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## RESEARCH ARTICLE

# Bioherbicidal potential of a strain of *Xanthomonas* spp. for control of common cocklebur (*Xanthium strumarium*)

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Several isolates of a previously unreported bacterial pathogen were discovered on common cocklebur seedlings in Chicot County, AR and Washington County, MS. Diseased plants in nature exhibited angular-shaped leaf spotting symptoms on leaf margins and central leaf areas. The isolates were cultured from diseased leaf tissue and tentatively identified as *Xanthomonas* spp., and their virulence on common cocklebur seedlings compared. The most virulent isolate (LVA987) was used in studies to define disease progression on cocklebur seedlings and to carry out a host range evaluation on various weeds and crop plants. High virulence was found on common cocklebur > marestail (*Conyza canadensis*) > giant ragweed (*Ambrosia trifida*) ≥ and common ragweed (*Ambrosia artemisifolia*). These results suggest this pathogen may be useful for the biological control of these important species of weeds. This is also highly relevant since all of these weeds have evolved resistance to one or more synthetic herbicides and are thus becoming more difficult to control with conventional herbicides.

**Keywords:** bioherbicide; biological control; *Xanthomonas* spp.; cocklebur; *Xanthium strumarium*

## Introduction

Common cocklebur (*Xanthium strumarium* L.) has been cited as an economically important weed in soybean [*Glycine max* (L.) Merr.] (Rushing and Oliver 1998; Webster 2001; Norsworthy 2003), cotton (*Gossypium hirsutum* L.) (Byrd and Coble 1991; Webster 2001) and peanut (*Arachis hypogaea* L.) (Royal, Brecke, and Colvin 1997) production. The aggressive growth habit of common cocklebur, both within and above the crop canopy, contributes to its weediness (Regnier, Stoller, and Nafziger 1989). Cocklebur can reduce yield even at a distance of 50 cm away from the soybean row (Henry and Bauman 1989). Heavy infestations of cocklebur can reduce yield by 50–80% in soybean (Barrentine 1974; Bloomberg, Kirkpatrik, and Wax 1982).

Common cocklebur has also developed resistance to some herbicides. Several biotypes of cocklebur were found resistant to imazaquin {2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-quinolinecarboxylic acid} (Barrentine 1994; Abbas and Barrentine 1995) with others resistant to monosodium methylarsonate (Haighler, Gossett, Harris, and Toler 1994; Abbas and Barrentine 1995).

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Before genetically modified glyphosate-resistant soybeans were adopted, common cocklebur was controlled with bentazon (3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide) and acetolactate synthase (ALS)-inhibiting herbicides (Barrentine 1974; Muyonga, DeFelice, and Sime 1996). Common cocklebur biotypes resistant to most ALS-inhibiting herbicides occur throughout the north-central and southern USA (Ohmes and Kendig 1999; Schmidt, Talbert, and McClelland 2004; Heap 2012). Glyphosate can effectively control common cocklebur (Wiesbrook, Johnson, Hart, Bradley, and Wax 2001), but early removal is crucial to prevent yield reduction which can occur as early as 4 weeks after planting (Barrentine 1974).

Due to the development of herbicide resistance and a trend towards a more chemically free environment, biological control using plant pathogens (bioherbides) has been considered (Hoagland 1990; Charudattan 1991, 2001, 2005; Rosskopf, Charudattan, and Kadir 1999; Weaver, Lyn, Boyette, and Hoagland 2007). Several disease organisms have been reported on common cocklebur (Anonymous 1970). Over a dozen fungal species have been reported to infect *Xanthium* spp. in the USA and Canada (Weaver and Lechowicz 1983). The obligate parasitic rust *Puccinia xanthii* Schw., occurs throughout the USA, southern Canada, portions of Europe and India, and infects species of *Xanthium* and *Ambrosia* (Conners 1967, Hasan 1974, Alcorn 1975, Jadhav and Somani 1978). The anthracnose-forming fungus *Colletotrichum orbiculare* causes stem and leaf lesions on *Xanthium spinosum* and under optimal conditions, kill plants in 14 days (Auld, Say, Ridings, and Andrew 1990). *Alternaria helianthi* (Hansf.), Tubaki and Nishih, has been evaluated as a bioherbicide for this weed (Quimby 1989; Abbas and Barrentine 1995; Abbas and Egley 1996; Sanyal, Bhowmik, and Abbas 2008). This fungal pathogen, isolated from sunflower (*Helianthus annuus* L.) (Quimby 1989), can also infect certain plants in the Asteraceae family (Allen, Brown, and Kochman 1983). Other fungal and bacterial pathogens have had some success in controlling *X. strumarium* in India (Deshpande 1982) and phytotoxins from seven fungi and bacteria could induce wilt in *X. strumarium* (Kalidas 1981). A powdery mildew that infects cocklebur in India has also been described (Sharma 1981).

