Registration of ‘B2’ Connecticut Broadleaf Cigar-Wrapper Tobacco Resistant to Fusarium Wilt, Tobacco Mosaic Virus, Cyst Nematodes, and Blue Mold

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ABSTRACT

‘B2’ Connecticut broadleaf cigar-wrapper tobacco (Nicotiana tabacum L.) (Reg. No. CV-124, PI 664300) is a male-sterile hybrid released by the Connecticut Agricultural Experiment Station and is resistant to Fusarium wilt, Tobacco mosaic virus (TMV), the tobacco cyst nematode [TCN; Globodera tabacum tabacum (Lownsbery and Lownsbery 1954) Stone 1973], and blue mold [caused by Peronospora tabacina de Bary Adam 1933 (= Peronospora hyoscyami de Bary 1863 f. sp. tabacina Skalicky 1964)]. Initial crosses were made in 1987 between VA-81 and PD4 flue-cured tobacco and three broadleaf inbreds resistant to Fusarium wilt [caused by Fusarium oxysporum Schlechtend: Fr. f. sp. nicotianae (J. Johnson) W.C. Snyder & H.N. Hans.] and TMV. F2 selections were backcrossed twice to broadleaf lines, inbred and selected across 12 generations using bulk modified single-seed descent with 2% of plants (20 plants of 1000) selected most years; selection for TMV and TCN resistance was conducted in greenhouse screens with fewer plants. Lines were field selected for reduced ozone weather fleck. One F12 selection was backcrossed again to the broadleaf cultivar ‘Scantic’ and inbred via pedigree selection across 10 generations with field and greenhouse selection. Progeny testing was used to select F8 plants homozygous for resistance to TCN and for hypersensitive TMV resistance from Nicotiana glutinosa L. A superior male-fertile inbred (MF-2) resulting from 27 generations of selection for broadleaf characteristics with 8 cycles of selection each for Fusarium wilt, TMV and TCN resistance was identified and crossed with a male-sterile CT broadleaf line resistant to TMV and Fusarium wilt (MS-1) to produce the F1 male-sterile hybrid B2.

A new cultivar of Connecticut broadleaf cigar-wrapper tobacco (Nicotiana tabacum L.) was developed with resistance to Fusarium wilt [caused by Fusarium oxysporum Schlechtend.: Fr. f. sp. nicotianae (J. Johnson) W.C. Snyder & H.N. Hans.], Tobacco mosaic virus (TMV), the tobacco cyst nematode [TCN; Globodera tabacum tabacum (Lownsbery and Lownsbery 1954) Stone 1973], and blue mold [caused by the Oomycete Peronospora tabacina de Bary Adam 1933 (= Peronospora hyoscyami de Bary 1863 f. sp. tabacina Skalicky 1964)]. This cultivar, ‘B2’ (Reg. No. CV-124, PI 664300), was developed at the Connecticut Agricultural Experiment Station’s (CAES) Valley Laboratory. Yields and sorting quality are equal to or better than those of the current standard wilt-resistant, TMV-resistant cultivar ‘C9’ (PI 556977; LaMondia, 2011).

Fusarium wilt has been the most persistent and important disease of broadleaf tobacco in the Connecticut River Valley during the last three decades. The most effective control of Fusarium wilt has been achieved through the development and use of wilt-resistant broadleaf tobacco cultivars (Lucas, 1975; LaMondia and Taylor, 1991). One wilt-resistant cultivar, C9, was released in 1991 (LaMondia and Taylor, 1992) and has been widely grown (up to 80% of the total acreage annually) throughout the valley. Wilt-resistant cultivars are not immune to Fusarium infection, however, and will develop wilt symptoms under high inoculum potential (LaMondia and Taylor, 1987). Resistance to F. oxysporum is conferred by an unknown number of small-effect genes that are quantitatively inherited (Gritton et al., 1965). C2 is a cultivar that was developed with resistance to TMV that coincidentally carried strong resistance to Fusarium wilt (Sand and Taylor, 1961). It was not commercially accepted but has been used as a source of wilt resistance in developing more acceptable (but somewhat less resistant)
broadleaf lines such as C9. Continuous growth of a single cultivar such as C9 over a number of years may eventually select for isolates able to cause disease on that cultivar. The development and production of additional wilt-resistant cultivars, which while still from the same C2 source of multigenic resistance, may have different numbers or combinations of the quantitatively inherited resistance genes, is desirable because it may prolong the effective use of plant resistance over time.

