

# Agronomic Performance of Flue-Cured Tobacco F<sub>1</sub> Hybrids Obtained with Different Sources of Male Sterile Cytoplasm\*

by

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## SUMMARY

Four cytoplasmic male sterile (cms) F<sub>1</sub> flue-cured hybrids cv. Wiślica × cv. Virginia Golta (VG), the male fertile analogue and the parental varieties were tested at two locations in Poland in a replicated field trial. The cms sources in the hybrids were *N. suaveolens*, *N. amplexicaulis*, *N. bigelovii* and a *N. tabacum* cms mutant. Under the slight to moderate pressure from black root rot present at the trial sites the hybrids showed a moderate tolerance of the disease characteristic of VG as opposed to medium strong susceptibility of Wiślica. Apart from the effect of black root rot tolerance the vegetative vigor of the hybrids (plant height, leaf size, earliness) was affected by cytoplasm source. The F<sub>1</sub> hybrid with *N. suaveolens* cytoplasm flowered approximately three days later than the remaining hybrids. Of the cms hybrids tested cms *N. bigelovii* produced the tallest plants with largest mid-position leaves. Yields of cured leaves were largely influenced by black root rot and were generally higher in VG and in the hybrids than in Wiślica. Leaf yields and curability were generally little affected by cms source under low pressure from black root rot. At the site with a relatively high level of black root rot infestation the yields of cms *N. suaveolens* were slightly lower but the percentage of light grades slightly higher compared to those of other cms hybrids. Cms *N. suaveolens* was the best hybrid in terms of money returns at the low black root rot field but it was the poorest hybrid performer under high pressure from the disease. Contents of nitrogen, sugars, nicotine and ash was little affected by source of cms. There was an increased incidence of potato virus Y (PVY) and white spots in cms *N. suaveolens* and, to a lesser extent, in cms *N. bigelovii* as compared to the remaining disease-free entries. [Beitr. Tabakforsch. Int. 21 (2004) 234–239]

## ZUSAMMENFASSUNG

Vier cytoplasmatisch bedingte männlich sterile (cms) F<sub>1</sub>-Hybriden des flue-cured Tabaks cv. Wiślica × cv. Virginia Golta (VG), die männlich fruchtbaren Analoge dieser Hybriden und die Elternformen wurden im Feldversuch an zwei Orten in Polen untersucht. Die Quellen der zytoplasmatischen männlichen Sterilität dieser Hybride waren *N. suaveolens*, *N. amplexicaulis*, *N. bigelovii* sowie die cms-Mutante von *N. tabacum*. Unter Einwirkung leichten bis mäßigen Infektionsdruckes von *Thielaviopsis basicola* Ferr. an beiden Orten waren die Hybriden tolerant gegenüber dieser für V. Golta charakteristischen Krankheit im Gegensatz zur mittleren Empfindlichkeit von cv. Wiślica. Neben der Empfindlichkeit gegenüber *Thielaviopsis basicola* wurden auch Wachstum, Entwicklung und Ertrag der Hybriden durch das Zytoplasma beeinflusst. Die F<sub>1</sub>-Hybride mit dem Zytoplasma von *N. suaveolens* blühte im Durchschnitt ungefähr drei Tage später als die anderen Hybriden. Unter den untersuchten Hybriden zeigten diejenigen mit dem Zytoplasma von *N. bigelovii* die höchsten Pflanzen mit den größten Mittelblättern. Der Ertrag an trockenen Blättern war stark durch das Auftreten von *Thielaviopsis basicola* bestimmt. Die Werte waren allgemein höher bei den Hybriden und bei der Sorte V. Golta als bei Wiślica. Der Einfluss des Zytoplasmas auf Blatterträge und Trocknungseignung war im allgemeinen geringer bei Pflanzen mit schwächerem *Thielaviopsis basicola*-Infektionsdruck. Bei starkem Krankheitsbefall dagegen waren die Blatterträge der Hybriden mit dem Zytoplasma von *N. suaveolens* kleiner und der Anteil von hellen Grades höher als bei den anderen cms-Hybriden. Auf dem Versuchsfeld mit schwachem *Thielaviopsis basicola*-Befall brachte die cms-Hybride *N. suaveolens* den höchsten finanziellen Ertrag, auf dem Versuchsfeld mit starkem Befall war der Ertrag jedoch unter den verglichenen Hybriden am