The subject of this paper is the discovery and characterisation of a leaf-spotting disease found on mature cocklebur in Mississippi and Arkansas. The specific objectives of this report were to isolate and examine this pathogen with respect to virulence, host range and possible interaction with adjuvant (surfactants) to obtain a formulation useful as a bioherbicide against common cocklebur. Knowledge of these basic parameters is essential for evaluating a plant pathogen as a bioherbicide for weed control.

## Materials and methods

### *Plant sources and propagation*

Cocklebur seeds were purchased from Azlin Seed Co. (Leland, MS, 38756). The burs were soaked in water for 7 d, then planted in a 1:1 potting mix of jiffy mix and soil (Jiffy Mix, Jiffy Products of America, Inc., Batavia, IL 60510), contained in plastic trays (25 × 52 cm). Germinated seeds were transplanted into 10 $cm^2$ plastic pots and grown under greenhouse conditions [28–32°C, 40–60% relative humidity (RH) with about 14-h day length] until the proper growth stage for each experiment was

attained. Other weed seeds were collected from sites in Stoneville, MS. Vegetable seeds were purchased from W. Atlee Burpee Seed Co., Warminster, PA., and crop seeds were purchased from Leland Feed and Seed Co., Leland, MS. Bermudagrass, centipedegrass, St. Augustinegrass and zoysiagrass were propagated from transplanted seedlings. Potato plants, propagated from tubers in 16.5-cm plastic pots, were inoculated at about 21 days of age. All plants were grown in a vermiculite/peat:soil mixture (Jiffy Mix:sandy loam; 2:1).

### *Isolation, culture and preparation of inocula*

Lesion areas of diseased leaf samples (Figure 1A) collected from common cocklebur seedlings in Chicot County, AR and Washington County, MI, were dissected into 0.525% sodium hypochlorite prepared with sterile, deionised water. After surface sterilisation, small leaf sections were placed on potato-dextrose agar. The plates were incubated for 24 h at 28°C. Edges of yellow-pigmented bacterial colonies (Figure 1B) were transferred to nutrient agar (NA) and incubated 3–5 days at 28°C under dark conditions. The bacterial isolates (Gram-negative rods) were sub-cultured on NA for an additional 5 d under the above conditions. Cells were rinsed from the plates using sterile water and an artist brush and diluted (heamocytometer) to a final concentration of $1.0 \times 10^8$ cells/ml. These preparations were used as inocula for each of the isolates and to test Koch's postulates on cocklebur (Koch 1893).

Cotton plants (cv. Stoneville 20) from Washington County, MS that exhibited similar disease symptoms (angular leaf spotting) were also collected to compare isolates with the cocklebur isolates above. These isolates were prepared and cultured as described above. These isolates were tested for pathogenicity on common cocklebur only.

### *Inoculation and virulence tests of* Xanthomonas *isolates*

The bacterial isolates were tested for virulence on common cocklebur by using a sterilised needle point to puncture the leaf cuticle after the inoculum droplet(s) had been placed on the leaf surface of seedlings in the one to two leaf stage. The needle was inserted through the droplet into the leaf tissue several times, thereby allowing

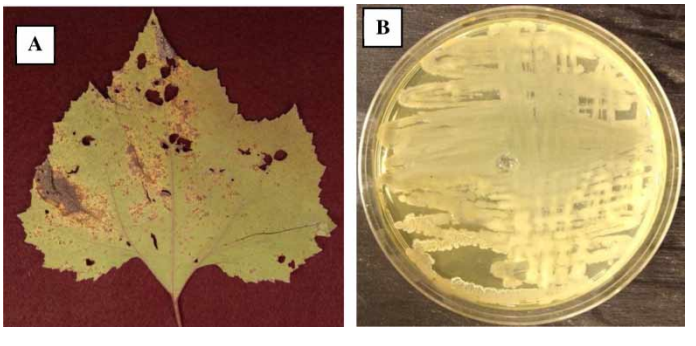


Figure 1. Isolation and culture of bacterial pathogen found on common cocklebur. (A) Leaf spot and phytotoxic effects. (B) Culture of bacterial pathogen (*Xanthomonas* isolate LVA987) in Petri dish culture.