The TCN was first discovered in Hazardville, CT in the early 1950s (Lownsberry and Lownsberry, 1954). It is now widely distributed in the tobacco production areas in the Connecticut River Valley of Connecticut and Massachusetts. A closely related nematode, Globodera tabacum solanacearum (Miller and Gray, 1972) Stone, 1973 suppresses the growth and yield of tobacco in Virginia and North Carolina (Komm et al., 1983; Melton et al., 1991). Nematode infection of roots can cause dramatic stunting, yield loss, and reduced leaf quality. A nonlinear damage function was developed to predict broadleaf tobacco yield losses based on preplant nematode densities in soil (LaMondia, 2002a). Losses can be as high as 40% at high nematode densities in Connecticut. Nematode-related losses have often been prevented by the use of preplant fumigation of soils. However, fumigant nematocide use is expensive, and the number of fumigants available for use has been reduced due to environmental considerations.

Tobacco mosaic virus is a common and important pathogen of wrapper tobacco in Connecticut. Virus infection can result in poor growth and mosaic symptoms, or green spot, symptoms on cured leaves that make them unsuitable for wrapper grades (LaMondia, 2008). Resistant cultivars containing genetic resistance derived from Nicotiana glutinosa L. conferred by a single dominant gene (N locus) result in a hypersensitive local lesion reaction and reduced virus infection (Lewis et al., 2005).

Blue mold has been a reoccurring problem in Connecticut and has caused severe epidemics and crop losses (LaMondia and Aylor, 2001; LaMondia, 2010). Resistance to the pathogen has been described and appears to be conferred by a single major gene in the wild species Nicotiana debneyi Domin and Nicotiana goodspeedii H.—M. Wheeler. The expression of resistance is reduced after the gene is transferred to cultivated tobacco, and segregation ratios indicate that resistance is additive and affected by a number of modifiers in the N. tabacum genome (Rufty et al., 1990). Clayton (1968) recognized four distinct levels of resistance in N. debneyi—derived lines, ranging from a high level of resistance (almost immunity) to a reduced level of resistance statistically superior to that of susceptible tobacco. A number of other Nicotiana species, including Nicotiana longiflora Cav., have also been described as sources of blue mold resistance (Clayton, 1945).

**Methods**

Initial crosses for cyst nematode resistance were made in 1987 between Connecticut broadleaf and two flue-cured tobacco lines obtained from cooperators in Virginia. The flue-cured breeding line VA-81 (Elliott et al., 1986) and cultivar PD4 (Currin et al., 1980) each carried resistance to TCN but were flue-cured types that were unmarketable as cigar-wrapper tobacco. Both VA-81 and PD4 were crossed with three selections of Connecticut broadleaf inbreds (breeding lines 1-1, 1-4, and 6-2). These inbreds were made at the CAES between ‘C2’ and three agronomically desirable broadleaf inbreds that were susceptible to all diseases, ‘Winn’, ‘Gogulski’ and ‘Gradowski’. C2 is a cultivar that was developed with resistance to TMV that coincidentally carried strong resistance to Fusarium wilt. It was used as a source of wilt resistance in developing broadleaf lines such as C9 (LaMondia and Taylor, 1992). C2 was developed by the CAES and released in 1961 (Sand and Taylor, 1961).