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geringsten. Der Gehalt der getrockneten Blätter an Stickstoff, Zuckern, Nikotin und Asche wurde wenig vom Zytoplasma beeinflusst. Bei der Hybride mit cms *N. suaveolens* und etwas geringer bei cms *N. bigelovii* war der Befall mit dem Kartoffel-Y-Virus (PVY) und weißen Flecken auf den Blättern stärker als bei den übrigen Hybriden. [Beitr. Tabakforsch. Int. 21 (2004) 234–239]

## RESUME

Quatre tabacs flue-cured hybrides  $F_1$  mâle-stériles cytoplasmiques (cms) issus du croisement entre les cv. Wislica  $\times$  cv. Virginia Golta, l'analogue mâle-fertile de ces hybrides et les variétés parentes ont été examinées lors d'essais répétés en plein champ dans deux localisations en Pologne. Pour l'obtention de ces hybrides, les espèces *N. suaveolens*, *N. amplexicaulis*, *N. bigelovii* et un mutant cytoplasmique de *N. tabacum* ont été utilisés comme sources de la stérilité mâle. Dans des conditions d'infection faible à modérée provoquée par *Thielaviopsis basicola* Ferr. présent sur les deux sites, les hybrides ont montré une tolérance modérée vis-à-vis de la pourriture noire des racines pour le cv. V. Golta par rapport au cv. Wislica moyennement sensible. En dehors de la tolérance vis-à-vis de *Thielaviopsis basicola*, la vigueur végétative (croissance, développement et précocité des récoltes) des hybrides a également été affectée par la source de mâle-stérilité cytoplasmique (cms). L'hybride  $F_1$  avec le cytoplasme de *N. suaveolens* a fleuri environ trois jours plus tard que les autres hybrides. Parmi les hybrides cms étudiés, le cms de *N. bigelovii* a produit les plantes les plus hautes ayant des feuilles médianes les plus grandes. Le rendement en feuilles séchées est influencé de façon importante par *Thielaviopsis basicola* et est généralement plus élevé chez les hybrides et avec la variété Virginia Golta qu'avec Wislica. Dans le cas d'une faible infestation par *Thielaviopsis basicola*, le rendement et le nombre de feuilles récoltables sont peu affectés par la source de cms. Dans des conditions d'infestation relativement importante, le rendement des feuilles de l'hybride avec le cms de *N. suaveolens* est légèrement plus faible, mais le nombre de grades clairs est légèrement supérieur par rapport aux autres hybrides cms. Dans le cas d'une faible infestation par *Thielaviopsis basicola*, l'hybride cms *N. suaveolens* apporte le meilleur rendement financier, mais dans des conditions d'une infection massive par cette maladie, il apporte le rendement le plus faible parmi les hybrides étudiés. La teneur en azote, sucres, nicotine et cendres des feuilles séchées est faiblement affectée par le cytoplasme. Le virus Y de la pomme de terre (PVY) et des taches blanches sur les feuilles se manifestent plus souvent chez l'hybride avec le cytoplasme de *N. suaveolens* et aussi, mais moins souvent, chez l'hybride avec le cytoplasme de *N. bigelovii*, que chez les autres hybrides. [Beitr. Tabakforsch. Int. 21 (2004) 235–239]

## INTRODUCTION

Confined for many years mostly to air-cured types the use of hybrid seed is now expanding to flue-cured cultivars. The advantages of hybrid varieties over conventional true-breeding lines are several. Given the availability of appro-

priate parental components a cultivar that combines desired characters can be developed very quickly in response to an emergency situation e.g. the outbreak of a disease. Another advantage is that the cytoplasmic male sterile (cms) seed gives the breeder full control of the reproduction of the cultivar and thereby both the breeder's commercial rights and the genetic integrity of commercially available cultivars are better safeguarded. However, there are two obstacles to widespread use of cms hybrids in flue-cured tobacco. One is to economics as related the production of hybrid seeds is certainly more costly than that of conventional selfed seed. The other is the concern that male sterile cytoplasm may have some undesirable effect on the agronomic performance of the hybrids and on the quality of the leaf. It has been known for many years that different sources of cms differ from one another in this respect. Those effects may be further complicated by specific cytoplasm-genotype interactions. Several sources of cms cytoplasm have been in practical use. In Poland, one of the pioneers in the development of flue-cured cms hybrids, a mutant *N. tabacum* cytoplasm, very close to *N. glauca*-type cytoplasm has been used since the early 70's. *N. suaveolens* and, to lesser extent, *N. undulata*-type cytoplasm have been the most popular sources of cms cytoplasm elsewhere. In our previous study (3) we found marked differences in the performance of different cms analogues of cv. Wislica – the principal flue-cured cultivar in Poland. Four best-performing cms analogues were used as maternal components to be crossed with cv. Virginia Golta (VG) – the German cultivar well adapted to cool climate conditions. Here we report on the performance of cms hybrids of cv. Wislica  $\times$  cv. Virginia Golta with particular reference to the effects produced by four types of alien cms cytoplasm.