the bacterial cells to come in direct contact within the inner vascular tissues. In a second inoculation method, abrasion (carborundum) was used to provide bacterial entry into the plant tissues. Carborundum wounding with fine sandpaper [Coated Abrasive Manufacturers Institute (CAMI), grit no. 220; 68 μ] was used to lightly abrade the leaf surface prior to administering the inoculum. Eight to ten plants were used in each of the inoculation tests. After wounding and inoculation of several leaves and stems of whole plants, they were placed in a dew chamber (100% RH, 25°C; 12 h), followed by transfer to a greenhouse (28–32°C, 40–60% RH, and a photoperiod of 14 h, at 1600–1800 μE $m^2$/s PAR at midday) for 10 d at which time disease ratings were accessed. Control plants received the wounding (carborundum and needlepoint) treatments, but only water was administered.

### *Tests of surfactants on virulence*

In attempts to circumvent the requirement for plant wounding to incite infection, the effects of two surfactants were investigated. Sterox (non-ionic, polyoxyethylene thioether) and Silwet L-77 (non-ionic, organosilicone) from Monsanto Chemical Corporation, St Louis, MO and OSi Specialties, Inc., Danbury, CT, respectively, were used. Preliminary tests indicated that these surfactants at 0.2% v/v provided ample wetting of the leaf surfaces and facilitated entry of the bacterium into plant tissues, but did not cause plant injury. Thus these concentrations were used to prepare formulations of the bacterial inocula thereafter.

### *Host range experiments*

About 60 plant species, including weeds, vegetables and field crops were utilised to evaluate and define the host-range of the most virulent isolate (LVA987). Seedlings were 10–14-day old when inoculated. Potato plants propagated from tubers in 16.5-cm plastic pots were inoculated at about 21 days old. Purple nutsedge plants were propagated from tubers collected near Stoneville, MS. Seedlings were sprayed until runoff with bacterial suspensions of $1.0 \times 10^8$ cells/ml containing Silwet L-77 (0.20%, v/v) and incubated in a dew chamber for 16 h at 25°C. Control plants for each species were treated with Silwet L-77 (0.2%, v/v) only and common cocklebur was included in all tests to verify pathogen virulence. Plants were rated 14 days after treatment for disease symptoms, that is, mortality, and dry weight reductions were recorded after plants (excised at the soil surface) were oven-dried at 80°C for 3 days. Dry-weight reductions for each species were evaluated using the *t*-test (Freund and Wilson 1997) to compare the treatment means with the means of the respective controls. The experiment was conducted twice with 3 sets of 12 plants for each experiment.

### *Disease progression*

Cocklebur seedlings were sprayed until runoff with bacterial suspensions of $1.0 \times 10^8$ cell/ml containing Silwet L-77 (0.20%, v/v) and incubated in a dew chamber for 16 h at 25°C. Disease progression or severity of the isolate was visually monitored at several intervals over a 15-day period based on a modified Horsfall and Barratt (1945) rating scale, assigning symptom expression from 0 to 10, with 0 being unaffected, and 2.0, 4.0, 6.0, 0.8 = 20, 40, 60 and 80% leaf and stem lesion

coverage/injury, respectively, and 10 = plant mortality. Symptomology was considered 'severe' at ratings of 8.0–10.

### *Experimental design and statistics*

Experiments were conducted twice with 3 sets of 12 plants for each experiment. Treatments (in triplicate) were arranged in a randomised complete block design and all experiments were repeated in time. Means were subjected to analysis of variance and were compared with Fisher's LSD ($P = 0.05$) only when the $F$-test from the analysis indicated significance. The $t$-test was used to separate means of data in the host range tests (Freund and Wilson 1997). Data from the disease progression studies were analysed using standard mean errors and best-fit regression analysis. All data were analysed using SAS (Version 9.1, SAS Institute, Inc., Cary, NC) (SAS 1999) statistical software.