The initial hybrids between Connecticut broadleaf cigar-wrapper types and flue-cured types were selfed to the F1, and selected plants were backcrossed twice to ‘Gogulski’ (CT86-4) broadleaf to restore broadleaf characteristics. Plants were selected for agronomic type annually under field conditions with a bulk system of modified single-seed descent in which only the top 2% of plants (20 plants of 1000 grown) were selected for each succeeding generation. To do this, all combinations of selected BC2 plants were bulked and grown in a field plot naturally infested with Fusarium wilt. Wilt-susceptible individuals had a high probability of mortality (susceptible plants grown in this field had approximately 90% mortality). In addition to wilt-resistance, agronomic type, and broadleaf characteristics, the lines were field selected for reduced sensitivity to weather fleck, which is caused by ozone, compared with the ozone-sensitive line Bel-W-3. One seedpod per selected plant was collected, and seed was bulked, mixed, and randomly selected for the next generation. Additionally, several hundred plants were selected for TMV and TCN resistance in greenhouse screens conducted during the winter. Six-week-old seedlings were inoculated with TMV, and those exhibiting a hypersensitive resistance response were selected. Seedlings in 128-cell trays were each inoculated with 5000 juveniles of TCN and those without white females visible on the roots after 6 wk were selected for the next generation (LaMondia, 1988). The most promising resulting inbred (F2 generation) was not of suitable wrapper leaf quality, so it was backcrossed again to the broadleaf cultivar ‘Scantic’ (PI 619163; LaMondia, 2002c), and F1 plants were again selfed to inbreds using pedigree selection across 10 generations with field and greenhouse selection for resistance to pathogens and tolerance of ozone damage and agronomic broadleaf tobacco characteristics. The resulting inbreds were progeny
tested to select plants with stable homozygous resistance to the TCN and were evaluated for Fusarium wilt and blue mold resistance. Individual plants in the F_1, F_2, F_3, F_4, and F_5 generations were selected for TMV resistance and cyst nematode resistance with a greenhouse seedling assay (LaMondia, 1991). Progeny testing was performed to select F_8 plants homozygous for *G. t. tabacum* resistance and for the dominant hypersensitive gene for resistance to TMV derived from *N. glutinosa*. A superior male-fertile inbred (MF-2) resulting from 27 generations of selection for broadleaf agronomic characteristics with eight cycles of selection each for Fusarium wilt, TMV, and TCN resistance was identified and crossed with a male-sterile broadleaf line with homozygous resistance to TMV and Fusarium wilt (MS-1) to produce the F_1 male-sterile hybrid B2.

The male-sterile parent (MS-1) was developed using a male-sterile Connecticut broadleaf line (CT87-33) of unknown pedigree. This plant was initially crossed with a TMV-resistant, Fusarium wilt-resistant inbred of C2 × ‘Sperry’ in 1987, continually backcrossed to that inbred, and maintained over time.

### Characteristics

B2 was evaluated for disease resistance, agronomic characteristics, and cured-leaf quality in experimental plots and under commercial conditions with cooperating farmers. Yields and sorting characteristics of B2 were compared to the wilt-resistant standard C9 on the Valley Laboratory Research Farm (Farm 1) and four commercial farms in CT from 2006 to 2010. Cured leaves were commercially graded into one of six grades representing wrapper, binder, or filler (stemming) quality in a blind test by commercial sorters. Binder and filler grades are of little or no economic value. The weight of cured wrapper leaves and percentage of the total yield in wrapper grades was determined; however not all data was collected for each year (Table 1). Leaf yield was numerically higher for B2 (2298.1 kg ha\(^{-1}\)) than for C9 (2197.2 kg ha\(^{-1}\)), but the means were not significantly different (paired \(t\) test \(P = 0.12\); Wilcoxon signed-rank test at \(P = 0.14\)). The percentage of leaves in wrapper grades was higher for B2 than for C9, 54.9 and 49.8% wrapper, respectively (nonparametric Wilcoxon signed-rank test at \(P = 0.04\)). Previous observations in field and greenhouse experiments as well as in commercial production have shown that expression of Fusarium wilt on wilt-resistant plants is often mild and that plants often outgrow early symptoms (LaMondia and Taylor, 1987, 1991). The incidence and severity of wilt symptoms were determined in 2006 and 2007 in a field with a high inoculum potential. Averaged across the 2 yr, wilt incidence was 2.0% for B2, 5.8% for C9, and 89.1% for the wilt-susceptible cultivar Gogulski. The severity of wilt symptoms on a scale of 0 to 4 (where 0 = no disease; 1 = off-color; 2 = 1 symptomatic leaf; 3 = 2 or more leaves with wilt symptoms; and 4 = plant death) averaged 2.4 for B2, 2.0 for C9, and 3.8 for Gogulski. Plant resistance is the only practical means of control for Fusarium wilt (Lucas and Shew, 1991).

Resistance to TCN and TMV were shown to be homozygous in the MF-2 line by progeny testing. Plant resistance is also the only practical means of control of TMV. The effects of TCN resistance are more economical and actually better than preplant soil fumigation (which costs approximately $500 per acre) because TCN populations that have been reduced by the fumigation subsequently rebound when a susceptible tobacco cultivar is grown. The single dominant gene in TCN-resistant B2 causes cyst nematodes to hatch, enter roots, and then die as a result of resistance, effectively reducing cyst nematode populations by more than 60% annually while still producing a tobacco crop (LaMondia, 1988, 2002b).