## MATERIALS AND METHODS

The breeding lines were  $F_1$  hybrids from crossing cv. Wislica and its cms counterparts with a German flue-cured genotype (VG) partially resistant to black root rot. The best performing alloplasmic lines of Wislica (3) were chosen as female components of the hybrids:

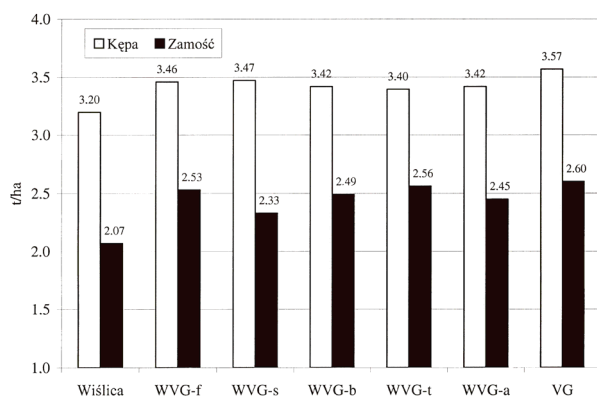
- WVG-f – Wislica  $\times$  VG (reference male fertile hybrid)
- WVG-s – Wislica cms *suaveolens*  $\times$  VG
- WVG-b – Wislica cms *bigelovii*  $\times$  VG
- WVG-t – Wislica cms *tabacum*  $\times$  VG
- WVG-a – Wislica cms *amplexicaulis*  $\times$  VG
- VG – the male parent
- Wislica – the female parent

The  $F_1$  hybrids had the same genomic make-up but differed in type of cms cytoplasm. In 2000 the entries were tested at two locations: Kępa Experiment Farm in Puławy and Institute of Agricultural Sciences Experiment Station in Zamość. At both locations the experiment design was randomized blocks with four replicates. Plot size was 18,8 m<sup>2</sup>, plants were spaced at 90  $\times$  40 cm, 50 plants per plot.

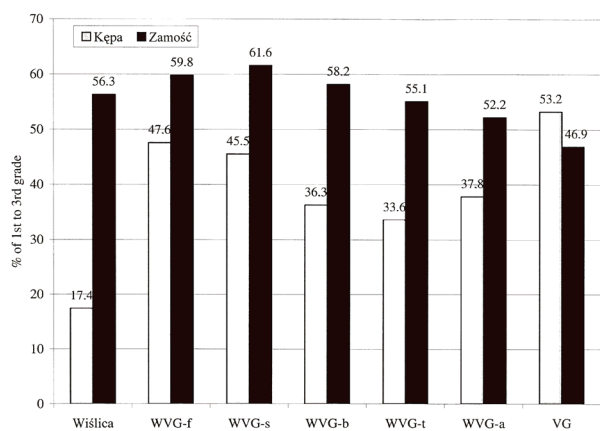
The following records were taken during plant growth: a) days to flowering – number of days from transplanting to the appearance of the first flower; b) plant height – from the base of the plant to the tip of the uppermost flower at full bloom; c) width and length of the midposition leaf (eighth leaf from the bottom of the plant); d) number of leaves on a plant.

**Table 1. Days to flower, plant height and leaf dimensions of the flue-cured tobacco F<sub>1</sub> hybrids and their parental cultivars (Wiślica and VG) – data from the Zamość site (2000).** Values that share the same superscript are not significantly different.

