OQn 11 DES2014 RPU Rec'd from Araine Clay wath ahont 6 mannage seconch for Franklen Lo $_{0}$, NC uniluding the follaning:

$\triangle$ OLIVIA GRUDUP - Nem to Buople Syptem
S. N. U = erhely STDNEFNOBLES 4 b 1860 d 1920
$\Delta$ JIm u
$\Delta$ R. $u=$ ?
$\triangle$ MARTHAT. $u=$ ?
Note - $9 x$ inlunely ther in an AF-AND cosple-RPM

 Frankein Cu, D.C. Entatis Recorda-Pace-Pance, Acriel/Chubedfor Re 1803
 Nitate chandio
(1) Calvin Pearce-1868-Heirs Harviett Upchunch cifle of a. Mfpchurch- Mowe sheeto in foder copy of one enclosed.
(2) Pomes_Pearce-1792-Sole of Est.-Buyers: Berjamin Upchurch tother-.
Boncl: Chiedrens of Cames Pricie bound to Hhomas Pay'- Buedy, Belsey, Penny, Patiei $t^{*}$ Willis pierce, Bondsmen. Fhoma's Pay, A Sichard Eypchunch $t$ Berj. Wheler.
(3) Dohn Pearce-1865-Mivision of Land of Dhn Pearce. 18 oct. 1865 . Phiccren or hevid at law of Ootni- Calvis Pearce, Pembertio pietchellot uvife, Lucinda, Clem alford $t$ wife Gane, Priscilla Afford, Gosph Pully twite Jannié, Clara ass Aasy Ambrose Elpchunch tuige

$\frac{\text { Franklin C., D.C. Estates Records- Hay, Shervol-Siel, }}{\text { Cohn D. }}$ Gohn D.-1781-1934. C.R.039.508.25
(4) Wotting of interiet.

Frankeri Co, D.C. Estates Becordo - Perom, Zhomas-Minnull. C.R.039.508.62

Ohecked for Price - Mothing of interest.
$\frac{\text { Fhankhiv Co, DN.C. Estotes PRecords-H6bts-A Iopheio. }}{1781-1934 \text {. C.R. } 039.508 .36}$
1781-1934 • C.R.039.508.36
(1) Calvin Hoplensi-1864. Sale - Buyero R.(1) upchurch $t$ othera.

FROM 1995 CD-ROM

| Unchurch Dorothy E | 1129 Snyder Ln | Bakersfield | CA $93304-3624$ | $805-831-3086$ |  |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Unchurch Lloyd | 4000 N Chester Ave | Bakersfield | CA $93308-1146$ | $805-393-8657$ |  |
| Unchurch Rob | 709 Kain Ave | Albany | CA | $94706-1604$ | $510-527-5195$ |
| Unchurch R $\uparrow$ |  | Anaheim | CA 92802 | $714-839-8500$ |  |
| Unchurch B | $1291 / 2$ Snyder Ln | Bakersfield | CA 93302 | $805-397-7534$ |  |
| Unchurch Dorothy E | Bakersfield | CA 93304 | $805-831-3086$ |  |  |

## FROM 1995 CD-ROM

Upchurch John
Upchurch Matthew S
Upchurch B
Upchurch R $\uparrow$
Upchurch Philip
Upchurch Philip Fax Line

212 May Ave
9200 Monte Vista Ave

5821 Vineland Av
5821 Vineland Av

Monrovia
Montclair
Newhall
Newhall
North Hollywood
North Hollywood

CA 91016-2230 818-358-2032
CA 91763-1750 909-625-0668
CA 91321 805-255-7921
CA 91321 805-253-1109
CA 91601-1322 818-763-3449
CA 91601-1322 818-763-9127

FROM 1995 CD-ROM


## FROM 1995 CD-ROM

Unchurch Lucy
Unchurch R
Unchurch Keith W
Unchurch Mark
Unchurch Mona

| 831 Fulton Ave | Vallejo | CA $94591-7950$ | $707-643-6501$ |  |
| ---: | :--- | :--- | :--- | :--- |
|  | Venice | CA 90291 | $310-827-2723$ |  |
| 161 Montana Ave | Victorville | CA $92394-5422$ | $619-246-3967$ |  |
| 1280 Hacienda Dr | Vista | CA | $92083-6451$ | $619-945-3576$ |
| 1250 Waxwing Dr | Vista | CA | $92083-3045$ | $619-726-3141$ |

Osee St 29 JUN 1992 shuley Mavie Lamh to RSM
Raheit Lunsford and 1988 (scoot (CO, VA) A.M.U, daw $\mathcal{P} R, U \uparrow \& C \cdot U$ of Shademose, $G A$. Ralutt Lunsford is san of Manse Lansford of scadf $L_{0}, V A$
$R \quad u$
(1) R. $U$ bill of sale, witnessed $b y$ sion $U$ and willie $d$ 16 FEB 1830 - Franklin Co, NC Deed Bk 26 pg 544 (41, 㫙204)
$R . U$
(8) Buyley fist
R. Ugalwurds

Pflugersulle, $+\times 78660$


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R . \quad 4
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(1) In 1980 Bugley fist.

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\text { Ronto b } \\
\text { Easley, SC } 29640
\end{array}\right\} \text { en AF }
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R. Ipchunct
$\left.\begin{array}{l}66 \text { Trapelo Rd } \\ \text { Belmont, mA O2178 }\end{array}\right\}$ h AF
Compts.
$R . U$.
(1) Adtren in Socremento CA Shone Bh TAN MAS) copred 185AN 197)
R.. Upchenct
$\left.\begin{array}{l}8601 \text { Fair Oaks Blul } \\ \text { Casmehael, CA } 95608\end{array}\right\}$ In AT Phone 944-3170
$R \quad U$
(1) Fen Astach 25 wert 2 th 18 mAYIF 15 - Franblin co NC- $u$ deed grantore
A. "1853 R.U. A C. 5 . foner - Boh 31 of 143 "
B. "1867 R. U. 大 s.m. U. - Book 33 pg 27 "
(2) Per Adtudi"26 wect th 18MAY1875-Fnanhem Co NC Graulsto " 1835 R. U. to Alue U. Bk 22 Pg 128."
(3) Per Aitach \#24 West th 18 mAY 1975-Froukhen Lo NVC Conneyemere " 1891 s.m.u to R.U. Bk 33 阬 $291 "$

# (C) My Rezoidz II NOV 1998 

UPCHURCH,R.A.
Marriage
Wife: E. J. RICHARDSON
Marriage Date: 6 Dec 1879
Recorded in: Oktibbeha, Mississippi
Source: FHL Number 900509 Dates: 1886-1895
$R, A \cdot U$

$$
\text { XID - } 2076
$$

$$
R . A \cdot U=X I D-2026=\text { RHODA } A . U \text {, whom see. }
$$



# ONC BIRTHS-SSEP 2005 -PETERSON-FRANKLIN CO-FAT = UPCHURLIV $1900-1932$ PART $I_{3}$ 



# NC BIRTHS-SSEP 2005 -PETERSON-FRANKLIFN CO-FAT = UPCHURCIV $1900-1937$ PART $I_{3}$ 


$R \quad B \quad u$
R. B. Unchurch
birth: 1898
North Carolina
Marriages, 1759-1979 marriage:
2 Jan
1943
Marriages, 1759-1979 marriage: 1943 Alamance, NC


FROM 1995 CD-ROM
Upchurch P L
Upchurch R B
Upchurch R L
Upchurch Robert
Upchurch Rosa B
Upchurch W D
Bedias
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TX 77831
TX 77831
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409-395-2931
409-395-8762
409-395-6061
409-395-2431
409-395-6201
409-395-6593
R.B. U. U
(1) Bayley Jist.
$\left.\begin{array}{l}\text { R.B. Ysolunch } \\ 828 \text { 165 th Ane NE } \\ \text { Belleme, wA } 98008\end{array}\right\} \ln A F$.
$R$. BENBOW $u$
(1) Addren in seatte, WA 1974 Phone Bh.
copreid 4 APR 1975
R. Benbow grhmech $\}$ ave $N$ AF
909. $4 \operatorname{StA}_{1}^{a^{2 v}} \mathrm{~N}$

CQhone 283-2211)
birth: 1892
R. Brack Upchurch

| North Carolina Marriages, |
| :--- |
| $1759-1979$ | marriage: | 28 Sep |
| :--- | :--- |
| 1913 |$\quad$| Near Apex, Wake, North |
| :--- |
| Carolina | spouse: Addie Fuller

R.C. Upchurch

North Carolina Marriages, 1759-1979
spouse: Dora A. Upchurch child: Rupert H. Upchurch

## R. C. Upchurch

North Carolina Marriages, 1759-1979
spouse: Dora Upchurch
child: William A. UpchurchTX Brithes
UPCHurch Fried
$R \quad C \quad u$
(1) 1920 CENSUS-WARE CO,NC
R.C.U 33 baut 1887
hodger unta:
Not Repartex