## Results and discussion

### *Identification and testing of isolates*

Three isolates of the disease found on common cocklebur were tested for infectivity on this host (Table 1). This bacterium was tentatively identified as a *Xanthomonas* spp. based on morphological and cultural characteristics. The bacterium was re-isolated and found to infect and kill common cocklebur seedlings, thus fulfilling Koch's postulates (Koch 1893) for disease identification. Generally all isolates incited infection when inoculation was via needlepoint or carborundum, however, the response was weak. When tested with surfactants without mechanical wounding, no infection occurred in the spray inoculum with Sterox, but Silwet L-77 greatly promoted infection. An isolate from a diseased cotton leaf (identified as angular leaf-spot; SVM987) and an isolate causing angular leaf-spot on cotton (ATCC 9924) did

Table 1. Effect of several *Xanthomonas* isolates and inoculation method on infection on common cocklebur.[a]

| | Inoculation method | | | |
|---|---|---|---|---|
| | Wound | | Spray | |
| *Xanthomonas* isolate | Needle | Carborundum | Sterox | Silwet L-77 |
| SVM787 | + | + | − | ++ |
| SVM887 | + | + | − | ++ |
| LVA987 | + | − | − | +++ |
| SVM987[b] | − | − | − | − |
| ATCC 9924[c] | − | − | − | − |
| Control ($H_2O$) | − | − | − | − |

[a]Infection responses to wounding and the surfactants: − = no effect; + = 20–33% of leaf surface infected; ++ = 34–66% of leaf surface infected; +++ = 67–100% of leaf surface infected (mortality).
[b]Isolated from infected cotton (identified as angular leaf spot).
[c]Angular cotton leaf-spot pathogen, *X. campestris* pv. *malvacearum*, obtained from American Type Culture Collection (ATCC), Manassas, VA, USA.

not cause infection on this weed tested under the same inoculation methods. Since the two isolates from cotton did not infect common cocklebur, the isolates from this weed differed from the cotton disease. Due to its high virulence when combined with Silwet L-77, isolate LVA987 was selected as the standard strain for further experiments. The role of surfactants in increasing the activity of plant pathogens in biological weed control has been well documented (Connick, Lewis, and Quimby 1990; Daigle and Connick 1990; Watson and Wymore 1990; Boyette et al. 1996; Boyette and Hoagland 2012). Surfactants may improve leaf wettability, improve spore deposition and retention and prolong water retention to overcome dew period requirements (Green, Stewart-Wade, Boland, Teshler, and Liu 1998).

### *Disease progression*

Analysis of disease progression of this *Xanthomonas* isolate (LVA987) over a 15-day period indicated that disease severity progressed rapidly when plants were sprayed with bacterium plus Silwet L-77 with a 50% (rating = 5.0) prior to 4 days (Figure 2). The leaf spotting lesions coalesced, and by 7–9 days after inoculation the leaves were extensively blighted. Thereafter, disease symptomology continued to increase, with mortality occurring about 12–15 days after treatment. For the bacteria/Silwet treatment, a second degree polynomial regression curve provided the best fit to the data. The relationship was best described by the equation $Y = 0.67 + 1.36X - 0.06Y + 0.01Y^2$, $R^2 = 0.96$. Little infection occurred when the organism was applied to non-wounded plant leaves in water without surfactant, and no disease occurred to plants treated with water only (regression equations not shown) (Figure 2).

### *Host range tests*

In greenhouse tests the bacterium had no effect on mortality or dry weight accumulation of 55 weed and crop plants tested encompassing nine plant families 14 d after inoculation (Table 2). However, in addition to cocklebur the pathogen

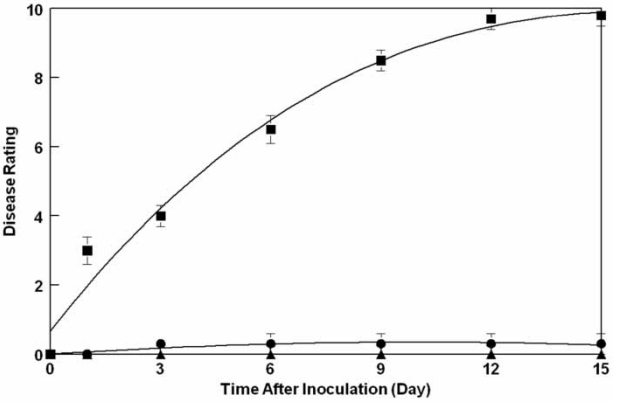


Figure 2. Disease progression on greenhouse-grown cocklebur seedlings over a 15-day period. Squares = *Xanthomonas* isolate LVA987 + Silwet; circles = *Xanthomonas* isolate LVA987 in $H_2O$; triangles = $H_2O$.