Cultivar	Days to flower	Plant height (cm)	Estimated leaf area (mid-position leaves) (cm <sup>2</sup> )	Length/width ratio (mid-position leaves)
Wiślica	85.5 <sup>b</sup>	109.0 <sup>c</sup>	501.0 <sup>a</sup>	1.96
WFG-f	81.2 <sup>a</sup>	153.2 <sup>b</sup>	965.4 <sup>b</sup>	1.93
WFG-s	85.3 <sup>b</sup>	131.3 <sup>a</sup>	815.0 <sup>b</sup>	1.96
WVG-b	82.4 <sup>a,b</sup>	151.1 <sup>a,b</sup>	859.2 <sup>b</sup>	2.03
WVG-t	82.2 <sup>a,b</sup>	133.9 <sup>a,b</sup>	733.2 <sup>a,b</sup>	2.15
WVG-a	82.5 <sup>a,b</sup>	145.5 <sup>a,b</sup>	737.0 <sup>a,b</sup>	2.04
VG	85.9 <sup>b</sup>	149.1 <sup>a,b</sup>	711.6 <sup>a,b</sup>	2.20
HSD at 0.05	3.9	20.6	278.0	non-significant



**Figure 1. Cured leaf yield of the flue-cured tobacco hybrids and its parents at two locations.** HSD (Kępa) = 0.58, HSD (Zamość) = 0.47 at 0.05 probability level.



**Figure 2. Percentage of first to third grade in the cured leaf crop of the flue-cured tobacco hybrids and its parents at two locations.** HSD (Kępa) = 25.9, HSD (Zamość) = 33.9 at 0.05 probability level.

For each of the above parameters means based on the measurements of 10 plants of each plot were used as plot values. Leaf area was calculated according to the formula: length × width × 0.6345 (10). Records were taken of incidence of some diseases. Plants were topped at early budding leaving approximately 18–20 leaves on a plant and suckers were controlled with Stomp. Leaves were reaped four times at Kępa and five times in Zamość. They were cured in a bulk curing barn (Puławy) and in a traditional barn (Zamość). Classification of the cured leaf was based on the grading system used in Poland involving grades 1 to 6, superior

grades 1 to 3 are referred to as light grades because of the prevalent light color (yellow to orange) as opposed to darker (brown) color of the inferior grades 3 to 6.

Where feasible, the data were subjected to analysis of variance (ANOVA) and the differences tested for significance using Tukey's honestly significantly different (HSD).

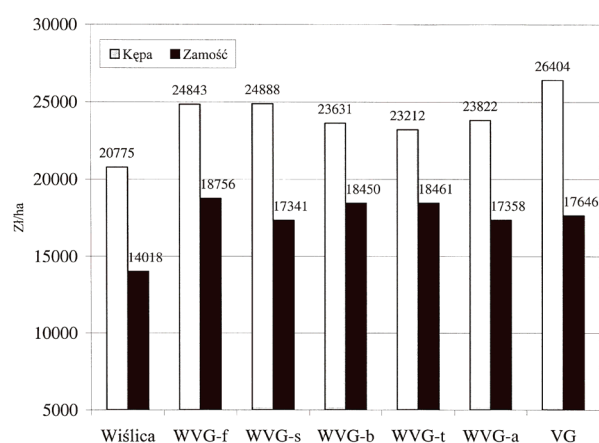
## RESULTS

### Growth and morphology

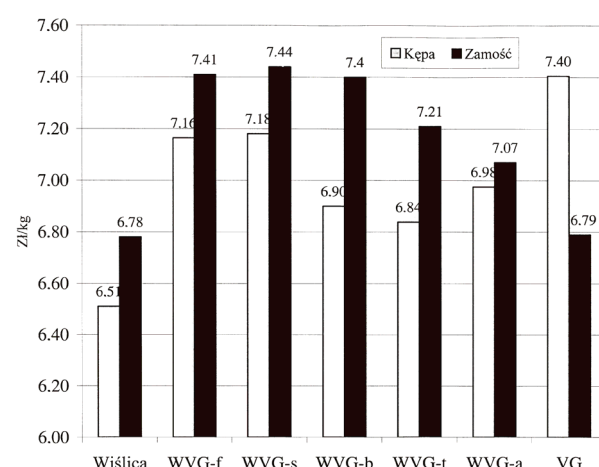
The F<sub>1</sub> hybrids and the parental lines became well established in the field at both locations. The initial growth of the hybrids and of the parental line (VG) was somewhat faster than that of Wiślica. At both locations the hybrids, with the exception of that with *N. suaveolens* cytoplasm (WVG-s), came to flower earlier than either Wiślica or VG (Table 1). Plant growth and particularly plant height and leaf size were, to a considerable degree, the reflection of the tolerance of black root rot. Under moderate to strong pressure from the disease observed at the Zamość site (Table 1) the plants of Wiślica were approximately 40 cm shorter than those of the tolerant line VG and also shorter than the hybrid plants. WVG-f (the fertile hybrid) and WVG-b (cms *N. bigelovii*) showed tolerance of black root rot similar to that of VG parent. Interestingly, the fertile hybrid produced leaves significantly larger than those of either parent which may be indicative of a heterosis effect. Narrowing of the leaves, a frequently observed effect of alien cytoplasm (9), was not recorded in any of the cms WVG hybrids.

