staminate flowers were at some distance from the crown. The first pistillate flowers were very close to the crown on L18-4 and L26-3 plants, intermediate on 'La Segunda' and 'La Primera' plants, at some distance in 'Borinquen' and 'Soler' plants, and farthest from the crown in Linea C Pinta plants.

The first mature fruits were earliest and closest to the crown on L18-4 and L26-3 plants. There was no difference in maturity among the other entries. Apparent discrepancies in the distance and number of nodes to the first pistillate flower and first mature fruit can be explained by the fact that not all pistillate flowers develop into fruit. In addition, pistillate flowers that develop on branches other than the one where the first pistillate flower appeared may set closer to the crown than the first one.

Results of these studies indicate that 3 distinct calabaza populations were included. L18-4 and L26-3, the bush types developed at CFREC-Leesburg, produced flowers closest to the crown, were earliest, but produced very small fruit. At the other extreme, 'Borinquen', Linea C Pinta, and 'Soler', developed in Puerto Rico, produced flowers farthest from the crown, were latest in fruit maturity, and produced medium to large flattened fruit. 'La Primera' and 'La Segunda' were intermediate in distance of flower production from the crown, intermediate in fruit maturity, and produced medium to large fruit.

The bush types offer promise in the goal to produce short-vined calabazas. Further backcrossing to large-fruited, long-vined types will be made to improve fruit size and shape and selection for intermediate vine length will be made.

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Proc. Fla. State Hort. Soc. 105:358-361. 1992.

DEVELOPMENT AND BREEDING OF HERBICIDE TOLERANT LETTUCE

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Additional index words. biotechnology, glyphosate, imazethapyr, *Lactuca sativa* L., sulfonylurea, weed control.

Abstract. **Weed control measures for lettuce, *Lactuca sativa* L. on south Florida high organic soils (>70%) can exceed $600 per acre due to the high use of manual labor. Available chemical herbicides are ineffective for broad spectrum control of weeds. It is the approach of the lettuce breeding program of the University of Florida to develop herbicide tolerant lettuce cultivars through classical and gene splicing methodology. Sulfonylurea herbicide resistance was derived from prickly lettuce, *L. serriola* L. and backcrossed into crisphead, cos, bibb and leaf lettuce that have desirable disease resistance and commercial quality. Inheritance of the herbicide resistance was through an incomplete dominance gene action. Good expression of resistance in lettuce was observed at 2 oz/acre rate of imazethapyr. Segregation ratios for backcrossing was 1:1, tolerant:susceptible. F_2 segregation for resistance:tolerant:susceptible was in a 1:2:1 ratio. Breeding lines of leaf, cos, crisphead and butterheads resistant and tolerant to sulfonylurea herbicides have been developed. Closed heading types are progressing slower than open head types due to multiple genes involved in heading. Glyphosate resistance was bioengineered into 'South Bay' lettuce through the use of *Agrobacterium tumefaciens* as the vector. A total of 73 individual family lines, each representing a independent transformation event have been generated. Seed were collected for each line. Initial screening of regenerated 'South Bay' crisphead lettuce with the incorporated gene have indicated good resistance to glyphosate at a use rate of 2 lb/acre. The mechanism of gene action is believed to be dominant. Homozygous lines have been identified and studies are continuing.**

Currently, it is standard procedure to evaluate individual vegetable crops for tolerance to various herbicides. The efficacy of weed control at various application rates is correlated to crop response. When efficacy and crop tolerance are identified, registration of the herbicide for the crop is pursued. A radically different approach is now being undertaken at the University of Florida. First, sources of herbicide resistance are identified, along with information on the herbicide in question. This approach allows the researcher to choose a specific herbicide which is highly efficacious and have minimal or no negative impact on non-target organism. When true breeding lines are developed having horticultural quality, registration for the herbicide would then be pursued. Two different group of herbicides have been selected thus far, glyphosate and sulfonylurea. Sulfonylurea herbicide resistance was originally discovered in a naturally occurring population of prickly lettuce, *Lactuca serriola* L. (Mallory et al., 1989) a wild weedy relative of the cultivated lettuce. Mallory et al. (1989) determined that the resistant biotype of prickly lettuce was resistant to eight different sulfonylurea compounds. Transfer of resistance should follow the classical approach based on mendelian genetics where the resistance gene is introgressed from a cross compatible relative through a backcrossing procedure.

Glyphosate is also an attractive herbicide because of broad spectrum weed control, low animal toxicity and very low environmental impact when used correctly. But in lettuce and its relatives, there are no reports of glyphosate resistance. However, there are glyphosate resistant genes from other sources (Shah et al., 1986). In the past decade, bioengineering of plants have become common practice (Rogers et al., 1986). The insertion of a foreign gene for herbicide resistance into crop plants have been a part of these studies. This report covers the screening and selection for homozygous resistant plant material.