OTEXAS BIRTH INDEX-PER C.J. PETERSON,JR 18 JUL 2005 1903-1997


## OFROM 1995 CD-ROM

| Upchurch Chris | 4173 180th Ave Se |
| :--- | :---: |
| Upchurch Thomas B | 9207 Ne 38th St |
| Upchurch Gene | 27610 86th E |
| Upchurch Brian | 2882 Sun Mountain Dr |
| Upchurch Craig | 2207 S 292nd St |
| Upchurch Phillip W | 308 Point Fosdick Pl Nw |
| Upchurch Brian | 9417 S 207th Pl |
| Upchurch R C in | 4125 Somerset Ln |
| Upchurch R D | 9417 S 207th Pl |
| Upchurch Robert C | 4125 Somerset Ln |
| Upchurch Robert E | 12509 Se 214th Pl |
| Upchurch Robert E | 12509 Se214th Pl |
| Upchurch Ben | 329 6th Ave |
| Upchurch L C | 201 2nd Ave S |
| Upchurch Sue M |  |
| Upchurch Chris |  |
| Upchurch Paul E |  |
| Upchurch J \& L | 7240 Littlerock Rd Sw |
| Upchurch Jim | 7240 Littlerock Rd Sw |
| Upchurch Jim | N4822 Ormond Rd |
| Upchurch Shelly | 4813 Dana Dr Se |
| Upchurch John |  |
| Upchurch Mark | 1536 Thayer Dr |
| Upchurch H C | 2328 Swl15th St |
| Upchurch Allan R | 3015 W Tilden St |
| Upchurch Lance |  |
| Upchurch Lisa | E13717 Riversd |
| Upchurch Eldon E | 8025 S Alaska |
| Upchurch Brian | 7812 59th W |
| Upchurch Rodney |  |


| Bellevue | WA 98008 | $206-643-4711$ |
| :--- | :--- | :--- |
| Bellevue | WA 98004-1345 | $206-455-2709$ |
| Buckley | WA 98321-9458 | $206-829-1003$ |
| Enumclaw | WA 98022-2269 | $206-825-5790$ |
| Federal Way | WA 98003-3822 | $206-946-0839$ |
| Gig Harbor | WA 98335-7822 | $206-851-8644$ |
| Kent | WA 98031-1414 | $206-852-5183$ |
| Kent | WA 98032-7617 | $206-852-3467$ |
| Kent | WA 98031-1414 | $206-859-0929$ |
| Kent | WA $98032-7617$ | $206-852-3467$ |
| Kent | WA $98031-2277$ | $206-631-7248$ |
| Kent | WA $98031-2277$ | $206-631-7248$ |
| Kirkland | WA $98033-5532$ | $206-827-5915$ |
| Kirkland | WA $98033-6510$ | $206-822-9147$ |
| Mercer Island | WA 98040 | $206-232-0964$ |
| North Bend | WA 98045 | $206-831-6986$ |
| Oak Harbor | WA 98277 | $206-675-3743$ |
| Olympia | WA 98503 | $206-456-1048$ |
| Olympia | WA $98512-7419$ | $206-786-1916$ |
| Olympia | WA $98512-7419$ | $206-786-9790$ |
| Otis Orchards | WA $99027-9731$ | $509-226-3258$ |
| Port Orchard | WA $98366-9534$ | $206-895-4186$ |
| Port Orchard | WA 98366 | $206-895-2517$ |
| Richland | WA $99352-2869$ | $509-943-1804$ |
| Seattle | WA $98146-3433$ | $206-246-8170$ |
| Seattle | WA $98199-1728$ | $206-283-7433$ |
| Seattle | WA 98109 | $206-281-4306$ |
| Spokane | WA $99216-0828$ | $509-926-6627$ |
| Tacoma | WA $98408-1039$ | $206-539-0864$ |
| Tacoma | WA $98467-2601$ | $206-474-6266$ |

Upchurch \& Associates
Upchurch Doris S
Upchurch Irvin
Upchurch J C
Upchurch Music Co Inc
Upchurch Paul
Upchurch Paul J
Upchurch R C A
Upchurch W W
7303 Abercorn St
806 Wilmington Island Rd
1415 Audubon Dr
806 Wilmington Island Rd
7303 Abercorn St
43 Navigator Ln
11934 Idlewood Dr
17 Herty Ave
5521 Waters Dr
Savannah
Savannah
Savannah
Savannah
Savannah
Savannah
Savannah
Savannah
Savannah

GA 31406-2446 912-354-0004
GA 31410-4503 912-897-4114
GA 31401-7882 912-232-3115
GA 31410-4503 912-897-4103
GA 31406-2446 912-352-3737
GA 31410-2136 912-897-0174
GA 31419-1812 912-927-1769
GA 31408-1315 912-964-4979
GA 31406-2038 912-355-2392
R.C.U

OIn 1980 Buyley fot.

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3610 \text { Montreab Creeh } \\
\text { Clasksten, GA } 30021
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\text { Noucross, IA } 30071
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R.C.Lupchunch 17 Hesty aue. Savarnal, Ha. 31408
Phoue book syet 1979.

## 1995 CD-ROM



## $\bigcirc$ FROM 1995 CD-ROM

Upchurch Chris
Upchurch Thomas B
Upchurch Gene
Upchurch Brian
Upchurch Craig
Upchurch Phillip W
Upchurch Brian
Upchurch R C
Upchurch R D
Upchurch Robert C
Upchurch Robert E
Upchurch Robert E
Upchurch Ben
Upchurch L C
Upchurch Sue M
Upchurch Chris
Upchurch Paul E
Upchurch J \& L
Upchurch Jim
Upchurch Jim
Upchurch Shelly
Upchurch John
Upchurch Mark
Upchurch H C
Upchurch Allan R
Upchurch Lance
Upchurch Lisa
Upchurch Eldon E
Upchurch Brian
Upchurch Rodney



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EROBERT PAFTLLA LIANURCN]
R.D.U.
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\text { R. D. U. } \\
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\text { Goffney, Sc } 29340
\end{array}\right\} \ln A F \text {. }
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$R \quad$ PhuTo CEM-ELLIS CO, TX
(1) $\longrightarrow$ Report of Edjar Dalton Groding who ingiecten the cemetery on ZOFES 1996 ( $R E C$ 'D 23FEB1996-R20
DETAILS QN MARKERS:


TNate: From the une bean that "fennie md f.it. Powese The idintly of ${ }^{ \pm} 2$ and $\# 3$ ric not yet hnown - RPU TCulled id 23 FES 1996 - He says the marher for llenjon bromnon ll reabe sumply $K$. B. Ugehurch uuth the dato shown as shightly dypernt from ny vocos For fennie marher readr jennie $\mathcal{Z}$, unfe of I.H. Pomens, dan of W.A.U and R.F.U.
(2) See Pinto, Tx Cemitery File - Entry 8 mor 1996

Anersian of boo cemeleny iecarded 10 mAN 1939 shoun R.E.Un \& Y APR 1854 \& 30 SEP 1897

| Upchurch Diane S | 3509 Lanell Dr |
| :--- | ---: |
| Upchurch Gary | 308 Brownlee Cir |
| Upchurch Janice Vowell Hairsty | 2352 Barksdale Blvd |
| Upchurch John \& Amy | 3509 Lanell Dr |
| Upchurch RE g | Hilly Rd |
| Upchurch M J | Crestvw Dr |
| Upchurch Max | Fire Twr Rd |


| Bossier City | LA | $71112-3710$ | $318-742-0704$ |
| :--- | :--- | :--- | :--- |
| Bossier City | LA | $71111-2043$ | $318-742-4116$ |
| Bossier City | LA | $71112-3202$ | $318-747-3749$ |
| Bossier City | LA | $71112-3710$ | $318-746-6099$ |
| Dubach | LA | 71235 | $318-777-3631$ |
| Ferriday | LA | 71334 | $318-757-3682$ |
| Grand Cane | LA | 71032 | $318-858-2548$ |

## FROM 1995 CD-ROM

| Upchurch Charley J | 111 E Washington St |
| :--- | :---: |
| Upchurch Mark | 701 E Main |
| Upchurch Clifton |  |
| Upchurch Charles M | 3500 St Joachim Ln |
| Unchurch R E | 10100 Cabana Club |
| Unchurch Marvin | 529 Caulks Hill Rd |
| Upchurch Edwin R | Moselle Rd |


| Richland | MO 65556 | $314-765-4380$ |
| :--- | :--- | :--- |
| Richland | MO 65556 | $314-765-3930$ |
| Rockaway Beach | MO 65740 | $417-561-4160$ |
| Saint Ann | MO $63074-2922$ | $314-429-5010$ |
| Saint Ann | MO $63074-1726$ | $314-427-0503$ |
| Saint Charles | MO $63304-7448$ | $314-928-5881$ |
| Saint Clair | MO 63077-1230 | $314-629-1913$ |

## FROM 1995 CD-ROM

| Upchurch D C |
| :--- |
| Upchurch John C Jr |
| Upchurch R E $\uparrow$ |
| Upchurch H C |
| Upchurch William C |

2086 Brockett Rd
3671 Bonnie Glenn Ln 115 Chimney Spring

Lakebluff Dr Rr 108 White Oak St

Tucker
Tucker
Tyrone
Waynesboro
Whitesburg

GA 30084-5514 404-934-2818
GA 30084-8302 404-934-3407
GA 30290-9666 404-487-1391
GA 30830 706-554-3488
GA $30185 \quad$ 404-832-2932
R.E. Mpchunch
phore book 1979

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\begin{aligned}
& \text { R.E.Lpchurch } \\
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\text { atlanta, Sa. } 30337
\end{array}\right\} \text { \& AF }
\end{aligned}
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R.E.U.
(1) Ginin on 1050215 suit to Ada Morgan ar a cogperitos that mould not morh. 7308 muncaster nill Rd Derwood, mD 20855

O see pento, TX Cemetery Recordr - In fot BMAR 1996 Gerald Bavily Tyler of RPD

ROMDPUTOKCEMETERF
Located five miles East of Maypearl, Ellis County, Texas, off F.M. \#308, on Mill Creek. Recorded by Frances Phillips on 10 Mar. 1979.