Table 2. Effects of *Xanthomonas* isolate LVA987 on various weed and crop species.

| Family and scientific name | Common name (cultivar in parenthesis) | Dry-weight | |
|---|---|---|---|
| | | Mortality (%) | Reduction (%) |
| Amaranthaceae | | | |
| *Amaranthus retroflexus* L. | Redroot pigweed | 0 | 0 |
| Asteraceae | | | |
| *X. strumarium* L. | Common cocklebur | 98** | 98** |
| *C. Canadensis* L. | Marestail | 80** | 90** |
| *Tagetes* sp. | Marigold (Petite Yellow) | 20* | 30* |
| *H. annuus L.* | Sunflower (Mammoth Gray Stripe) | 45* | 70** |
| | Wild Sunflower | 55* | 70** |
| *Ambrosia artemisiifolia* L. | Common ragweed | 60* | 70** |
| *A. trifida* L. | Giant ragweed | 65** | 70** |
| *Zinnia elegans* Jacq. | Zinnia (Sombrero) | 50* | 55* |
| *Lactucca sativa* L. | Lettuce (Little gem) | 55* | 60* |
| *Taraxacum officinale* L. | Dandelion | 58* | 62* |
| Brassicaceae | | | |
| *Brassica rapa L.* | Turnip (Seven Top) | 0 | 0 |
| *Raphanus sativus* L. | Radish (Cherry Belle) | 0 | 0 |
| Chenopodiaceae | | | |
| *Beta vulgaris* L. | Beet (Detroit Dark Red) | 0 | 0 |
| *Chenopodium amaranticolor* Coste and Reynier | Lambsquarters | 0 | 0 |
| Convolvulaceae | | | |
| *Ipomoea lacunosa* L. | Morning glory, pitted | 0 | 0 |
| Cucurbitaceae | | | |
| *Cucumis melo* L. | Cantaloupe (Hale's Best Jumbo) | 0 | 0 |
| *Cucumis sativus* L. | Cucumber (Straight 8) | 0 | 0 |
| *Curcubita pepo* L. | Pumpkin (Jack-O'-Lantern) | 0 | 0 |
| *Curcubita pepo* var. *melopepo* (L.) Alef. | Squash (Yellow Crookneck) | 0 | 0 |
| *Citrullus vulgaris* Schrad. | Watermelon (Charleston Gray) | 0 | 0 |
| Cyperaceae | | | |
| *Cyperus rotundus* L. | Nutsedge, purple | 0 | 0 |
| Fabaceae | | | |
| *A. hypogaea* L. | Peanut (Improved Spanish) | 0 | 0 |
| *Cassia occidentalis* L. | Coffee senna | 0 | 0 |
| *G. max* (L.) Merr. | Soybean | | |
| | (Braxton) | 0 | 0 |
| | (Cajun) | 0 | 0 |
| | (Crawford) | 0 | 0 |
| | (Forrest) | 0 | 0 |
| *Phaseolus vulgaris* L. | Bean, garden | | |
| | (Kentucky Wonder) | 0 | 0 |
| | (Henderson Bush) | 0 | 0 |

Table 2 (*Continued*)