Materials and Methods

Glyphosate:

Transfer and incorporation of the resistant gene was accomplished through the use of cointergrated and binary *Agrobacterium tumefaciens* vectors containing the glyphosate resistant gene and a kanamycin resistant marker. Transformation of lettuce was accomplished by the method described by Enomoto et al. (1990) and modified by Torres (unpublished data) to maximize number of transformed plants. Basically the procedure used excised cotyledons from 'South Bay' lettuce. The seeds were surface sterilized and germinated aseptically. Lettuce seedlings, 24-72 hr post-germination were found to produce the most plantlets after transformation. The cotyledons were co-incubated for 24 hr in a bacterial suspension of *Agrobacterium* having the plasmid containing the glyphosate resistant gene. The cotyledons were then plated onto a tissue culture medium containing kanamycin to selectively screen for transformed cells. Leaf and shoot tissue that arose were excised and place on to a rooting medium. Plantlets with roots were then transplanted into 0.5 gal pots containing sterilized potting medium and placed in a greenhouse to produce seeds.

The R_0 generation was the designation for plants that resulted from the transformation event. In this experiment, the R_0 plants were allowed to mature and flower. R_1 seeds were harvested from the R_0 plants and were tested for resistance to a commercial source of glyphosate (Round-up). Twenty-seven plants of each R_1 line were grown in a seedling flat and treated with glyphosate at a rate of 2 lb/acre when seedlings were three weeks old. Segregation ratios were noted for normal, stunted, and dead plants, two and four weeks post-treatment. Stunted plants were those plants that were affected by the herbicide and often displayed lanceolate shaped leaves. These plants would either out grow the symptoms or die within five weeks post treatment. All normal plants were transplanted and grown for seed production. The R_2 plants were treated similar to the R_1 plants. All plant lines that were nonsegregating and resistant, were considered to be homozygous resistant. These lines will be used for further studies.

Sulfonylurea:

Imazethapyr (Pursuit) was selected as the sulfonylurea herbicide used for screening herbicide resistance. Transfer of the resistant character was conducted by backcrossing the resistant selections to the recurrent parent (Allard, 1960) having commercially acceptable qualities (Figure 1).