UPCHURCH, ReF. $\quad 4$ Apr 1854/30 Sep.t. 1897

LITTLE, Mrs. M.E. - died 18 Aug. 1889 - wf of C.O.
 dt.of W. W. \& R-J.UPCHURCH:
KILIINGSWORTH, Mary Bell - 18 Mar 1870/8 Jan 1887 - wf of J.B.
GIENN, A.E. - 31 Dec 1869/15 Nov 1891 - wf of J.C.
UPCHURCH, N.B: - 19 May 1839/7 Dec 1893 - husb of M.S.
UPCHURCH, Sarah Mo- 27 Aug 1829/22 Febl894;
UPCHURCH, M.S. - 21 Mar 1845/ 16 Oct 1885-wf of W.B.?
Troter same of the abere ad hnown $w \cdot 1 t \cdot U=$ ulham theny $u$, $I$
I. Richard $u_{7}$ IIt-Head yf Clas
A. Caluen U, I

1. Rechand K, IV
a. wulham tenng $U$, I sud

TQuetton: Cowd
$i$. Gmney Suevenyer $U^{\prime \prime}$ formie"
R.E.f.Un he mo 5.A. Pomen - RPUS Richand U, II??-RPU


Marriage Date: 15 Jan 1890
Source: FHL Number 1006514

Recorded in: Grimes, Texas
Dates: 1889-1899
(1) FROM 1995 CD-ROM

Upchurch Brenda W Upchurch HF Upchurch Hollis Upchurch J B Upchurch M A Upchurch Marty P Upchurch R F $\uparrow$

| 109 Ben Hill St | La Grange |
| :---: | :--- |
| 903 Park Ave | La Grange |
| 860 Stewart Rd | La Grange |
| 38 Stewart Rd | La Grange |
| Mt Zin Rd | La Grange |
| 598 Stewart Rd | La Grange |
| 74 Stewart Rd | La Grange |
| 53 S Oak Dr | La Grange |

GA 30240-2607 706-882-4064
GA 30240-5015 706-882-6077
GA 30240-8133 706-882-7529
GA 30240-8129 706-884-5487
$\begin{array}{lll}\text { GA } & 30240 & 706-882-6257 \\ \text { GA } & 30240-8131 & 706-884-2586\end{array}$
GA $30240-8129$ 706-882-2505
$\begin{array}{lll}\text { GA } & 30240-8129 & 706-882-2905 \\ \text { GA } & 30240-8479 & 706-882-7907\end{array}$
(2) In 1980 Bayley Jitt
R.F.U介

(3) 1984 Tayler Jut
R.F.Upluuds
stemart Rd Rt 4
La Gnarge, GA 30240

$$
(404) 882-2905
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ORPU Extint of 3 NOV 1892 WILKES CO, NC DEED BK 31 阵 223 [Dued 9 in dtr 24 APR 1999 Lemir Tumotty Fersir to kAMS

RECIE F.U 末 unfe PHOEBE F.U ("PHEBE", "PHOEBY") sell 100 Acer for ${ }^{\$ 190}$ an water of whtuto oak Baard, o an county Line A LEVI colisivs. Bolts hua t unfe suin sued an $X$. Hife examined seporabof Adjain lave of J. F. GENTRY, AUSTAN LYANC?), ROBERT WOODRUFF \& R.F.UT[RECEEF. U\}
R.F. Ipotureh

Shan lork 1580 .

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871 \text { soldel Stage del. } \\
\text { Raluig2,NC } 27603
\end{array}\right\} \text { AF }
$$

R.F. $U$
(1) Ses Noter $g$ R PU 18 NOV 1978 Vent to musice brown en the 1920 "Raotle" (Ansuch foi Rallgh we (Hy's School)
gage 62 R.F.U inaron foothall teans.

01984 Taylon list
R. M. Lepchureh

4 wibon st.
manchastir, ISa. 31816

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(404) 846-2866
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R. G. U
(1) In 1980 Bayley fit.

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\text { manchesto, GA } 31816 .
\end{array}\right\} \text { InAF }
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R. G. U.

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(1) See Pachet from Ben fanell Si- 22 moy 1980 E5- R.G.U. in child of Bobll, ge of melic J. U. of charbe/ Thomor bay 4 clan.
R. G. U.
(DPer Adtach \#24 wht Jt, 18 mAY 1975-uphhuch with-Wehelo NC contt toove "R.G.U. क力 Frovin J.U. 16 MAR 1901 - bec D, Py 457, Fib z $2180^{2}$
R.G.U K XID-3013 = ROBERT ERANAM $U$, whom see copied for Robeit GrahnurlNOTE: The artoched article from ured sacince 46: 139-146 TAN-FES 1998 includer a reference $D$ an artole by R.G. 4 r

# Prevention of fungal diseases in transgenic, bialaphos- and glufosinate-resistant creeping bentgrass (Agrostis palustris) 

Chien-An Liu<br>Heng Zhong<br>Department of Crop and Soil Sciences, Michigan<br>State University, East Lansing, MI 48824

Joseph Vargas
Department of Botany and Plant Pathology,
Michigan State University, East Lansing, MI 48824

## Donald Penner

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#### Abstract

The antifungal activity of the herbicides bialaphos and glufosinate, the active moiety of bialaphos, was assessed. Bialaphos showed a higher level of in vitro antifungal activity against Rhizoctonia solani, Sclerotinia homoeocarpa, and Pytbium aphanidermatum than glufosinate. Glufosinate suppressed the mycelial growth of R. solani and $S$. homoeocarpa, but it had no inhibitory effect on P. aphanidermatum up to the highest concentration in our testing regimes. Various concentrations of bialaphos solutions were applied to transgenic, bialaphos- and glufosinate-resistant creeping bentgrass inoculated with fungal pathogens. Bialaphos applications were able to significantly reduce symptomatic infection by $R$. solani and S. homoeocarpa on transgenic plants. Bialaphos significantly inhibited $P$. aphanidermatum, but not to the same degree that $R$. solani and $S$. homoeocarpa were inhibited. These results indicate that bialaphos may provide a means for the simultaneous control of weeds and fungal pathogens in turf areas with transgenic, bialaphos-resistant creeping bentgrass.


Nomenclature: Bialaphos, L-2-amino-4-(hydroxymethylphosphinyl)butanoic acid-L-alanyl-L-alanine; creeping bentgrass, Agrostis palustris Huds. AGSPL; glufosinate.

Key words: Transformation, plant disease, weed control, brown patch, dollar spot, Pythium blight, Fusarium blight, take-all patch, AGSPL.

Creeping bentgrass is the most commonly used Agrostis species on golf putting greens and similar closely cut turf areas in North America (Beard 1982). Among the existing cultivars of creeping bentgrass, 'Penncross' is one of the major ones and is being used increasingly on many golf course greens. Due to close mowing, heavy fertilization, intense irrigation, and constant bruising from traffic and divoting, turfgrass diseases are a greater problem on golf courses than on most other types of turf (Smiley 1983). Creeping bentgrass is susceptible to a variety of diseases such as brown patch (Rhizoctonia spp.), dollar spot (Sclerotinia homoeocarpa), Pythium blight (Pythium spp.), Fusarium blight (Fusarium roseum and $F$ tricinctum), and take-all patch (Gaeumannomyces graminis) (Smiley 1983).

Bialaphos, ${ }^{1}$ a nonselective and broad-spectrum contact herbicide, is a tripeptide composed of two L-alanine residues and an analog of glutamate known as phosphinothricin or glufosinate (Ogawa et al. 1973; Suzuki et al. 1973). Glufosinate inhibits the glutamine synthetase (GS) of bacteria and plants (Bayer et al. 1972; Leason et al. 1982). Bialaphos, a precursor of glufosinate produced by some strains of Streptomyces, had little or no inhibitory activity in vitro (Ogawa et al. 1973; Tachibana et al. 1986a). In both plants and bacteria, the active glufosinate moiety is released by intracellular peptidases that remove alanine residues from bialaphos. The GS plays an important role in assimilation of ammonia, both in plants and bacteria (Joy 1988; Miflin and Lea 1977). Inhibition of GS, by either bialaphos or glufosinate, causes a rapid build-up of intracellular ammonia levels and an associated disruption of chloroplast structure, resulting in inhibition of photosynthesis and plant cell death (Tachibana et al. 1986b). Bayer et al. (1972) reported that bialaphos had a certain antibiotic effect on Escherichia coli and Bacillus subtilis, which was neutralized by the addition
of glutamine. Experiments with GS isolated from E. coli confirmed that glufosinate inhibited the GS in competition with glutamate as a varying substrate. Leason et al. (1982) determined that the activity of GS was inhibited by glufosinate in peas (Pisum sativum L.), and they demonstrated that the mode of action was the same in bacteria and plants.

