, where $Y = 0.684 + 0.176\ X - 0.002\ X^2 + 0.006\ X^3$; $R^2 = 0.94$; ■ = R4 (glyphosate-resistant), third degree polynomial, where $Y = 0.639 + 0.168\ X - 0.002\ X^2 + 0.006\ X^3$; $R^2 = 0.95$; ▲ = C3 (glyphosate-susceptible), third degree polynomial, where $Y = 0.699 + 0.158\ X - 0.008\ X^2 + 0.009\ X^3$; $R^2 = 0.98$. Open symbols represent 4-week old plants: ○ = C7 (glyphosate-resistant), third degree polynomial, where $Y = 0.050 + 0.127\ X - 0.002\ X^2 + 0.003\ X^3$; $R^2 = 0.98$;; □ = R4 (glyphosate-resistant), third degree polynomial, where $Y = 0.065 + 0.147\ X - 0.002\ X^2 + 0.004\ X^3$; $R^2 = 0.98$; Δ = C3 (glyphosate-susceptible), third degree polynomial, where $Y = 0.086 + 0.150\ X - 0.003\ X^2 + 0.003\ X^3$; $R^2 = 0.98$]. Error bars represent ± 1 SEM.

Our results are important since this is the first report that demonstrates glyphosate-resistant weeds can be controlled with the bioherbicide MV. Future research in progress will address field testing to further define the parameters of using MV as a bioherbicide for this troublesome weed. We will also investigate the possibility of synergistic interactions of glyphosate with MV on glyphosate-resistant Palmer amaranth in glyphosate-resistant crops since we have previously demonstrated synergistic interactions of MV and glyphosate on several other weeds (10,11).

ACKNOWLEDGEMENTS

We thank Ken Stetina for culturing and preparing the fungal mycelia product and Robin Jordan for technical assistance during this project.

REFERENCES

1. Abbas, H.K., Tak, H., Boyette, C.D., Shier, W.T. and Jarvis, B.B. (2001). Macrocyclic trichothecenes are undetectable in kudzu (*Pueraria montana*) plants treated with a high-producing isolate of *Myrothecium verrucaria*. *Phytochemistry* **58:** 269-276.
2. Anderson, K.I. and Hallett, S.G. (2004). Herbicidal spectrum and activity of *Myrothecium verrucaria*. *Weed Science* **52:** 623-627.
3. Anonymous. http://eol.org © Missouri Botanical Garden, 4344 Shaw Boulevard, St. Louis, MO, 63110 USA; accessed 9-12-12.
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 *b* in lichens and higher plants. *Environmental and Experimental Botany* **2**: 85-100.
5. Bensch, C.N., Horak, M.J. and Peterson, D. (2003). Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. *Weed Science* **51**:37-43.
6. Boyette, C.D., Abbas, H.K. and Walker, H.L. (2001). Control of kudzu with a fungal pathogen derive dfrom *yrothecium verrucaria*. U.S. Patent No: US 6,274,534.
7. Boyette, C.D., Walker, H.L. and Abbas, H.K. (2002). Control of kuduz with a fungal pathogen derived from *Myrothecium verrucaria*. *Biocontrol Science and Technology* **11**: 677-684.
8. Boyette, C.D., Walker, H.L. and Abbas, H.K. (2002). Biological control of kudzu (*Pueraria lobata*) with an isolate of *Myrothecium verrucaria*. *Biocontrol Science and Technology* **12**: 75-82.