Resistance to blue mold was evaluated in replicated field plots. Field plots consisted of single rows of 10 plants with three replications. Plants were examined during the season and at harvest after cutting stalks to determine the number of blue mold lesions per individual plant. Data were analyzed by analysis of variance and Fisher’s least significant difference test. In 2003, the MF-2 parent of B2 was compared with the blue mold–susceptible cultivar Scantic and the resistant dark Cuban cultivar ‘H2000’. MF-2 was comparable with H2000, having fewer plants with lesions and fewer lesions per plant (on only those plants that had blue mold) than Scantic (Table 2). Blue mold resistance was evaluated for three advanced F_2 lines—B2, B3 and B6—and compared with the standard cultivar.

### Table 1. Percentage of leaf wrapper grades and cured leaf yields of B2 and C9 broadleaf tobacco across 5 yr under commercial production conditions in CT.

<table>
<thead>
<tr>
<th>Year</th>
<th>Grower</th>
<th>Cultivar</th>
<th>Cured leaf yield kg ha(^{-1})</th>
<th>Percentage wrapper †</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>1</td>
<td>C9</td>
<td>2245.2</td>
<td>57.0</td>
</tr>
<tr>
<td>2006</td>
<td>1</td>
<td>B2</td>
<td>2359.5</td>
<td>60.0</td>
</tr>
<tr>
<td>2006</td>
<td>2</td>
<td>C9</td>
<td>2210.4</td>
<td>59.9</td>
</tr>
<tr>
<td>2006</td>
<td>2</td>
<td>B2</td>
<td>2362.9</td>
<td>60.9</td>
</tr>
<tr>
<td>2007</td>
<td>1</td>
<td>C9</td>
<td>—</td>
<td>43.6</td>
</tr>
<tr>
<td>2007</td>
<td>1</td>
<td>B2</td>
<td>—</td>
<td>46.5</td>
</tr>
<tr>
<td>2008</td>
<td>3</td>
<td>C9</td>
<td>2117.4</td>
<td>51.7</td>
</tr>
<tr>
<td>2008</td>
<td>3</td>
<td>B2</td>
<td>2185.8</td>
<td>53.1</td>
</tr>
<tr>
<td>2008</td>
<td>4</td>
<td>C9</td>
<td>—</td>
<td>47.4</td>
</tr>
<tr>
<td>2008</td>
<td>4</td>
<td>B2</td>
<td>—</td>
<td>54.9</td>
</tr>
<tr>
<td>2009</td>
<td>3</td>
<td>C9</td>
<td>2092.7</td>
<td>39.3</td>
</tr>
<tr>
<td>2009</td>
<td>3</td>
<td>B2</td>
<td>2017.6</td>
<td>53.8</td>
</tr>
<tr>
<td>2010</td>
<td>5</td>
<td>C9</td>
<td>2320.3</td>
<td>—</td>
</tr>
<tr>
<td>2010</td>
<td>5</td>
<td>B2</td>
<td>2564.6</td>
<td>—</td>
</tr>
</tbody>
</table>

†Percentage of leaves in wrapper grades determined in commercial sorting facilities with blind samples.

### Table 2. Blue mold resistance evaluation for the susceptible cultivar Scantic, the resistant cultivar H2000, and the MF-2 breeding line in field plots, 2003.

<table>
<thead>
<tr>
<th>Line</th>
<th>Plants with lesions n</th>
<th>Lesions per plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scantic (susceptible)</td>
<td>100 a†</td>
<td>133 a</td>
</tr>
<tr>
<td>MF-2</td>
<td>30 b</td>
<td>2 b</td>
</tr>
<tr>
<td>H2000 (resistant)</td>
<td>50 b</td>
<td>2 b</td>
</tr>
</tbody>
</table>