Glufosinate resistance has been achieved by introducing a gene coding for a detoxifying enzyme, phosphinothricin acetyltransferase (PAT), into several plant species (Akama et al. 1995; Casas et al. 1993; Christou et al. 1991; De Block et al. 1987, 1989; Somers et al. 1992; Spencer et al. 1990; Wan and Lemaux 1994; Zhong et al. 1996). The gene, designated bar, was found in strains of Streptomyces that produced bialaphos and was isolated from S. hygroscopicus (Murakami et al. 1986). The bar gene product protected these strains from the action of their own antibiotic by metabolizing glufosinate to an inactive, acetylated derivative (Thompson et al. 1987).

We have generated transgenic creeping bentgrass showing high levels of bar gene expression, which resulted in both glufosinate and bialaphos resistance. It was reported that bialaphos treatment of transgenic rice (Oryza sativa L.) plants expressing the bar gene could prevent infection by the sheath blight fungal pathogen ( $R$. solani) (Uchimiya et al. 1993). Glufosinate in vitro reduced colony radius of and sclerotia production by both $R$. solani AG-1 IA and IB, the etiologic agent of Rhizoctonia foliar blight of soybean [Glycine max (L.) Merr.] (Black et al. 1996). Since R. solani is also the etiologic agent of brown patch, one of the severe foliar fungal diseases of creeping bentgrass, it was the objective of this research to determine whether glufosinate and bialaphos had potential for protecting the bialaphos- and glufosinate-resistant transgenic creeping bentgrass against fungal diseases. If the application of these herbicides could
be shown to reduce plant damage due to fungal infection, a novel and economical usage for the herbicide would be to provide weed control in turf areas with bialaphos- and glu-fosinate-resistant creeping bentgrass while simultaneously and cumulatively reducing levels of fungal pathogens.

## Materials and Methods

## Regeneration and Analysis of Transgenic Plants

Friable embryogenic callus was initiated from mature seeds of creeping bentgrass cultivar Penncross following the method described by Zhong et al. (1993). The embryogenic calli were subjected to microprojectile bombardment ( $\mathrm{He} /$ 1000, Bio-Rad) with the plasmid pTW-a (Zhong et al. 1996). This plasmid contains the bar gene, driven by the CaMV 35 S promoter and terminated with the nos $3^{\prime}$ region. It also contains the $\operatorname{pin} 2$ gene, driven by the $\operatorname{pin} 2$ promoter and the rice Actl intron and terminated with the pin2 $3^{\prime}$ region.

The bombarded calli were cultured and selected on a callus induction medium (Zhong et al. 1993) containing 5 $\mathrm{mg} \mathrm{L}^{-1}$ bialaphos or $15 \mathrm{mg}^{-1}$ glufosinate for 12 wk after bombardment. Plantlets were regenerated on a regeneration medium (Zhong et al. 1993) containing the same amount of corresponding selective agent for 1 or 2 mo before being transferred to soil in the greenhouse. The plants in the greenhouse were sprayed with $2.4 \mathrm{~g} \mathrm{~L}^{-1}$ of glufosinate-ammonium ${ }^{2}$ in a spray volume of $150 \mathrm{~L} \mathrm{ha}^{-1}$.

The genomic DNA, RNA, and protein purified from leaf samples of greenhouse-grown, independently transgenic plants were analyzed by Southern blot hybridization (Southern 1975), Northern blot hybridization (Wadsworth et al. 1988), and thin-layer chromatography (D'Halluin et al. 1992; Spencer et al. 1990), respectively.

## In Vitro Test

The sensitivity of different pathogens to glufosinate and bialaphos was first tested in vitro by culturing fungi on glu-fosinate- or bialaphos-supplemented medium. Isolates of $R$. solani, S. homoeocarpa, and P. aphanidermatum were cultured on potato (Solanum tuberosum L.) dextrose agar medium (PDA) as stock plates. Glufosinate and bialaphos were dissolved in double-distilled water and filter-sterilized (Nalgene filter) before adding into molten PDA ( 60 C ) medium after sterilizing at 121 C for 15 min . A dilution series was used to obtain the needed concentrations. The amended medium was mixed thoroughly and poured into $10-\mathrm{cm}$-diam petri dishes.

Inoculum for the study, which consisted of 7 -mm-diam agar plugs of fungal mycelia, was taken from stock plates and transferred onto freshly prepared PDA. Plates were incubated for at least 1 wk under lights at 25 C . When mycelia had completely covered the plates, plugs were aseptically transferred to fresh PDA twice. Inverted plugs were then transferred to the center of test plates (one plug per plate) containing 20 ml PDA medium supplemented with various concentrations of glufosinate or bialaphos ranging from 0 to $600 \mathrm{mg} \mathrm{L}^{-1}$. Test plates with inoculum were wrapped with parafilm and placed under the light at 25 C . There were 15 replications in each treatment.

Radial growth (mm) of mycelia on bialaphos- and glu-


Figure 1. Transgenic plants (upper portion) and untransformed control plants (lower portion) 2 wk after foliar spray of glufosinate.
fosinate-supplemented medium was measured 4 d after incubation and compared with that on nonamended medium. This measurement was used as an indicator of the sensitivity of the fungus to glufosinate or bialaphos. Percent inhibition was plotted as a function of the bialaphos or glufosinate concentration. Linear regression was used to determine the $\mathrm{ED}_{50}$ value (effective dose for a $50 \%$ response). Tukey's Honestly Significant Difference test at $\mathrm{P}=0.05$ was employed to perform the statistical analysis to detect differences in the mean values of radial length.

## Greenhouse Test

Twelve grams of dry wheat (Triticum aestivum L.) seeds were autoclaved twice for 30 min in a $125-\mathrm{ml}$ glass flask containing 25 ml of distilled water. Five $7-\mathrm{mm}$-diam PDA plugs with actively growing mycelium of $R$. solani, S. homoeocarpa, or P. aphanidermatum were aseptically transferred to the sterile seeds. The fungal culture was incubated under lights at 25 C for 1 wk after the inoculation.

To obtain uniform plants for fungal inoculation, 3-moold healthy transgenic (all from the same transgenic event) and nontransgenic control creeping bentgrass cultivar Penncross that were grown in $10-\mathrm{cm}$ plastic pots were trimmed (about 2.5 cm in height) then fertilized with Peter's 20-20201 wk before the pathogen inoculation. To inoculate the creeping bentgrass, about 500 mg of wheat seed inoculum was placed on each pot (Powell 1993).

Various concentrations of bialaphos ( 200 to $2,400 \mathrm{mg}$ $\mathrm{L}^{-1}$ ) were applied to the plants using a hand sprayer either 3 h before or 2 d after fungus inoculation. After the bialaphos application, plants were wrapped with plastic bags with holes to raise the humidity. Transgenic and nontransgenic control plants that were not treated with bialaphos were also subjected to the same inoculation procedures. There were 10 replications in each treatment.

Visual ratings of plant damage were taken on a 0 to 10 scale ( $0=$ no damage, $10=$ all dead $)$. Data were analyzed using the $F$-test at $\mathrm{P}=0.05$ and Tukey's Honestly Significant Difference test at $\mathrm{P}=0.05$.

| Fungus | Herbicide | Linear regression ${ }^{\text {b }}$ | Correlation coefficient | $\mathrm{ED}_{50}$ |
| :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | (mg L-1) |
| R. solani | Bialaphos | $Y=0.91 X+44.96$ | 0.72 | 5.54 |
|  | Glufosinate | $Y=0.11 X+17.86$ | 0.97 | 292.18 |
| S. bomococarpa | Bialaphos | $Y=0.28 X+40.75$ | 0.88 | 33.04 |
|  | Glufosinate | $Y=0.17 X+4.09$ | 0.98 | 270.06 |
| P. aphanidermatum | Bialaphos Glufosinate | $Y=0.037 X-4.28$ | 0.79 | $1,467.18$ |

[^0]
## Results and Discussion

## Regeneration and Analysis of Transgenic Plants

The selectable marker gene (bar) was transferred into creeping bentgrass by microprojectile bombardment of embryogenic callus. A total of 37 independent bialaphos- or glufosinate-resistant callus lines were recovered 3 mo after bombardment of embryogenic callus (Figure 1). Southern hybridization analysis, Northern hybridization analysis, and thin-layer chromatography analysis confirmed the integration of bar, the expression of bar, and the functional activity of phosphinothricin acetyltransferase, respectively, in all of 37 independent herbicide-resistant transgenic plant lines.

## In Vitro Test

Fungal pathogens, R. solani, S. homoeocarpa, and $P$. aphanidermatum, were inoculated and cultured on PDA medium supplemented with various concentrations of bialaphos or glufosinate to assess their responses to these two inhibitors of glutamine synthetase. Bialaphos exhibited inhibitory activity in vitro against the growth of $R$. solani, $S$. homoeocarpa, and P. aphanidermatum that was superior to glufosinate, as reflected by their $\mathrm{ED}_{50}$ values (Table 1; Figure 2). Increasing concentrations of bialaphos and glufosinate increased their inhibitory activity, with the highest rates resulting in the smallest colonies of mycelium. In our greenhouse test, the three pathogens also showed different responses to bialaphos and glufosinate.