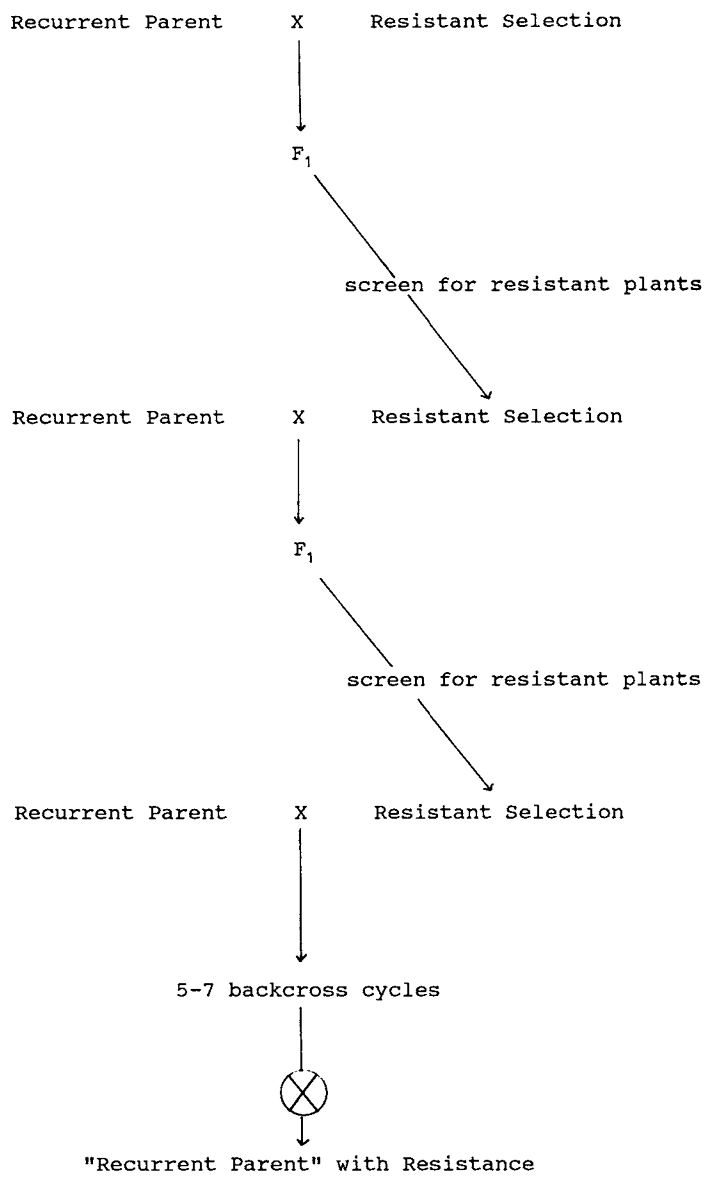

Figure 1. Diagram of backcross breeding procedure used to transfer sulfonylurea herbicide resistance to a commercially acceptable lettuce type.

Crisphead, 'South Bay'; cos, 'Tall Guzmaine'; butterhead, 'Florida Buttercrisp'; and leaf, 'Slobolt' were selected as the recurrent parent. Inheritance of resistance was reported to be controlled by an incomplete dominant factor that could be selected for in each backcrossing generation (Mallory et al., 1989). Due to the incomplete dominant factor of gene expression, segregation ratio in each backcross generation there would be one tolerant:one susceptible. The F_2 segregation would be 1:2:1, resistant:tolerant: susceptible. The selection scheme was to identify the tolerant plants from each backcross population using imazethapyr at the application rate of 2 oz/acre three weeks after sowing. All tolerant plants were transplanted into 6-inch azalea pots and allowed to flower. Plants that flowered were backcrossed to the recurrent parent and the process was repeated. Plants with the best horticultural characteristics were selected as parents. The selected plants were used as the pollen parent in crosses to the recurrent parents. Seeds were harvested and the procedure was repeated until a plant selection has all the necessary horticultural characteristics for cultivar release.

Results and Discussion

Glyphosate:

Screening evaluations of transformed 'South Bay' lettuce lines indicate the successful transfer and expression of a foreign gene using *Agrobacterium tumefaciens* as a vector. There were significant variations in the expression of glyphosate resistance between the individual transformation events (Table 1). No lines were found to be completely resistant, or completely susceptible, noting the exception of the non-transformed 'South Bay' control which was susceptible. This indicates that the kanamycin screening procedure used to identify transformed cells, (i.e., herbicide resistance) from tissue culture was adequate and non-transformed plants were not selected. Plants segregated into 3 response classes; normal, tolerant, and susceptible. Normal plants were considered to be resistant and generally did not slow down in growth nor display signs of herbicide toxicity when compared to untreated, non-transformed 'South Bay' control lines. Tolerant plants were those plants that displayed herbicide injury but not immediate death, the most common symptom being stunting. Leaves of stunted plants were lanceolate shaped, thick and with a rough dull surface. Leaf chlorosis were found on some lines. Tolerant plants were further subdivided into two class, those that eventually die and those that eventually grow out of the symptoms (data not shown). Susceptible plants were those that died within 2 weeks of glyphosate applications.

There were several possible explanations for the tolerant response. Less than optimal glyphosate dose. Incomplete dominance of gene expression and a heterozygous effect occurred. Expression of resistance may also have been affected by number of copies of the gene vectored into the transformed cell (Lewin, 1980). Resistance was conferred by the over production of EPSP synthase (Shah et al., 1986). Two copies of the gene may infact provide twice the EPSP synthase activity. Gene position effect was also a possibility (Lewin, 1980). Depending on the position on the chromosome where the plasmid containing herbicide resistance was inserted, its expression could be affected. As homozygous resistant lines were selected, further testing could be done to answer these questions.

Sulfonylurea:

Transfer of sulfonylurea resistance was successfully accomplished to cultivated lettuce lines. The cross compatibility between *L. sativa* and *L. serriola* was very good and there was no problem in transferring sulfonylurea resistant gene into the cultivated lettuce background. The gene expression for resistance was incomplete dominance (Mallory et al., 1989), thereby allowing for selection in each backcross generation without the need for a selfing generation, if care was taken in the evaluation. Due to the incomplete dominance of resistance, the homozygous dominant plants were the only group to have a normal phenotype after herbicide treatment (Table 2). The heterozygous dominant plants had partial expression of resistance exhibited as stunted plants and leaf chlorosis. These symptoms were outgrown in several weeks.

Although 2 different methods were used to incorporate herbicide resistant genes into lettuce, both methods resulted in resistant plants. Discussion over choice of methods would be based on many factors, including availability of vectors with herbicide resistant genes or germplasm, the state of technology available, and funding for

Table 1. Segregation of glyphosate resistant plants in the R_1 generation. Lines treated with 2 lb/acre glyphosate.