| Family and scientific name | Common name (cultivar in parenthesis) | Dry-weight | |
|---|---|---|---|
| | | Mortality (%) | Reduction (%) |
| *Pisum sativum* L. | Pea, English | | |
| | (Early Alaskan) | 0 | 0 |
| *Senna obtusifolia* (L.) Irwin and Barneby | Sicklepod | 0 | 0 |
| *Sesbania exaltata* (Raf.) Rydb. ex A. W. Hill | Hemp sesbania | 0 | 0 |
| *Vigna sinensis* (Torner) Savi. | Cowpea (California Pinkeye Purple Hull) | 0 | 0 |
| Malvaceae | | | |
| *Abelmoschus esculentus* (L.) Moench | Okra (Clemson Spineless) | 0 | 0 |
| *Abutilon theophrasti* Medic. | Velvetleaf | 0 | 0 |
| *Anoda cristata* (L.) Schlecht. | Spurred anoda | 0 | 0 |
| *G. hirsutum* L. | Cotton | | |
| | (Stoneville 453) | 0 | 0 |
| | (Stoneville 506) | 0 | 0 |
| *Sida spinosa* L. | Prickly sida | 0 | 0 |
| Poaceae | | | |
| *Brachiaria platyphylla* (Griseb.) Nashe | Broadleaf signalgrass | 0 | 0 |
| *Cynodon dactylon* (L.) Pers. | Bermudagrass (Tifway 328) | 0 | 0 |
| *Digitaria ciliaris* (Retz.) Koel. | Crabgrass, southern | 0 | 0 |
| *Echinochloa crus-galli* (L.) Beauv. | Barnyardgrass | 0 | 0 |
| *Eremochloa ophiuroides* (Munro) Hack. | Centipedegrass | 0 | 0 |
| *Leptochloa* panicoides (L.) | Sprangletop, Amazon | 0 | 0 |
| *Poa annua* L. | Annual bluegrass | 0 | 0 |
| *S. viridis* (L.) Beauv. | Green foxtail | 0 | 0 |
| *Sorghum bicolor* (L.) Moench | Sorghum, grain | 0 | 0 |
| *Sorghum halepense* (L.) Pers. | Johnsongrass | 0 | 0 |
| *Stenotaphrum secondatum* (Walt.) Ktze. | St. Augustinegrass | 0 | 0 |
| *Zea mays* L. | Corn (Trucker's Favorite) | 0 | 0 |
| *Zoysia matrella* (L.) Merr. | Zoysiagrass | 0 | 0 |
| Solanaceae | | | |
| *Capsicum frutescens* L. | Pepper, green (California Wonder) | 0 | 0 |
| *Datura stramonium* L. | Jimsonweed | 0 | 0 |
| *Lycopersicon esculentum* Mill. | Tomato | | |
| | (Marglobe) | 0 | 0 |
| | (Tiny Tim) | 0 | 0 |
| *Nicotiana tabacum* L. | Tobacco | | |
| | (Kentucky 26) | 0 | 0 |

Table 2 (*Continued*)

| Family and scientific name | Common name (cultivar in parenthesis) | Dry-weight | |
|---|---|---|---|
| | | Mortality (%) | Reduction (%) |
| *Solanum ptycanthum* Dun. | Nightshade, eastern black | 0 | 0 |
| *Solanum tuberosum* L. | Potato | | |
| | (Kennebec) | 0 | 0 |
| | (Red LaSoda) | 0 | 0 |

*Significant at the 95% level; **significant at the 99% level according to the *t*-test.

infected several other important weeds. Compared to cocklebur the range of virulence was: common cocklebur (98%) marestail (*Conyza canadensis*) (80%) > giant ragweed (*Ambrosia trifida*) (65%) ≥ common ragweed (*Ambrosia artemisifolia*) (60%) mortality, with concomitant reductions in dry weight.

## Conclusions

Although most plant pathogens studied as bioherbicides have been fungi, some bacterial phytopathogens have been examined (Charudattan 2001). For example, rhizobacteria, such as *Pseudomonas fluorescens* have been shown to have suppressive action against certain grasses. A granular formulation containing *P. fluorescens* cells was evaluated as a soil-applied bioherbicide for green foxtail (*Setaria viridis* L.) control (Caldwell, Hynes, Boyetchko, and Korber 2012). Phytotoxins of certain *P. fluorescens* (Gurusiddaiah, Gealy, Kennedy, and Ogg 1994) and *Pseudomonas syringae* strains (Gealy, Gurusiddaiah, Ogg, and Kennedy 1995; Gealy, Gurusiddaiah, and Ogg 1996) inhibited the germination and early root growth of downy brome (*Bromus tectorum*). Bacterial strains of various *Xanthomonas* spp. have also been evaluated as bioherbicides. *Xanthomonas badrii* was reported as a pathogen on cocklebur in India (Patel, Kulkarni, and Dhande 1950). Several pathovars of *Xanthomonas campestris* have been identified on a wide range of plants including crops and weeds (Anonymous 1970). *X. campestris* pv. *poae* was reported to effectively control annual bluegrass (Imaizumi, Nishino, Miyabe, Fujimori, and Yamada 1997), and there was a direct dose response of bacterial concentration with annual bluegrass control (Imaizumi, Tateno, and Fujimori 1998). A commercial bioherbicidal product (Camperico®) consisting of dried bacterial cells was developed (Nishino and Tateno 2000). Successful control using this product requires mowing (wounding) of the bluegrass prior to application.