Rhizoctonia solani was very sensitive to the addition of bialaphos into the PDA medium (Table 1; Figure 2). Even


Figure 2. In vitro response of Rhizoctonia solani (R), Sclerotinia bomoeocarpa $(\mathrm{S})$, and Pythium aphanidermatum ( P ) to bialaphos (B)- or glufosinate ( G )amended potato dextrose agar. The radial growth (millimeters) of the colony measured 4 d after inoculation was used to represent the mycelial growth of the pathogen for 15 replications.
at a concentration of $1 \mathrm{mg} \mathrm{L}^{-1}$, the radial mycelial growth of $R$. solani was significantly suppressed compared to that on PDA medium without bialaphos ( $31.6 \pm 0.7$ vs. 40.0 $\pm 0.0 \mathrm{~mm}$ ) (Figure 2). Only about 5 mg of bialaphos per liter was needed to reduce the fungal growth by $50 \%$ (Table 1). There was no significant growth of $R$. solani observed for up to 2 wk after the initial inoculation when the concentration of bialaphos was $60 \mathrm{mg} \mathrm{L}^{-1}$ or higher (data not shown).

Glufosinate was also very effective in the suppression of the mycelial growth of R. solani (Figure 2). However, glufosinate was not as effective as bialaphos in inhibiting the growth of $R$. solani on PDA, as reflected by the $\mathrm{ED}_{50}$ values (Table 1). The growth of mycelium was significantly reduced at the concentration of $25 \mathrm{mg} \mathrm{L}^{-1}$ compared to that on PDA without glufosinate ( $35.0 \pm 0.7$ vs. $40.0 \pm 0.0$ mm ) (Figure 2). There was still some mycelial growth of $R$. solani observed 4 d after the initial inoculation, even at a concentration as high as $600 \mathrm{mg} \mathrm{L}^{-1}$ of glufosinate in PDA.

The mycelial growth of $S$. homoeocarpa was sensitive to bialaphos and glufosinate, though its responses were different from those of $R$. solani. Higher concentrations of bialaphos ( $20 \mathrm{mg} \mathrm{L}^{-1}$ ) and glufosinate ( $100 \mathrm{mg} \mathrm{L}^{-1}$ ) were necessary to significantly reduce the mycelial growth of $S$. homoeocarpa. The $\mathrm{ED}_{50}$ value of bialaphos for $S$. bomoeocarpa was higher than $R$. solani ( 33.04 and $5.54 \mathrm{mg} \mathrm{L}^{-1}$, respectively). More than $200 \mathrm{mg} \mathrm{L}^{-1}$ of bialaphos amendment was necessary to completely suppress mycelial growth of $S$. homoeocarpa on PDA medium. However, S. homoeocarpa was more sensitive to higher concentrations of glufosinate (between 25 and $600 \mathrm{mg}^{-1}$ ) than $R$. solani. The $\mathrm{ED}_{50}$ value for glufosinate of $S$. homoeocarpa was lower than that of $R$. solani ( 270.06 and $292.18 \mathrm{mg} \mathrm{L}^{-1}$, respectively). In general, bialaphos or glufosinate amendment of PDA inhibired mycelial growth of R. solani and S. homoeocarpa, with the highest concentration resulting in the least mycelium growth.

Compared with R. solani and S. homoeocarpa, P. aphanidermatum was the least sensitive to both bialaphos and glufosinate (Figure 3). At least $500 \mathrm{mg} \mathrm{L}^{-1}$ of bialaphos supplement was needed to significantly reduce mycelial growth on amended PDA (Figure 2), and even then, P. aphanidermatum mycelia covered the whole petri dish 1 wk after the initial inoculation. The presence of glufosinate had no effect on inhibition of $P$. aphanidermatum up to the highest concentration ( $600 \mathrm{mg} \mathrm{L}^{-1}$ ) amended in PDA (Figure 2). However, the supplement of bialaphos and glufosinate had an


Figure 3. The inhibition of the growth of Rhizoctonia solani (A, B, and C), Sclerotinia homoeocarpa (D, E, and F), and Pythium aphanidermatum (G, H, and I) 4 d after the initial inoculation on PDA without (A, D, and G) or with $400 \mathrm{mg} \mathrm{L}^{-1}$ bialaphos (B and E), $350 \mathrm{mg} \mathrm{L}^{-1}$ bialaphos (H), 400 mg $\mathrm{L}^{-1}$ glufosinate ( C and F ), and $600 \mathrm{mg} \mathrm{L}^{-1}$ glufosinate (I).
inhibitory effect on growth of $P$. aphanidermatum when the visual density of mycelia, instead of the measurement of radial length of mycelium, was employed as the indicator to represent growth of $P$. aphanidermatum (Figures 3G-I).

It is intriguing to observe that different fungal pathogens showed the same trend in responses toward bialaphos and glufosinate in vitro. Bialaphos is a tripeptide precursor of glufosinate, an analog of glutamate, in which two alanine residues are linked to the glufosinate. While glufosinate is an inhibitor of glutamine synthetase from both plants and bacteria, the intact tripeptide had little or no inhibitory activity in vitro (Bayer et al. 1972; Tachibana et al. 1986a).

Though the glufosinate used in this study was a racemic mixture of D - and L -isomers, in which the D -isomer is inactive and the L -isomer is the active GS inhibitor, it is still difficult to explain the significant differences shown in the magnitude of $\mathrm{ED}_{50}$ values.

## Greenhouse Test

Various concentrations of bialaphos were applied to the transgenic creeping bentgrass expressing the bar gene to assess the effects of bialaphos on development of three different pathogens (Table 2).

Table 2. Effects of bialaphos applied 3 h before or 2 d after the pathogen inoculation on the development of R. Solani, S. homoeocarpa, and $P$. aphanidermatum in transgenic creeping bentgrass.

| Pathogen | Time of spraying | Disease rating ${ }^{2}$ |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Concentration of bialaphos ( $\mathrm{mg} \mathrm{L}^{-1}$ ) |  |  |  |  |  | Uncreared ${ }^{\text {b }}$ |  |
|  |  | 200 | 400 | 600 | 800 | 1,200 | 2,400 | Trans- | Non-trans- |
| R. solani | 3 h before 2 d after | $\begin{aligned} & 0.4 \mathrm{~b} \\ & 0.5 \mathrm{~b} \end{aligned}$ | $\begin{aligned} & 0.3 \mathrm{~b} \\ & 0.5 \mathrm{~b} \end{aligned}$ | $\begin{aligned} & 0.2 \mathrm{~b} \\ & 0.2 \mathrm{~b} \end{aligned}$ | - | - | - | 7.8 a | 8.2 |
| S. homoeocarpa | 3 h before 2 d after | $\begin{aligned} & 0.6 \mathrm{~b} \\ & 1.3 \mathrm{~b} \end{aligned}$ | $\begin{aligned} & 0.4 \mathrm{~b} \\ & 0.9 \mathrm{~b} \end{aligned}$ | $\begin{aligned} & 0.2 \mathrm{~b} \\ & 0.7 \mathrm{~b} \end{aligned}$ | $\begin{aligned} & 0.1 \mathrm{~b} \\ & 0.5 \mathrm{~b} \end{aligned}$ | - | - | 5.2 a | 6.3 |
| P. aphanidermatum | 3 h before <br> 2 d after | $\begin{aligned} & 4.3 \mathrm{bcd} \\ & 5.8 \mathrm{ab} \end{aligned}$ | 3.6 bcd 5.6 abc | 3.4 bcd $5.1 \mathrm{a}-\mathrm{d}$ | 3.2 bcd 4.8 bcd | 2.8 bcd 4.4 bcd | $\begin{aligned} & 2.1 \mathrm{~d} \\ & 3.1 \mathrm{bcd} \end{aligned}$ | 8.3 a | 9.0 |

${ }^{2}$ Visual rating of plant damage on a 0 to 10 scale ( $0=$ no damage, $10=$ all dead).
${ }^{\mathrm{b}}$ Untreated indicates there was no bialaphos application either before or after the pathogen inoculation. Trans-: transgenic; Nontrans-: nontransgenic.
${ }^{c}$ There was no bialaphos application at this concentration for the corresponding pathogen inoculation.

Bialaphos had a very significant effect on the suppression of $R$. solani development when the disease rating was taken 1 wk after fungal inoculation. Transgenic plants not treated with bialaphos showed typical disease symptoms and had a significant amount of plant damage (Figure 4A). The disease began to develop 2 d after pathogen inculation. When bialaphos was applied 3 h before inoculation of $R$ solani on transgenic plants, disease symptoms were rarely observed 1 wk after inoculation, and there was only minimal plant damage due to pathogen infection. With $200 \mathrm{mg} \mathrm{L}^{-1}$, about one-tenth of the recommended herbicide rate to kill common weeds, the bialaphos significantly reduced plant damage (Figure 4B). Even when applied 2 d after pathogen inoculation, bialaphos could still significantly restrain growth of mycelium and development of brown patch disease on transgenic plants (Table 2). The untreated control plants, either transgenic or nontransgenic, were severely damaged by the infection of $R$. solani. The grass blades became water soaked and dark at first but soon became dry and withered and turned brown. The disease was able to develop continuously even after plastic bags were taken off, and many of the untreated plants were dead 3 wk after pathogen inoculation (data not shown). There was no statistically significant difference observed between two different timings of bialaphos application ( $\mathrm{F}=0.29<\mathrm{F}_{0.05(1,63)}=4.00$ ).