Line #	Number of plants: Normal	Stunted	Dead
A-1	9	19	0
A-4	0	24	3
A-6	0	17	8
A-8	0	8	9
A-10	0	6	14
A-11	17	2	4
A-13	8	1	3
A-15	20	5	1
A-16	0	7	6
A-17	13	12	4
A-21	18	7	0
South Bay[z]	0	14	5

[z]All stunted plants died within 4 weeks of glyphosate application.

Table 2. Segregation of sulfonylurea resistance in the F_2 generation. Lines treated with 2 oz/acre imazethapyr.

Line #	Number of plants: Homozygous resistant (normal)	Heterozygous resistant (stunted)	Homozygous susceptible (dead)
S1044	6	14	7
S1045	5	13	8
S1046	6	13	7
S1047	6	17	4
S1048	6	15	6
S1049	3	16	8
S1050	6	14	7
S1051	7	17	3
S1052	4	16	6
S1053	3	18	6
Summer Bibb	0	0	27
South Bay	0	0	27
L. serriola	27	0	0

the project. Gene splicing offers the advantage of placing a single or very few genes into the target organism. This had the benefit that very little of the genetic background of the target cultivar was disturbed and resistant lines could quickly be released. Because of the lack of undesirable genes, progress to a homozygous line could be accomplished within 3 generations. On the other hand, backcrossing procedure was less costly but may take longer to achieve due to the many cycles of crossing and selections to achieve horticultural quality. It may take five to seven generation cycles to accomplish providing no tight linkages were involved. Backcrossing from a wild relative adds many undesirable characters. This research opened a new era in the way that weed control in vegetables was approached. Now, the best herbicides for weed control may be selected while having the least possible impact on the environment and the applicator.

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Proc. Fla. State Hort. Soc. 105:361-363. 1992.

EFFECT OF MIXED INFECTION AND IRRIGATION METHOD ON COLONIZATION OF TOMATO ROOTS BY *TRICHODERMA HARZIANUM* AND *GLOMUS INTRARADIX*

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Additional index words. Subirrigation, overhead irrigation.

Abstract. **Inocula of the fungal biocontrol agents *Trichoderma harzianum* and/or *Glomus intraradix* were used in an experiment to determine their ability to simultaneously colonize tomato [*Lycopersicon esculentum* (Mill.) 'Piedmont'] roots. Untreated tomato seeds were planted in styrofoam plug trays containing noninfested soil or soil inoculated with one or both fungi. Seedlings were watered using either overhead irrigation or subirrigation. Both fungi effectively colonized the same root system, but *T. harzianum* reduced colonization by *G. intraradix*. When compared with overhead irrigation, subirrigation reduced colonization by *G. intraradix* but not *T. harzianum*. *T. harzianum* increased plant height and dry weight.**

Interest has increased over the last 3 decades in developing alternative strategies for the management of soilborne plant diseases using beneficial microorganisms (Cook, 1990). Fusarium crown and root rot [*Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL)] has consistently been the most serious soilborne disease of tomato in southwest and east central Florida (Jones et al., 1990; Sonoda, 1976). FORL apparently colonizes soil most aggressively when competing microorganisms have been eliminated through soil sterilization (Marois and Mitchell, 1981), making it an excellent candidate for management through biological control. Amendment of soil with either of 2 fungi, *Trichoderma harzianum* or *Glomus intraradix* (a vesicular-arbuscular mycorrhizal fungus), reduced the severity of crown rot in tomatoes (Marois and Mitchell, 1981; Sivan et al., 1987). In addition, both fungi stimulated the growth of tomato plants (Caron et al., 1986; Chet, 1990). In recent research, vesicular-arbuscular mycorrhizal fungi appeared to be closely associated with a number of beneficial rhizosphere microorganisms (Lindermann and Paulitz, 1990).

The simultaneous use of *T. harzianum* and *G. intraradix* may produce a synergistic effect leading to greater control of disease than that afforded by either agent alone. However, mixed infection of tomato plants by *T. harzianum* and *G. intraradix* has not been previously reported. This research was conducted to examine the capacity of *T. harzianum* and *G. intraradix* to simultaneously colonize tomato roots. A second objective was to compare the effects of overhead irrigation vs. subirrigation on the infection process. (Subirrigation is commonly used by large vegetable transplant growers in Florida to reduce foliar diseases by lowering leaf wetness).

Materials and Methods

The research was conducted in a greenhouse at the Southwest Florida Research and Education Center, Im-

Florida Agricultural Experiment Station Journal No. N-00707. The use of trade names in this publication does not imply either endorsement or criticism of these products by the authors or the University of Florida.