9. Boyette, C.D., Hoagland, R.E. and Abbas, H.K. (2006). Evaluation of the bioherbicide *Myrothecium verrucaria* for weed control in tomato (*Lycopersicon esculentum*). *Biocontrol Science and Technology* **17**: 171-178.
10. Boyette, C.D., Reddy, K.N. and Hoagland, R.E. (2006). Glyphosate and bioherbicide interaction for controlling kudzu (*Pueraria lobata*) with an isolate of *Myrothecium verrucaria*. *Biocontrol Science and Technology* **16**: 1067-1077.
11. Boyette, C.D., Hoagland, R.E., Weaver, M.A. and Reddy, K.N. (2008). Redvine *(Brunnichia ovata)* and trumper creeper *(Campsis radicans)* controlled under field conditions by a synergistic interaction of the bioherbicide, *Myrothecium verrucaria,* with glyphosate. *Weed Biology and Management* **8**: 39-45.
12. Boyette, C.D., Weaver, M.A., Hoagland, R.E., and Stetina, K.C. (2008). Submerged culture of a mycelia formulation of a bioherbicidal strain of *Myrothecium verrucaria* with mitigated mycotoxin production. *World Journal of Microbiology and Biotechnology* **24**: 2721-2726.
13. Bradow, J.M. and. Connick, W.J., Jr. (1987). Allelochemicals from Palmer amaranth, *Amaranthus palmeri* S. Wats. *Journal of Chemical Ecology* **13**:185-202.
14. Bradow, J. M. and Connick, W.J., Jr. (1988). Seed germination inhibition by volatile alcohols and other compounds associated with *Amaranthus palmeri* residues. *Journal of Chemical Ecology* **14**:1633-1648.
15. Charudattan, R. (2005). Ecological, practical, and political inputs into selection of weed targets: What makes a good biological control target? *Biological Control* **35**: 183-196.
16. Connick, W.J., Jr., Bradow, J.M., Legendre, M.G., Vail, S.L. and Menges, R.M. (1987). Identification of volatile allelochemicals from *Amaranthus palmeri* S. Wats. *Journal of Chemical Ecology* **13**:463-472.
17. Culpepper, A.S., Grey, T.L., Vencill, W.K., Kichler, J.M., Webster, T.M., Brown, S.M., York, A.C., Davis, J.W., and Hanna, W.W. (2006). Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. *Weed Science* **54**: 620-626.
18. Gaines, T.A., Zhang, W., Wang, D., Bukun, B., Chisholm, S.T., Shaner, D.L., Nissen, S.J., Patzoldt, W.L., Tranel, P.J., Culpepper, A.S., Grey, T.L., Webster, T.M., Vencill, W.K., Sammons, R.D., Jiang, J.M, Preston, C., Leach, J.E., and Westra, P. (2010). Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. *Proceedings of the National Academy of Science, U.S.A.* **107**: 1029-1034.
19. Gaines, T.A., Ward, S.M., Bukun, B., Preston, C., Leach, J.E. and Westra, P. (2012). Interspecific hybridization transfers a previously unknown glyphosate resistance mechanism in *Amaranthus* species. *Evolutionary Applications* **5**: 29-38.