Bialaphos applied either 3 h before or 2 d after pathogen inoculation was also very effective in preventing development of $S$. homoeocarpa on transgenic creeping bentgrass (Table 2). The disease damage on transgenic, bialaphos-resistant plants, after bialaphos application, was significantly less than that on transgenic plants not treated with bialaphos (Figures 4C and 4D). The difference between the two application times was statistically significant ( $\left.\mathrm{F}=8.23>\mathrm{F}_{0.05}(1,81)=3.98\right)$, and there was more plant damage caused by the infection of $S$. homoeocarpa when bialaphos was applied to transgenic plants 2 d after pathogen inoculation.

The development of S. homoeocarpa on transgenic and nontransgenic control plants not treated with bialaphos was not as rapid and severe as that of $R$. solani. Most untreated control plants showed typical disease symptoms when covered with plastic bags, and when the plastic bags were removed, a few of them were able to recover from the damage caused by infection of $S$. homoeocarpa 2 wk after the disease rating.

The bialaphos application was slightly more effective
in protecting against infection of $R$. solani than against infection of $S$. homoeocarpa. There was more disease damage to transgenic plants from $S$. homoeocarpa than from $R$. solani after the bialaphos application. However, disease development was significantly restrained and most plants were able to completely recover from the infection.

Bialaphos applied 3 h before or 2 d after pathogen inoculation was effective in prevention of disease development of either $R$. solani or $S$. homoeocarpa. The lowest applied rate of bialaphos ( $200 \mathrm{mg} \mathrm{L}^{-1}$ ) sufficiently suppressed the disease development of both fungal pathogens at either application time (Table 2).

The results also showed that a single application of bialaphos could suppress the development of Pythium blight, though not as effectively as with $R$. solani and S. bomoeocarpa (Table 2). When $200 \mathrm{mg} \mathrm{L}^{-1}$ of bialaphos was applied 3 h before the pathogen inoculation, it significantly restrained infection of $P$ aphanidermatum and reduced the amount of plant damage 1 wk after the initial inoculation. Greater disease control was achieved when a higher concentration of bialaphos was applied to transgenic plants. A concentration of $800 \mathrm{mg} \mathrm{L}^{-1}$ of bialaphos was needed to significantly reduce the damage caused by Pythium when the application was made 2 d after inoculation. Transgenic plants sprayed with bialaphos before inoculation appeared to have greater protection against infection by P. aphanidermatum $\left(\mathrm{F}=25.57>\mathrm{F}_{0.05}(1,153)=3.96\right)$. However, regardless of application time and concentration of bialaphos, Pytbium infection was severe and caused more plant damage than the other pathogens tested did when disease symptoms were examined 1 wk after initial inoculation (Figures 4E and 4F).

The in vitro sensitivity data helped explain some of the efficacy trends observed in greenhouse studies. Bialaphos applied to transgenic bialaphos-resistant creeping bentgrass, even at the lowest rates, suppressed disease development. Bialaphos was most significant in suppressing $R$ solani development. Both spraying regimes provided significant suppression of R. solani.

The $S$. homooocarpa was sensitive to in vivo application of bialaphos; however, application before pathogen inoculation provided greater $S$. homoeocarpa control. If bialaphos was applied 2 d after pathogen inoculation, it still provided good plant protection and was able to significantly restrain disease symptoms of $S$. homoeocarpa.


Figure 4. The application of bialaphos for prevention of infection by Rhizoctonia solani (A and B), Sclerotinia homoeocarpa (C and D), and Pythium aphanidermatum ( E and F ) in transgenic, bialaphos-resistant creeping bentgrass 1 wk after application. $\mathrm{A}, \mathrm{C}$, and E : transgenic plant not sprayed with bialaphos showed severely damaged by pathogen infection. B and D: transgenic plant sprayed with $200 \mathrm{mg} \mathrm{L}^{-1}$ of bialaphos 3 h before pathogen inoculation showed little damage from infection of $R$. solani and $S$. homoeocarpa. F: transgenic plant sprayed with $400 \mathrm{mg} \mathrm{L}^{-1}$ of bialaphos 2 d after pathogen inoculation showed limited damage from infection of P. aphanidermatum.

Though not as effective as for $R$. solani and S. homoeocarpa, bialaphos still limited plant damage due to infection by P. aphanidermatum. The timing of bialaphos application was also important in suppressing disease symptoms of Pythium. Greater reduction in plant damage was obtained when bialaphos was applied 3 h before pathogen inoculation.

Interactions between herbicides and plant pathogens have been well documented (Altman 1985; Ben-Yephet et
al. 1983). The main cause of this phenomenon is that biological activity of pesticides may extend beyond their target organisms. Plant diseases caused by fungal pathogens have been reported to increase after herbicide treatment (Altman 1981; El-Khadem et al. 1979) or decrease (Cohen et al. 1986; Grinstein et al. 1979, 1984). More research needs to be done, not only to assess the applicability of antifungal activity of bialaphos toward other
fungi, but also to investigate the mechanism of its inhibitory effect.

Bialaphos has mainly been used as a broad-spectrum contact herbicide and as a selective agent in plant transformation experiments. However, it has been reported that it could be used as an effective selective agent to improve the transformation frequencies of Cercospora kikuchii, a fungal pathogen of soybean (Upchurch et al. 1994). This report and the results of our in vitro study, where bialaphos showed significant inhibitory effects toward $R$. solani and $S$. homoeocarpa, suggest that bialaphos could be used as an efficient fungicide for a variety of fungal pathogens.

Bialaphos and glufosinate dissipate very rapidly in soil and surface water, even before they can be transported to lower soil layers, after application (Hoerlein 1994). The active ingredient and its transitory metabolites were readily soluble in water, did not evaporate, and did not accumulate in fatty tissue of fish or other animals. Therefore, there was almost no accumulation of detectable residues in any environmental compartment. Application of $200 \mathrm{mg} \mathrm{L}^{-1}$ of bialaphos, which is about one-tenth the recommended concentration to kill untransformed turfgrass plants, was enough to significantly reduce plant damage due to infection of both R. solani and S. homoeocarpa. The low rate at which bialaphos was effective presents a novel and economical means for control of some fungal pathogens. The fact that bialaphos is a relatively environmentally sound herbicide, coupled with the results presented in this paper, indicates that it may be possible to combat fungal infections and weed infestations simultaneously in fields of transgenic, bialaphosresistant creeping bentgrass.

## Sources of Materials

${ }^{1}$ Bialaphos is the active ingredient of the commercial herbicide Herbiace® marketed by Meiji Seika Kaisha, Japan.
${ }^{2}$ Glufosinate-ammonium, $1.2 \%$ Ignite product of AgrEvo, 2711 Centerville Road, Wilmington, DE 19808.

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## Current Issues in Symbiotic Nitrogen Fixation

Proceedings of the 15th North American Symbiotic Nitrogen Fixation Conference held at North Carolina, USA, from August 13-17, 1995

Edited by G.H. Elkan, Dept. of Microbiology, North Carolina State University, Raleigh, USA;
R.G. Upchurch, Dept. of Plant Pathology, North

Carolina State University, Raleigh, USA
developments in plant and soil sciences 72 Reprinted from PLANT AND SOIL, 186:1
In the 100 years since the legume-Rhizobium symbiotic nitrogen fixation interaction was first described, interest in this field has grown rapidly. The types of studies have been cyclical in nature, involving a cross-section of disciplines. The availability of cheap nitrogenous fertilizers caused much of the biological nitrogen fixation research to become more theoretical in the developed world. The high cost of energy, coupled with environmental concerns and the interest in sustainable agriculture, has stimulated research in symbiotic nitrogen fixation. The development of modern genetic techniques has resulted in interdisciplinary research on plant-microbe interactions controlling nitrogen fixation. This has resulted in a better understanding of environmental factors influencing the nodulation process, chemical signalling between the symbiotic partners and the nature of the specificity between host plant and microsymbiotant.