The host specificity of plant pathogenic bacterial strains is generally high and well characterised, and numerous pathovars have been defined in many species of these pathogens. A pathovar is a bacterial strain or set of strains with the same or similar characteristics, that is differentiated at the sub-specific level from other strains of the same species or subspecies on the basis of distinctive pathogenicity to one or more plant hosts (Dye et al. 1980). The *Xanthomonas* genus contains about 30 species pathogenic to over 400 different plants including many crops (Hayward 1993). It has been shown that plant pathogenic bacteria can occur on non-host plant surfaces without inciting disease symptomatology. For example, the causal agent of bacterial

leaf spot of lettuce (*Lactuca* spp.), *X. campestris* pv. *vitians*, colonised plant surfaces of several weed species belonging to members of the Asteraceae family, as well as some in the Chenopodiaceae, Malvaceae, Polygonaceae and Portulacaceae families (Toussaint, Benoit, and Carisse 2012). The number of bacteria on plant surfaces varied significantly among these different weed species and significantly more were recovered on lettuce than on plants in the Chenopodiaceae, Polygonaceae and Portulacaceae families. Although these latter families were not proven as 'true' hosts of *X. campestris* pv. *vitians*, they may play a role in the epidemiology by harbouring the pathogen, thus providing initial inoculum for infection (Toussaint et al. 2012). In studies with our *X. campestris* isolate (LVA987), lettuce was also affected, along with several other members of the Asteraceae (Table 2).

Asteraceae is an economically important family comprising important crop (e.g., sunflower) and several horticultural species (e.g., marigold, cone flowers, daisies, chrysanthemums, dahlias and zinnias) used in home gardens or in commercial production. In our study, Asteraceae species including marigold, zinnia, sunflower, lettuce and several weeds exhibited varying degrees injury when challenged with *X. campestris* (isolate LVA987). Because of this susceptibility, infectivity of these plants might occur if contacted by drift or other off-target dispersal of bacterial inoculum from field application of this pathogen used for biological weed control. Thus, as with field-scale application of herbicides and other compounds that alter plant growth, biological control agents should be applied using proper safeguards to protect non-target species. We did not examine any of the plants in our host range experiments for bacterial colonisation, but this could perhaps be the subject of future investigations.

Certain surfactants can promote efficacy in fungal and bacterial pathogens used as bioherbicides (Zidak, Backman, and Shaw 1992; Weaver et al. 2007; Sanyal et al. 2008; Boyette and Hoagland 2012). For example, Silwet L-77 has been reported to provide enhanced wetting of plant foliage and to increase stomatal infiltration of aqueous solutions (Field and Bishop 1988; Zabkiewicz and Gaskin 1989) in several different plant species. The extremely low oil–water surface tension (20 dynes $cm^{-1}$) created by Silwet L-77 has been shown to facilitate direct penetration by bacterial cells of *P. syringae* pv. *phaseolicola* van Hall, into kudzu [*Puerara montana* var. *lobata* (Willd.) Ohwi] stomata, thereby enhancing infection of kudzu by this bacterial pathogen (Zidak et al. 1992). Thus, there is a need to investigate the compatibility as well as the utility of surfactants for pathogen-weed systems. Future studies will consider other surfactants and adjuvants in an effort to find agents that will increase efficacy and perhaps alter the host range of this pathogen as reported previously for other bioherbicides (Bowling, Vaughn, Hoagland, Stetina, and Boyette 2010; Boyette, Bowling, Vaughn, Hoagland, and Stetina 2010; Boyette, Gealy, Hoagland, Vaughn, and Bowling 2011). Histological studies will determine the mode of entry into plant tissues facilitated by surfactants, that is, through stomates, hydathodes or natural wounds. Other studies will elucidate the environmental requirements of this pathogen to achieve optimal weed control, and to evaluate its weed control efficacy of glyphosate resistant weeds such as marestail and giant ragweed under greenhouse and field conditions. Knowledge of these basic parameters is essential for evaluating this plant pathogen as a bioherbicide for cocklebur control.

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