20. Gomez, K.A., and Gomez, A.A. (1984). *Statistical Procedures for Agricultural Research*. John Wiley and Sons, New York. p 791.
21. Gossett, B.J., Murdock, E.C. and Toler, J.E. (1992). Resistance of Palmer amaranth (*Amaranthus palmeri*) to the dinitroaniline herbicides. *Weed Technology* **6**: 587-591.
22. Heap, I. (2013). The international survey of herbicide resistant weeds; available on-line at www.weedscience.com (accessed February 3, 2013).
23. Hiscox, J.D. and Israelstam, G.F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany* **57**: 1332-1334.
24. Hoagland, R.E. (2001). Microbial allelochemicals and pathogens as bioherbicidal agents. *Weed Technology* **15**: 835-857.
25. Hoagland, R.E., Weaver, M.A. and Boyette, C.D. (2007). *Myrothecium verrucaria:* Bioherbicide and strategies to reduce its non-target risks. *Allelopathy Journal.* **19**: 179-192.
26. Hoagland, R.E., T.S. McCallister, C.D. Boyette, M.A. Weaver and R.V. Beecham. (2011). Effects of *Myrothecium verrucaria* on morning-glory (*Ipomoea*) species. *Allelopathy Journal* **27**: 151-162.
27. Hoagland, R.E., Jordan, R.H. and Teaster, N.D. (2013). Bioassay and characterization of several Palmer amaranth biotypes with varying tolerances to glyphosate. *American Journal of Plant Sciences*, In review.
28. Horak, M.J. and Peterson, D.E. (1995). Biotypes of Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) are resistant to imazethapyr and thifensulfuron. *Weed Technology* **9**: 192-195.
29. Horak, M.J. and Loughin,T.M. (2000). Growth analysis of four *Amaranthus* species. *Weed Science* **48**: 347-355.
30. Horsfall, J.G. and Barratt, R.W. (1945). An improved grading system for measuring diseases. *Phytopathology* **35**: 655.
31. Klingman, T.E. and Oliver, L.R. (1994) Palmer amaranth (*Amaranthus palmeri*) interference in soybeans (*Glycine max*). *Weed Science* **42**: 523-527.
32. Menges, R.M. (1988). Allelopathic effects of Palmer amaranth (*Amaranthus palmeri*) on seedling growth. *Weed Science* **36**:325-328.
33. Monks, D.W. and Oliver, L.R. (1988). Interactions between soybean (*Glycine max*) cultivars and selected weeds. *Weed Science* **36**:770-774.
34. Moran, P.J. and Showler, A.T. (2007). *Phomopsis amaranthicola* and *Microsphaeropsis amaranthi* symptoms on *Amaranthus* spp. under South Texas conditions. *Plant disease* **91**: 1638-1646.
35. Norsworthy, J.K., Griffith, G.M Scott, R.C. Smith, K.L and Oliver, L.R. (2008). Confirmation and control of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) in Arkansas. *Weed Technology* **22**:108-113.
36. Paredes-Lopez, O. (1994). *Amaranth Biology, Chemistry and Technology*. CRC Press, Boca Raton. 223 pp.
37. Rosskopf, E.N., Yandoc, C.B. and Charudattan, R. (2005). Genus-specific host range of *Phomopsis amaranthicola* (Sphaeropsidales), a bioherbicide agent for *Amaranthus* spp. *Biocontrol Science and Technology* **16**: 27-35.
38. Sauer, J.D. (1957). Recent migration and evolution of the dioecious amaranths. *Evolution* **11**: 11-31.
39. Sellers, B.A., Smeda, R.J. Johnson,W.G., Kendig, J.A., and Ellersieck, M.R. (2003). Comparative growth of six *Amaranthus* species in Missouri. *Weed Science* **51**: 329-333.
40. Sonoskie, L.M., Webster, T.M., Culpepper, A.S. and Kichler, J. (2011). The biology and ecology of Palmer amaranth: Implications for control. Extension Article. http://www.caes.uga.edu/applications/ publications / files/pdf/C%201000_1.PDF
41. Sprague, C.L., Stoller, E.W., Wax, L.M. and Horak, M.J. (1997). Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) resistance to selected ALS-inhibiting herbicides. *Weed Science* **45**:192-197.
42. Steckel, L. E. 2007. The dioecious *Amaranthus* spp.: here to stay. *Weed Technology* **21**: 567-570.
43. Teaster, N.D. and Hoagland, R.E. (2013). Characterization of glyphosate resistance in cloned *Amaranthus palmeri* plants. *Weed Biology and Management*, in review.
44. Vencill, W. K, Grey, T.L. Culpepper, A.S. Gaines, T.A. and Westra, P. (2008). Herbicide-resistance in the Amaranthaceae. *Journal of Plant Disease Protection, Special Issue* **21**: 41-44.
45. Walker, H.L. and Tilley, A.M. (1997). Evaluation of an isolate of *Myrothecium verrucaria* from sicklepod (*Senna obtusifolia*) as a potential mycoherbicide agent. *Biological Control* **10**: 104-112.

46. Weaver, M.A., Lyn, M.E., Boyette, C.D. and Hoagland, R.E. (2007). Bioherbicides for weed control. In: Non-chemical Weed Management (Eds., M. Upadhyaya and R. Blackshaw) pp. 93-110. CAB International, New York, USA.
47. Wise, A. M., Grey, T.L., Prostko, E.P., Vencill, W.K. and Webster, T.M. (2009). Establishing the geographical distribution and level of acetolactate synthase resistance of Palmer amaranth (*Amaranthus palmeri*) accessions in Georgia. *Weed Technology* **23**: 214-220.
48. Yang, S. and Jong, S.C. (1995). Host range determination of *Myrothecium verrucaria* isolated from leafy spurge. *Plant Disease* **79**: 994-997.
49. Yang, S. and Jong, S.C. (1995). Factors influencing pathogenicity of *Myrothecium verrucaria* isolated from *Euphorbia esula* on species of *Euphorbia*. *Plant Disease* **79**: 998-1002.
50. Yang, S. and Brenner, D. (1998). Virulence of *Myrothecium verrucaria* on species of amaranth (*Amaranthus*). *Abstracts, Weed Science Society of America* **38**: 43.