This volume summarizes the diverse research efforts in biological nitrogen fixation by presenting a collection of papers in the areas of physiology and metabolism, taxonomy and evolution, genetics and ecology.
Contents and Contributors: Preface. 1. Advances in the Positional Cloning of Nodulation Genes in Soybean; G. Caetano-Anollés, P.M. Gresshoff. 2. Molecular Analysis of Actinorhizal Symbiotic Systems: Progress to Date; B.C. Mullin, S.V. Dobritsa. 3. Diversity of Rhizobia Isolated from Various Hedysarum Species; B.D. Kishinevsky, et al. 4. Diazotrophic Endophytes: Progress and Prospects for Nitrogen Fixation in Monocots; E.W. Triplett. 5. Drought-Avoidant Soybean Germplasm Maintains Nitrogen-Fixation Capacity under Water Stress; P. Patterson, C.M. Hudak. 6. Phylogeny and Taxonomy of Rhizobia; J.P.W. Young. 7. Compariso of Rhizobitoxine-Induced Inhibition of $\beta$-Cystathionase from Different Bradyrhizobia and Soybean Genotypes; K. Xiong, J.J. Fuhrmann. 8. Assimilation of nod Gene Inducer ${ }^{14} \mathrm{C}$-Naringenin and the Incorporation of Labelled Carbon Atoms Into the Acyl Side Chain of a Host-Specific Nod Factor Produced by Rhizobium leguminosarum by. viciae; J.R. Rao, et al. 9. Corresponding 16 S rNA Gene Segments in Rhizobiaceae and Aeromonas Yield Discordant Phylogenies; B.D.

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## R.I. Upchurch

North Carolina
Marriages, 17591979
birth: 1876
marriage: 14 Oct Clayton, Johnston, North Carolina
G.B. Upchurch, Sallie
parents: Upchurch
spouse: Bettie Wood

## G.B. Upchurch

spouse: Sallie Upchurch
North Carolina Marriages, 1759-1979
child: R.I. Upchurch
R. J. Unchurch
spouse: Lula Upchurch
North Carolina Marriages, 1759-1979 child: Otis Unchurch

| R. J. Upchurch |  |  |
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| North Carolina Marriages, <br> $1759-1979$ | birth: $\quad$1873 <br> 26 Dec | Franklin, North <br> 1894 |
| Carolina |  |  | spouse: Lulu Burnett

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## UPCHURCH, R. J. :

## Marriage

Wife: Mary P: SANDERS
Marriage Date: 13 Mar $1879 \quad$ Recorded in: Grimes, Texas
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-R Kinney UPCHURCH-2489 BIRTH-PARENTS: 1900 U.S. Census, Moore CO, NC; 30 Jun 1900; Line 28; Fam \#312, p97a; FHC film 1241207; NOTE: Age 11, DOB May 1889, illiterate, day laborer.
BIRTH-PARENTS: 1910 U.S. Census, Hoore CO NC; 1910; ; P227B, Fam \#153; National Archives, San Bruno CA, T624 Roll 1119; NOTE: Age 18, single.
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Birth Date
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Martha Upchurch<br>spouse: J. M. Upchurch<br>North Carolina Marriages, 1759-1979 child: R. L. Upchurch

## R. L. Upchurch

North Carolina Marriages, 17591979
birth: 1894 Greensboro, Nc 09 Feb Gilmer Twnsp, Guilford, 1924 North Carolina
J. M. Upchurch, parents: Martha Upchurch spouse: Addie Mc...Aige
$R \quad L \quad u$
OMy Record I NOV 1998
UPCHURCH, R. . $\therefore$
Marriage
Wife: C. C. GREEN
Marriage Date: 20 Jul 1882
Source: FHL Number 985181

Recorded in: Hill, Texas
Dates: 1876-1886
R- L. UPCNURCA
(1) R.L. U. bited on canserpondent an U. gencalogy Yy Ada Morgon in mut 1050 R 75 - R.L.U, P. O.Bor 136, Bediaz, TX 77831

## O <br> FROM 1995 CD-ROM



| Carnegie | PA | $15106-2110$ | $412-279-4015$ |
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| Chester | PA | $19013-3043$ | $610-876-8369$ |
| Chester | PA | $19013-3634$ | $610-876-1468$ |
| Downingtown | PA | 19335 | $610-269-2792$ |
| Edinburg | PA | 16116 | $412-667-0815$ |
| Elkins Park | PA | $19117-1002$ | $215-572-1476$ |
| Harrisburg | PA | $17111-4899$ | $717-541-9435$ |
| Kutztown | PA | 19530 | $610-683-3810$ |
| Lansdowne | PA | $19050-4061$ | $610-622-1840$ |
| Middlebury Center | PA | 16935 | $717-376-2489$ |
| New Freedom | PA | $17349-9702$ | $717-235-4571$ |
| New Kensington | PA | 15068 | $412-335-7728$ |
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| Stroudsburg | PA | 18360 | $717-421-2934$ |
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## FROM 1995 CD-ROM

| Upchurch R L | Gaslihg Taps |
| :--- | ---: |
| Upchurch John M | 550 Braebridge Rd |
| Upchurch Phillip W | 406 Sorrento Dr |
| Upchurch David | 220 Hillcrest Rd |
| Upchurch John | 1719 Oak Hills St |
| Upchurch Richard |  |
| Upchurch Homer |  |


| Albany | MO 64402 | $816-726-5730$ |  |
| :--- | :--- | :--- | :--- |
| Ballwin | MO | $63021-6702$ | $314-256-0505$ |
| Ballwin | MO | $63021-6425$ | $314-394-3593$ |
| Belton | MO | $64012-1889$ | $816-331-4379$ |
| Cape Girardeau | MO | $63701-2929$ | $314-335-2184$ |
| Cape Girardeau | MO 63701 | $314-264-2757$ |  |
| Charleston | MO 63834 | $314-683-3327$ |  |

FROM 1995 CD-ROM

| Unchurch Harlan | Grant City |  | Martinsville | MO 64467 |
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| Upchurch Eva R | Old Hartsvil Rr | Carthage | TN 37030 | $615-735-9102$ |  |
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| Upchurch Phillip | 406 Moore Ln | Carthage | TN 37030 | $615-735-0986$ |  |
| Upchurch R L 1 | Upchurch Rd | Carthage | TN 37030 | $615-735-0410$ |  |
| Upchurch Ray | 107 Morris Ave | Carthage | TN | $37030-1217$ | $615-735-2928$ |
| Upchurch Scott | 504 Cormack Ave | Carthage | TN | $37030-1112$ | $615-735-1911$ |
| Upchurch/john R | 1491 Cedar Grove Rd | Chapel Hill | TN $37034-7044$ | $615-294-5634$ |  |

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| Upchurch P L | Bedias | TX 77831 | $409-395-2931$ |
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| Upchurch R L | Bedias | TX 77831 | $409-395-6061$ |
| Upchurch Robert | Bedias | TX 77831 | $409-395-2431$ |
| Upchurch Rosa B | Bedias | TX 77831 | $409-395-6201$ |
| Upchurch W D | Bedias | TX 77831 | $409-395-6593$ |

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| Upchurch Clyde | 6240 Parsifal Pl | Las Vegas |
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| Upchurch Jason G | 2549 Starburst Dr | Las Vegas |
| Upchurch Michael |  | Las Vegas |
| Upchurch William A | 856 Shrubbery Ln | Las Vegas |
| Upchurch Neil | 2980 Middlecoff Ct | Reno |
| Upchurch R L |  | Reno |
| Upchurch Roberta M | 4035 Houston Dr | Reno |
| Unchurch Tracy |  | Reno |
| Unchurch Lisa |  | Stateline |


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| NV | $89110-2137$ | $702-453-6453$ |
| NV | 89509 | $702-857-1222$ |
| NV | 89501 | $702-831-1578$ |
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## OFROM 1995 CD-ROM


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flone bork jeer 1980.
R. Merriman

North Carolina Marriages, 17591979
birth: 1872
marriage: ${ }_{1895}^{22 \mathrm{Dec} \text { Williams, Chatham, }}$ 1895 North Carolina
$\begin{array}{ll}\text { parents: } & \begin{array}{l}\text { W.... Upchurch, S. } \\ \text { M. Upchurch }\end{array} \\ \text { spouse: } & \text { Haskie G. Council }\end{array}$
W.... Upchurch
spouse: S. M. Upchurch
North Carolina Marriages, 1759-1979 child: R. Merriman Upchurch

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ETHEL V. ROBBNS UPCHURCH
TEXAS DIVORCE REGARDS

-Fram th $260 c T 2000$ foln Cowan Farwe it RP4

- Item In 210cT2000 The Dallas Monning Neus (SAT) Dalloz, TX. RPA records shou ther foinily fits in the lepehueh, family tree as follons:
I. JAMES EVERETT $a$, SR of the Richand U, II / Clahuen $U$, I Sulchan - Rechard U, IV Lime
A. EVEBETTE 4 , JR [JAMES EVERETT U, JR] \& IFAUE 1925 (Waxahachie, Selin Co, TX) d 190CT 2000 (Dallas, $T X$ ) (Bur - Dallon - Ft woith Nat'l Cemi.) ad () THELMA - - who suswier in harcontes, Dillar $C 0, T X, G \in C ; Z G E C$

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B. B.O. U of weatherfars, Parker $c_{0}, 7 x$
C. Wilvon $U$ of Attanita, Cas $C_{0}, i x$
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E. Eectrude U [GERTRUDE NTNN U U) Inne md 1)- iNIGPEN m $\angle A$
F. Jillian $U$. mod ()-Goodnight - They line in Cirreana, TX.
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(2) See $2 t 14$ SEP 1983 folm shmer $u$ ARB4
R.O.U 4 had anitials only - no attor name.