### Yields of cured leaves

The data on yield and value of the cured leaf crop are given in Figures 1 to 4. Yields of the hybrids and their parents were largely affected by location (Figure 1). They were much higher at Kępa than at Zamość regardless of cultivar. The reason for this was two-fold: higher soil fertility and lower pressure from black root rot at the Kępa site. At both sites the hybrids and the male parent (VG) yielded significantly higher than Wiślica. At the Kępa site differences in yield among the hybrids were practically non-existent, at Zamość the yields of WVG cms *N. suaveolens* (WVG-s) were slightly depressed compared to other cms hybrids and to the male fertile hybrid although the difference was not significant. Grades of cured leaf were generally better at the



**Figure 3. Crop value of the flue-cured tobacco hybrids and its parents at two locations.** HSD (Kępa) = 4083, HSD (Zamość) = 3312 at 0.05 probability level.



**Figure 4. Unit crop value of the flue-cured tobacco hybrids and its parents at two locations.** HSD (Kępa) = 0.62, HSD (Zamość) = 0.84 at 0.05 probability level.

Zamość site largely because there was a problem with excessive nitrogen supply at Kępa (Figure 2). Wiślica seemed to be particularly sensitive to over-fertilization, percentage of higher grades of VG was not affected by location (actually, it was slightly higher at Kępa) the hybrids were intermediate in this respect. In terms of the percentage of higher grades the best curability was found in WVG-f (the fertile hybrid) and WVG-s (cms *N. suaveolens*).

Compared to the best entries WVG-b performed well at Kępa but slightly worse at Zamość. All the hybrids and the male parent (VG) surpassed Wiślica in terms of money value per unit area regardless of site (Figure 3). As with yield and curability, the differences between the normal fertile hybrid and the alloplasmics (cms) were small. WVG-s (cms *N. suaveolens*) gave the best money returns at Kępa (equal to those of WVG-f, but slightly worse than those of VG), WVG-f, WVG-b and WVG-t were the best performers at Zamość. The highest zloty/kg index was recorded for WVG-f, WVG-s and WVG-b at Zamość and for VG at Kępa (Figure 4). At both sites the hybrids gave better money returns per kg than Wiślica.

### Basic chemical components of cured leaves

The cured leaves of the hybrids and of VG tended to be heavier and to have more body than those of Wiślica but the differences generally were below significance level (Table 2). The contents of major chemical constituents were generally intermediate between the parents (Table 2). Total nitrogen contents was not significant in WVG-t and WVG-a compared to the remaining hybrids. Total sugars were very high in VG but moderate in the hybrids, being similar to Wiślica, except WVG-s which was significantly higher than Wiślica. Nicotine was low in VG and moderately high in Wiślica and in the hybrids.

### Disease incidence

The entries were scored for the following leaf diseases: potato virus Y (PVY), blue mold and white spots (Table 3). Assessments of black root rot damage were made at Puławy-Kępa (light to moderate pressure from black root rot), Zamość (moderate to high black root rot pressure) and at Dorbozy (black root rot field under very high black root rot infestation level) (Table 3). Symptoms of PVY (chlorosis, mosaic, mottling, rarely vein necrosis) was recorded consistently on some plants of WVG-s and, very sporadically, on WVG-b. White spots, which most likely have been caused by *Cercospora*, developed on a small percentage of plants of the same entries and also on those of WVG-a. Since none of such symptoms were recorded on WVG-f or on the parental lines they seem to be cytoplasm-related. However, in most hybrids affected, with the exception of WVG-s, the incidence of the two diseases was very low. All the hybrids showed moderate susceptibility to

**Table 2. Some physical and chemical characteristics of the flue-cured leaves from WVG F<sub>1</sub> hybrids and their parents.** Values that share the same superscript are not significantly different.

