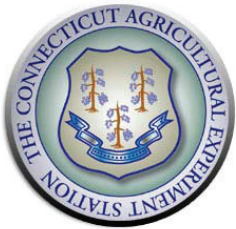


*The  
Connecticut  
Agricultural  
Experiment  
Station,  
New Haven*



*Bulletin 1031*

*May 2011*

[www.ct.gov/caes](http://www.ct.gov/caes)

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# A New Connecticut Broadleaf Cigar Wrapper Tobacco with Resistance to Multiple Pathogens

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A new cultivar of Connecticut broadleaf cigar wrapper tobacco (*Nicotiana tabacum* L.) was developed with resistance to Fusarium wilt, caused by the fungus *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *nicotianae* (J. Johnson) W.C. Snyder & H.N. Hans., Tobacco Mosaic Virus (TMV), the tobacco cyst nematode, *Globodera tabacum tabacum* (Lownsbery and Lownsbery) Stone and blue mold, caused by the Oomycete pathogen *Peronospora tabacina* Adam (*Peronospora hyoscyami* de Bary). This cultivar, 'B2', has been developed at the Connecticut Agricultural Experiment Station's Valley Laboratory. Yields and sorting quality are equal to or better than the current standard wilt-resistant, tobacco mosaic virus (TMV)-resistant cultivar 'C9'. Limited quantities of seed are available to growers and scientists.

Until Fusarium wilt-resistant broadleaf tobacco cultivars were released (LaMondia and Taylor, 1991), losses due to this disease reached 20% of the acreage in Connecticut. C9, a wilt-resistant cultivar released in 1991 (LaMondia and Taylor, 1992), has been widely grown (up to 80% of the total acreage annually) throughout the Valley, and has enabled tobacco production in infested soils with minimal losses. However, the pathogen is persistent in soils and continues to affect susceptible broadleaf tobacco even after three decades. Resistance to *F. oxysporum* is conferred by an unknown number of small-effect genes that are quantitatively inherited (Gritton et al., 1965).

The tobacco cyst nematode (TCN) was first discovered in Hazardville, CT in the early 1950's (Lownsbery and Lownsbery, 1954) and is now widely distributed in both shade and broadleaf tobacco production areas in the Connecticut River Valley of Connecticut and Massachusetts. 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 pre-plant nematode densities in soil (LaMondia, 2002c). Losses can be as high as 40 percent at high nematode densities in Connecticut. Nematode management has relied for years on nematicide use, but non-fumigant nematicides are no longer available and fumigant nematicide use is expensive and also being reduced due to environmental considerations. Nematode resistance would be an effective, inexpensive and environmentally benign means of control.

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* 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, caused by the Oomycete pathogen *P. tabacina*, has been a re-occurring problem in Connecticut and has caused severe epidemics and crop losses (LaMondia, 2010; LaMondia and Aylor, 2001). Resistance to the pathogen has been described and appears to be conferred by a single major gene in the wild species *N. debneyi* and *N. goodspeedii*. 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.

Plant resistance to major pathogens is the most economical, environmentally responsible, and often most effective way to control multiple tobacco diseases. The development of tobacco resistance to TMV in the 1950's, to ozone damage (weather fleck) in the 1960's, black shank in the 1970's, and Fusarium wilt in the 1980's and early 1990's effectively controlled serious diseases which each threatened to seriously impact or even eliminate cigar wrapper tobacco production in the Connecticut River Valley. We are building on this work by developing resistance to the cyst nematode and to blue mold.

#### BREEDING AND SELECTION

Initial crosses for cyst nematode resistance were made in 1987 between two flue-cured tobacco lines obtained from cooperators in Virginia. The breeding line VA-81 (Elliot et al., 1986) and cultivar PD4 (Currin et al., 1980) each carried resistance to the tobacco cyst nematode but were unmarketable as cigar wrapper tobacco. Both VA-81 and PD4 were crossed with three selections of Connecticut broadleaf inbreds. These inbreds were made at the CAES between 'C2' and three agronomically desirable broadleaf inbreds that were susceptible to all diseases. C2 is a cultivar that was developed with resistance to TMV that coincidentally carried resistance to Fusarium wilt. It was used as a source of wilt resistance in developing broadleaf lines such as C9. 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 F2. Selected plants were backcrossed twice to CT broadleaf to restore broadleaf characteristics and then inbred and selected over 12 generations. Plants were selected for agronomic type annually under field conditions using a bulk system of modified single seed descent in which only the top 2% of plants (20 plants of 1,000 grown) were selected for the next generation. One seed pod per plant was collected and seed bulked, mixed and randomly selected for the next generation. Additionally, plants were selected for TMV and TCN resistance in greenhouse screens. The lines were field selected for reduced sensitivity to weather fleck caused by ozone. The most promising resulting inbred (F12 generation) was not of suitable quality, so it was backcrossed again to the broadleaf cultivar 'Scantic' (LaMondia, 2002b) and F1 plants were again selfed to inbreds using pedigree selection over 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 F2, F5, F7 and F9 generations were selected for TMV resistance and cyst nematode resistance using a greenhouse seedling assay (LaMondia, 1991). Progeny testing was performed to select F8 plants homozygous for *G. t. tabacum* resistance and the dominant hypersensitive gene for resistance to tobacco mosaic virus derived from *Nicotiana glutinosa*. A superior male-fertile inbred (MF-2) resulting from 27 generations of selection for broadleaf agronomic characteristics with 8 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 resistant to Fusarium wilt (MS-1) to produce the F1 male-sterile hybrid B2.