## FROM 1995 CD-ROM

| Upchurch Ethel | 1236 W Water St | Weatherford | TX | $76086-2952$ |
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| 8pchurch Margaret | 208 Dean Rd | Weatherford | TX | $76087-9074$ |
| $817-596-4351$ |  |  |  |  |
| Upchurch R O | 208 Dean Rd | Weatherford | TX | $76087-9074$ |
| Upchurch R O | 208 Dean Rd | Weatherford | TX | $76087-9074$ |

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R.P. Apchucel

Bux 599
Livingston, AL 35470 \}
Bagly list 1980, $A-63,3-10, B-6-81$

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| $\bigcirc$ NCMARRI | S EPO Female | PETERSON- Dave Mcdonald | $\text { LEE } \underset{27}{\text { Feb } 1910}$ | Lee |
| , Lowney Upchurch | Male | Mattie Mcdonald | 14 Nov 1916 | Lee |
| William A Upchurch | Male | Almura Edwards | 14 Jan 1920 | Lee |
| Kurley U Upchurch | Male | Sulu Campbell | 26 Nov 1925 | Lee |
| Upch | Male | Hellen Ḃell | 25 Feb 1926 | ee |
| R B Upchurch | Male | Maude Crews | 23 Oct 1928 | Lee |
| R P Upchurch \$ | Male | Minnie ${ }^{\text {P Pattishall }}$ | 31 Aug 1929 | ee |
| Will Upchurch | Male | Mamie Mcneill | 11 Apr 1935 | Lee |
| .Jessie M Upchurch | Female | Wm Howard Espey | 15 Apr 1936 | Lee |
| Rondo B Upchurch | Male | Nancy J Scoggins | 10 Nov 1935 | Lee |
| Winston Upchurch | Male | Clara Esther Brown | 18 Feb 1946 | Lee |
| Sarah J Upchurch | Female | Nelson Alex Patterson | 30 Sep 1950 | Lee |
| Meta Upchurch | Female | Hamp Beck | 5 Mar 1951 | Le |
| Betty Upchurch Wyatt Gray Upchurch | Female <br> Male | Henry Albert Garner Mary Frances Garner | 5 Dec 1952 6 Feb 1954 | Lee |
| Wyatt Gray Upchurch |  |  | 16 Jun 1957 | Lee |
| Alvah Melvin Upchurch | Male | Meta Amanda Morgan |  |  |
| Milton Upchurch | Male | Pauline Marks | 23 Oct 1957 | Lee |
| Arliss Lindsay Upchurch | Male | Sidney Ann Epps | 14 Jun 1958 | Lee |
| Anita Upchurch | Female | Joseph Reid Spivey | 4 Nov 1966 | Lee |
| Mary Lillian Upchurch | Female | John Joseph Bradley | 5 Apr 1969 | Lee |
| Arliss Lindsay Upchurch | Male | Nancy Oldham Garner | 14 Aug 1971 | Lee |
| Linda Dianne Upchurch | Female | Ronald Eric Vaughn | 18 Aug 1973 | Lee |
| Mary Lillian Upchurch | Female | Michael Anthony Barnard | 15 Jun 1975 | Lee |
| Claude Upchurch III, | Male | Carolyn Deborah Maxwell | 23 Dec 1976 | Lee |
| 'Vickie Lynn Upchurch | Female | Ronald Scott Gaster | . 1 Jul 1978 | Lee |
| Linda Fay Upchurch | Female | Henry Lee Johnson | 27 May 1980 | Lee |
| Rhonda Lorraine Upchurch | Female | J Carlos Zarate | 18 Jan 1984 | Lee |
| Robert Mitchell Upchurch | Male | Lessie Gearldine Millhouse | 29 Dec 1984 | Lee |
| Alvah Mark Upchurch | Male | Donna Sharlene Morrison | 23 Feb 1984 | Lee |
| Cassandra Ann Upchurch | Female | Stevie Kyle Sellers | 1 Apr 1986 | Lee |

R. P. UPCHDRCH
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\text { Ralergh, NC } 27608
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| R Pearson Upchurch |  |  |  |
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| North Carolina <br> Marriages, 1759-1979 | $\text { marriage: }{ }_{1931}^{27} \text { Jun }$ | Raleigh, Wake, North Carolina | spouse: <br> Georgette M Schwartz |

IX Busthr UPCHURCH ROBERT HAMMETT
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## FROM <br> 1995 <br> CD-ROM

Upchurch Anthony N Upchurch Anthony N Upchurch Anthony N
Upchurch Arthur B
Upchurch Donald
Upchurch John
Upchurch Michael D \& S
Upchurch R R $\uparrow$
Upchurch Roy $F$
Upchurch Susan
Upchurch Wray D

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2135 Primrose Place Ln Sw
2135 Primrose Place Ln Sw
3264 Creekview Dr N
650 Oakland Rd
1211 Providence Dr Sw
391 Davis Mill Rd
1731 Atkinson Rd
2101 Deer Run
951 Hounds Ridge Ct Nw

Lawrenceville Lawrenceville Lawrenceville Lawrenceville Lawrenceville Lawrenceville Lawrenceville Lawrenceville

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GA 30244-3735 404-963-2091
GA 30244-6170 404-978-8221
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GA 30243-5605 404-963-8837
GA 30244-5935 404-985-9186
GA 30243-6319 404-682-9250
R.R. Ipchunch

Phone boak 1979
R.R. Lpchuret

351 Lainis milf Rd.
Laurenceville, Ya 30245 SAAA

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R.R. Upeluwch, fo P* 1 Boy 482 B Buichonon Dom, Ix 18609
R.S.Lupchurch
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| Upchurch R T 1 | 6th St | Munfordville | KY | 42765 | $502-524-5682$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Upchurch J | 1359 Grand Av | Newport | KY | $41071-2543$ | $606-441-2915$ |
| Upchurch Gary A | 10116 Boone St | Owensboro | KY | $42301-9570$ | $502-229-4989$ |
| Upchurch Steve | 2322 Ponder Pl | Owensboro | KY | $42301-4225$ | $502-685-2247$ |
| Upchurch Charles | 308 Ruoff Dr | Paducah | KY | $42003-9446$ | $502-898-8658$ |
| Upchurch Paul | 6408 Crossbrook Dr | Pewee Valley | KY | $40056-9120$ | $502-241-9878$ |
| Upchurch Kelly W | 100 N Fairview Ave | Richmond | KY | $40475-1916$ | $606-624-5824$ |
| Upchurch William W | 1407 Providence Rd | Richmond | KY | $40475-1155$ | $606-624-1896$ |

Phare book 1980
R.T. Apchuch
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MAR R T. WPCHURCH, SR
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R. talmage u
(1) Info from RP4 9FEB 1978 Phove call to famer fenviz $U$ in Dallar $T X$ - hir commente se ottes $U_{2}^{\prime}$ in Dallar IX phone hooh:
$R$.Talmage $U$
928 North valley Redge pure $\}$ in $A F$ Dallas, TX 15220
"A mell hnown sengone in a quaste
(2) Phone book 1979

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(3) RPU in Dallar TX SFEB 15j8-called R.Talmoge 4 928 N. vallyy Redge in 223-8294. No

## FROM 1995 CD-ROM

| Upchurch Katie C | ------...- | Bailey | MS | 39320 | 601-737-5494 |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Upchurch Thomas |  | Bailey | MS | 39320 | 601-737-8869 |
| Upchurch D M | 301 Central St | Belzoni | MS | 39038-3603 | 601-247-2489 |
| Upchurch John N | Anatol By Twnhs | Biloxi | MS | 39530 | 601-388-3787 |
| Upchurch Joe | 132 Bentley Dr | Brandon | MS | 39042-8740 | 601-825-4690 |
| Upchurch Teresa |  | Byhalia | MS | 38611 | 601-838-5220 |
| Upchurch Carol | 109 N Sabine St | Charleston | MS | 38921-1624 | 601-647-2580 |
| Upchurch John |  | Clarksdale | MS | 38614 | 601-627-9158 |
| Upchurch R W $\uparrow$ | 100 Parker Dr | Clinton | MS | 39056-4539 | 601-924-7922 |

R.W. hpofuch

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5. M. W. Up-churcht 2623 Chintos Ave. gackson hriss. phone FL5-8013
(1) Mg Recode II NOV 1998

## UPCHURCH, R. W.

Wife: Amelia J. Whens
Marriage Date: 24 Jul 1886
Recorded in: Harris, Texas
Source: FHL Number 25225
Dates: 1881-1891

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\left.\begin{array}{l}
\text { R.W. Ipctureh } \\
2623 \text { clinton ave. } \\
\text { Jackson, MS } 35209
\end{array}\right\} \text { AF AF }
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[^0]:    ${ }^{2}$ The radial growth (millimeters) of the colony measured 4 d after inoculation was used to represent the mycelial growth of the pathogen for 15 replications.
    ${ }^{\mathrm{b}}$ Percent inhibition $(Y)$ was plotted as a function of bialaphos or glufosinate concentration $(X)$.
    ${ }^{c}$ There was no inhibition of Pythium aphanidermatum by glufosinate up to the highest concentration ( $600 \mathrm{mg} \mathrm{L}^{-1}$ ) amended in potato dextrose agar.