Entry	Weight/ 10 leaves (g)	Weight/ 1 dm <sup>2</sup> leaf (g)	% Stem	Total N (%)	Total sugars (%)	Nicotine (%)	Ash (%)
Wiślica	72.2 <sup>a</sup>	0.85	25.2	1.94 <sup>b</sup>	21.3 <sup>a</sup>	1.56 <sup>a</sup>	13.5 <sup>cd</sup>
WVG-f	82.5 <sup>ab</sup>	0.81	24.8	1.72 <sup>ab</sup>	24.1 <sup>ab</sup>	1.43 <sup>a</sup>	12.9 <sup>bc</sup>
WVG-s	94.3 <sup>b</sup>	0.96	25.6	1.73 <sup>ab</sup>	27.5 <sup>b</sup>	1.35 <sup>a</sup>	11.9 <sup>ab</sup>
WVG-b	77.4 <sup>ab</sup>	0.89	24.6	1.74 <sup>ab</sup>	23.0 <sup>a</sup>	1.53 <sup>a</sup>	12.7 <sup>bc</sup>
WVG-t	84.6 <sup>ab</sup>	0.94	28.0	1.87 <sup>b</sup>	24.2 <sup>ab</sup>	1.28 <sup>a</sup>	12.8 <sup>bc</sup>
WVG-a	83.7 <sup>ab</sup>	0.88	26.6	1.93 <sup>b</sup>	21.6 <sup>a</sup>	1.47 <sup>a</sup>	14.2 <sup>e</sup>
VG	93.4 <sup>b</sup>	1.00	24.2	1.44 <sup>a</sup>	34.1 <sup>c</sup>	0.76 <sup>b</sup>	10.8 <sup>a</sup>
HSD (Tukey) at 0.05	19.71	n.s.	n.s.	0.32	4.1	0.48	1.3

n.s. = non-significant.

**Table 3. Incidence of diseases on the flue-cured hybrids and their parents**

Entry	PVY (% of diseased plants)		White spots (% of diseased plants)		Blue mold <sup>a</sup>		Black root rot (% of damaged roots)		
	Zamość	Kępa	Zamość	Kępa	Zamość	Kępa	Dorbozy	Kępa	Zamość
Wiślica	0	0	0	0	1–2	0	100	25–35	40–65
WFG-f	0	0	0	0	3	2	65–85	5–10	20–25
WFG-s	8.5	6	5.5	6	3	2	70–80	5–10	20–30
WVG-b	2	1	10.5	1	2	2	70–85	10–15	20–30
WVG-t	0	3	0	2	2–3	2	70–80	10–15	20–25
WVG-a	1	0	3	0	2–3	2	70–80	10–15	20–35
VG	0	0	0	0	3	2	60–80	5–10	15–25

<sup>a</sup> Scores based on a 5-point scale: 1 = no symptoms; 2 = localized spots, no sporulation; 3 = medium severe, up to 50% of leaf affected; 4 = severe, more than 50% of leaf affected, 5 = systemic.

blue mold which was similar to that of VG but higher than that of Wiślica. Likewise, the hybrids showed level of tolerance of black root rot similar to that of VG which was considerably higher than that of Wiślica, especially under low to moderate pressure from the disease. Under very high pressure (site at Dorbozy) the hybrids and VG made very poor growth due to extensive root damage and their performance was little different from that of Wiślica.