†Numbers followed by different letters are significantly different (ANOVA–LSD).
C9 in replicated plots in 2007. B2 was more resistant than the other hybrid lines and exhibited significantly fewer plants with lesions and lesions per plant (on only those plants that had blue mold) than C9 (Table 3). In 2009 and 2011, B2 was compared with the susceptible Connecticut broadleaf cultivar C9, two burley tobacco cultivars—one susceptible (‘KT 200’) and one with low to moderate levels of resistance (‘KT 206’)—and two burley tobacco cultivars (‘NC 2000’ and ‘NC 2002’) with medium to high resistance to blue mold. Disease was rated on a scale of 0 to 5, where 0 = no lesions, 3 = most plants with at least one lesion, and 5 = all plants with multiple lesions. When compared with NC 2000 and NC 2002, the low to moderately resistant KT 206, and the susceptible KT 200 and C9 in the same experiment, B2 had moderate blue mold resistance that was intermediate to that of the NC cultivars and the KT cultivars (Table 4). The KT 200 cultivar was the most susceptible in this experiment. Results were similar when the experiment was repeated in 2011. The blue mold resistance in B2 was likely conferred by the VA-81 or PD4 parents and carried through the selection process along with TCN resistance because no resistance or tolerance to blue mold was observed in any other line used in the development of either parent. The exact pedigree of VA-81 is unknown; however, TCN resistance was transferred from *N. longiflora* to *N. tabacum* along with wildfire resistance through BVA S23 for VA-81 (Crowder et al., 2003) and B-21 for PD4. PD4 was described as having tolerance to blue mold (Curtin et al., 1980). Clayton (1945) described *N. longiflora* as susceptible to *P. tabacina* when young but resistant after plants were 6 to 8 wk old. This may not be totally unexpected because resistance genes can be clustered together on certain chromosomes and can be carried along with selection for resistance to other diseases. For example, resistance to *G. t. tabacum* and *G. t. solanacearum* may be linked to wildfire resistance caused by *Pseudomonas tabaci* Wolf & Foster, Stevens (Spaoff et al., 1971). Wildfire resistance was transferred from *N. longiflora* to the breeding line TL 106, which had a pair of chromosomes from the wild species (Clayton, 1947). *Nicotiana longiflora* was resistant to *G. t. solanacearum* in pot experiments (Baalawy and Fox, 1971). The Cuban dark-fired cultivar H2000 was bred for blue mold resistance but was also resistant to TCN in our tests.

The male-sterile F₁ hybrid B2 is highly resistant to Fusarium wilt, TMV, and TCN. B2 is also moderately resistant to blue mold leaf spot. This resistance is not enough to stand alone but will complement fungicide management. During field testing in 2010, B2 fields treated with standard fungicide programs for blue mold control were observed to be free of disease at cutting, whereas the C9 standard treated with fungicides often exhibited moderate levels of disease. Blue mold resistance would act to reduce the number of fungicide applications required to control the disease and may serve as a means of fungicide-resistance management. The flue-cured tobacco cultivars with resistance to *G. t. solanacearum* used as sources of TCN resistance were intolerant to nematode infection and required the use of a nematicide to maintain the growth and yield of resistant plants grown in nematode-infested soils (Komm et al., 1983). We selected inbreds under field conditions for growth and yield in the presence of damaging population levels of *G. t. tabacum* to avoid severe problems with intolerance to nematode infection. We did not observe intolerance to nematode infection in B2 in either experimental or commercial tobacco fields.

### Discussion

B2 Connecticut broadleaf cigar-wrapper tobacco is a male-sterile hybrid broadleaf cigar-wrapper tobacco with resistance to four of the most common and destructive pathogens of tobacco in Connecticut, including Fusarium wilt, TMV, TCN, and blue mold. This new cultivar should allow crop production with reduced losses to disease and reduced pesticide inputs. The use of an inbred with growers saving their own seed from year to year often results in genetic drift and the loss of resistance to pathogens over time. The use of a male-sterile hybrid that does not produce seed will result in a stable, uniform cultivar with no genetic drift over time.

### Availability

Seed will be released through the Connecticut Agricultural Experiment Station. A seed company has been contracted with nonexclusive rights to produce commercial seed under license agreement. Small quantities of B2 seed for research purposes are available from J. A. LaMondia under a Plant Variety Transfer Agreement to be administered by the
Connecticut Agricultural Experiment Station. If B2 is used in the development of other commercial hybrids, users should contact the Connecticut Agricultural Experiment Station to discuss a royalty contract. Seed has been deposited in the National Plant Germplasm System, but no seed will be distributed by the NPGS without written permission for 20 yr from the date of publication, at which time seed will also be available from NPGS.

Acknowledgments

References