#### EVALUATION AND PERFORMANCE

B2 was evaluated both for disease resistance and agronomic characteristics and cured leaf quality in both 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 and a commercial farm in Enfield, CT from 2006 to 2009. Cured leaves were commercially graded into one of six grades representing wrapper, binder, or filler quality. The percent of the total yield in wrapper grades was determined for each year (Table 1). Yields and leaf quality for B2 were as good as or better than the current standard cultivar C9 over all four years.

Resistance to *F. oxysporum* is quantitatively inherited (Gritton et al., 1965). Previous observations in field and greenhouse experiments as well as in commercial production have shown that wilt expression on wilt-resistant plants is often mild and that plants often outgrow early symptoms (LaMondia and Taylor, 1987; LaMondia and Taylor, 1991). The incidence and severity of wilt symptoms were determined in 2006 and 2007 in a field with a high inoculum potential. Averaged over the two years, wilt incidence was 2.0% and 5.8% for B2 and C9, and 89.1% for the wilt-susceptible cultivar Gogulski. Wilt symptom severity on a scale of 0 to 4 (where 0 = no disease and 4 = plant death) was 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. Resistance to the tobacco cyst nematode 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 for TMV. The effects of TCN resistance are more economical and actually better than preplant soil fumigation (which costs approximately \$500 per acre) as tobacco cyst nematode populations that have been reduced by the fumigation subsequently rebound when growing a susceptible tobacco cultivar. 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; LaMondia, 2002a).

Blue mold resistance 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 Differences Test. In 2003, the MF-2 parent of B2 was compared to the blue mold susceptible cultivar Scantic and the resistant dark Cuban cultivar 'H2000'. MF-2 was comparable to H2000 with 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 lines, B2, B3 and B6 and compared to the standard cultivar 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, B2 was compared to the susceptible cultivar C9, two burley tobacco cultivars with low levels of resistance ('KT 200' and 'KT 206') and two medium-level resistant burley tobacco cultivars ('NC 2000' and 'NC 2002'). 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 to the medium-level blue mold resistant tobacco cultivars NC 2000 and NC 2002, the low-level resistant KT 200 and KT 206, and susceptible C9 in the same experiment, B2 had moderate blue mold resistance intermediate to the NC cultivars and the KT cultivars (Table 4). The KT 200 cultivar was the most susceptible in this experiment. This resistance was likely conferred by the VA-81 or PD-4 parents and carried through the selection process along with TCN resistance. This may not be totally unexpected as resistance genes are often clustered together on certain chromosomes and can be carried along with selection for resistance to other diseases. For example, the Cuban dark-fired cultivar H2000 was bred for blue mold resistance but was

also resistant to the TCN in our tests. Fusarium wilt resistance was carried into commercial tobacco (such as C2) from the wild tobacco parent along with TMV resistance (Sand and Taylor, 1961), and resistance to *G. t. tabacum* and *G. t. solanacearum* may be linked to wildfire resistance caused by *Pseudomonas tabaci* Wolf & Foster, Stevens (Spasoff, 1971). Wildfire resistance was transferred from *Nicotiana 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 male-sterile F1 hybrid B2 is highly resistant to Fusarium wilt, TMV and the TCN. B2 is also moderately resistant to blue mold leaf spot, caused by the downy mildew pathogen *Peronospora tabacina*. 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 of nematode infection, and required nematicide use 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.

Our goal of developing a male-sterile hybrid broadleaf cigar wrapper tobacco with resistance to most of the major pathogens of tobacco in Connecticut, including Fusarium wilt, TMV, the tobacco cyst nematode and blue mold, has culminated in the development of B2. This 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.

**ACKNOWLEDGMENTS**

The author wishes to thank S. Waldron, W. Dunn, S. Jarmoc and Fairview Farm for excellent cooperation in commercial scale field trials and cured leaf quality analysis, and J. Canepa-Morrison, M. Salvas, S. Lamoureux, J. Preste and R. Horvath for technical assistance.

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**Table 1.** Percent leaf wrapper grades, and cured leaf yields of B2 and C9 broadleaf tobacco over four years under commercial production conditions in CT.

<u>Line</u>	2006	2006	2007	2008	2009
	<u>lb/acre</u>	<u>% wrapper</u>	<u>% wrapper</u>	<u>% wrapper</u>	<u>% wrapper</u>
C9	2003	57	44	53	39
B2	2105	60	47	52	54

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

<u>Line</u>	<u>% plants w/ lesions</u>	<u>lesions / plant</u>
Scantic (S)	100 a*	133 a
MF-2	30 b	2 b
H2000 (R)	50 b	2 b

\* Numbers followed by different letters are significantly different (ANOVA – LSD).

**Table 3.** Blue mold resistance evaluation for the susceptible (S) cultivar C9 and advanced F1 hybrid lines in field plots. 2007

<u>Line</u>	<u>% plants w/ lesions</u>	<u>Lesions/plant</u>
B2	10%	0.2 a*
B3	50%	1.6 bc
B6	43%	0.8 b
C9 (S)	70%	2.1 c

\* Numbers followed by different letters are significantly different (ANOVA – LSD).

**Table 4.** Blue mold resistance evaluation for the susceptible (S) cultivar C9, the low-level resistant (LR) burley cultivars KT 200 and KT 206, the medium-level resistant (MR) burley cultivars NC2000 and NC2002 and the F1 hybrid broadleaf line B2 in field plots. 2009

<u>Line</u>	<u>Blue mold rating*</u>
NC 2000 (MR)	1.7 a**
NC 2002 (MR)	2.0 a
B2	3.0 ab
KT 206 (LR)	3.3 ab
C9 (S)	3.5 b
KT 200 (LR)	4.7 b

\* Rating 0-5; 0=no lesions, 5=all plants with multiple lesions.

\*\* Numbers followed by different letters are significantly different (ANOVA –LSD).







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