## DISCUSSION

For the commercial production of cms hybrids the native cytoplasm of the maternal component of the hybrid must be replaced with an alien or mutant cytoplasm that effectively blocks the pollen production. In tobacco, the majority of known alien cytoplasm substitutions produce male sterility (2), but the morphological expression of the trait and, more importantly, its effects on plant growth and development may vary quite substantially (2). *Nicotiana suaveolens* has been acknowledged as the source of cytoplasm in tobacco that is associated with few, if any, adverse effects on agronomic performance (1,8,11,12). Indeed, probably the majority of commercialized F<sub>1</sub> tobacco hybrids are based on that type of cytoplasm. There are, however, other types of cms cytoplasm currently in use. In Poland, *N. tabacum* mutant cytoplasm (4) was used in the 70's and continues to be used in some newly developed F<sub>1</sub> hybrids. Judged by external flower morphology, the same type of cms cytoplasm or a closely related one, from *N. glauca*, is used in Hungarian-bred F<sub>1</sub> hybrids. Neither of these two cms has been associated in the past with any deleterious effects (5,7). However, in the previous study (2) the cytoplasm of *N. glauca* seemed to have a slightly depressing effect on the yield of cured leaves of cv. Wiślica as compared to the male fertile counterpart and the best performing male sterile counterparts (cms *N. bigelovii*, cms *N. suaveolens*, cms *N. tabacum*, cms *N. amplexicaulis*). Hence *N. glauca* cms was not included among the female cms components of the hybrids assessed in this study. The performance of the four cms sources in the Wiślica × V. Golta genotypic milieu was essentially similar to that in Wiślica in the sense that no major depressing effects of alien cytoplasm were found. However, there were some deviations from the pattern observed in the previous study involving the genotype of Wiślica. The most notable of these was the days to flower. In the study of Wiślica alloplasmics, cms *N.*

*suaveolens* came to flower as early as fertile Wiślica, cms *N. tabacum* and cms *N. amplexicaulis* but later than cms *N. bigelovii*. In this particular case the observations agreed with those by CHAPLIN (6) concerning the early flowering of *N. bigelovii* alloplasmics. In this study involving Wiślica × V. Golta hybrids it flowered later than the remaining entries including the male fertile hybrid. At the same location (Zamość) it showed also the lowest yields, although at the other site (Puławy) it produced yields equal to those of the remaining entries both in terms of weight of cured leaves and money returns from unit area. The Zamość field was peculiar in that it had a relatively high pressure from black root rot. The somewhat weakened performance of cms *N. suaveolens* (slightly delayed growth and slightly reduced yields, thought not significant) may have been the reflection of impaired tolerance to the disease as compared with the other cytoplasm counterparts. That the disease response of the alloplasmics involving cms *N. suaveolens* was altered relative to the other alloplasmics and relative to the fertile analogue was clearly observed in the case PVY and *Cercospora*-attributed white leaf spots. The elevated incidence of PVY symptoms in *N. suaveolens* alloplasmics was one of the most consistently observed cytoplasm-related traits both in the previous study with Wiślica (3) and in this study with Wiślica × V. Golta hybrids. Not included in this study, but data from another site with an unusually high pressure from PVY shows an incidence of nearly 12% of diseased cms *N. suaveolens* Wiślica × V. Golta hybrids and approximately 3% of cms *N. bigelovii* hybrids of the same hybrid formula. Since it has never been reported in literature the increased susceptibility to some diseases as observed in this and in the previous study seems unlikely to be the general trait related to some types of alien cytoplasm. A more plausible explanation is that it is a result of the particular cytoplasm interacting with the particular nuclear genome and maybe with the particular pathogen type. However, as the otherwise superior type of cms cytoplasm is involved the phenomenon should be studied in more depth.

## CONCLUDING REMARKS

- Except for cms *N. suaveolens* all F<sub>1</sub> hybrids, including the reference male fertile hybrid, flowered approximately 3 days earlier than either parent and approximately three days earlier than cms *N. suaveolens*.

- b) The cms hybrids *N. suaveolens* and cms *N. tabacum* showed a tendency to produce shorter plants than the remaining hybrids including the reference male fertile hybrid.
- c) Mid-position leaves tended to be smaller in cms hybrids, especially in cms *N. tabacum* and in cms *N. amplexicaulis*, than in the reference male fertile hybrid.
- d) Yields of the F<sub>1</sub> hybrids were generally little affected by type of cytoplasm.
- e) Curing performance (percentage of higher leaf grades) was highest in male fertile and cms *N. suaveolens* and slightly depressed in the remaining cms hybrids, especially at the site with higher incidence of black root rot.
- f) Money returns from unit area planted to F<sub>1</sub> hybrids did not seem to vary with type of cytoplasm nor did physical and chemical leaf characteristics.
- g) The cms hybrid *N. suaveolens* and, to lesser extent, cms *N. bigelovii* showed some susceptibility to PVY and to white leaf spots (mostly *Cercospora*-related), the remaining entries including the parents being free of the symptoms of either disease.

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