The PVY collaborative experiment 1996-2002: a global synthesis of results.

VERRIER, Jean-Louis (*), DOROSZEWSKA, Teresa (**).
(*)ALTADIS- Institut du tabac, Bergerac, France.
(**) Institute of Soil Science and Plant Cultivation, Pulawy, Poland.

Summary.
A synthesis based on observations accumulated since the start of this collaborative work, which involved 151 trials in 27 countries between 1996 and 2002, highlights the following results:

- assessment of different PVY resistance sources in *N. tabacum*, with regard to the global frequency of breakage of these resistances,
- world distribution, frequency and trends of the tobacco disease caused by necrotic PVY strains.

Results are discussed by referring to knowledge's concerning the PVY disease, namely:

- consequences of the PVY disease on the tobacco leaf chemical equilibrium,
- understanding of the resistance mechanism conferred by the « va » gene,
- possibilities of control through breeding,
- other possibilities of control.

Application of this knowledge in the view of GAP programs is considered. New trends of the collaborative experiment are proposed.

Introduction.

Among tobacco pathogens, Potato Virus Y is the most widespread, occurring in every tobacco producing country (5, 10). PVY infection affects yield and leaf quality. The cured leaf chemical balance is modified, with a greater importance of total nitrogen, which has been shown to be associated with an increased smoke mutagenicity (36).

Since PVY is transmitted by aphids upon the non-persistent mode, the use of insecticides to eliminate aphids has a low efficiency for controlling the virus. Other methods to prevent virus transmission by aphids have been proposed, and have been found efficient to some extent in the case of PVY in tobacco (6, 19). However, they have direct or indirect costs and risks: their application relies on the economic and agronomic context.

Another set of means consists on targeting the virus itself. The use of molecules that have direct antiviral effect has not been developed. Compounds that are triggering the plant defence mechanisms are partly efficient for some viruses (33). A third approach, cross-protection, has been studied for PVY in tobacco (16). None of these methods have been applied to PVY in tobacco.

Genetic modification of tobacco varieties is efficient to protect tobacco against PVY (34). Despite this, for many reasons this approach has not been developed so far, so that conventional non-GMO resistant varieties are the only way of controlling PVY.

Several *Nicotianae* show resistance to PVY. Among them, *N. africana* bears an interesting resistance, resulting in a quasi immunity to the virus (11,13). However, the transfer of the gene(s) involved in *N. tabacum* is still not complete (12, 21).

Inside *N. tabacum*, a few resistance factors have been identified. Most of them have been shown to be allelic and recessive, meaning that only one locus, called "va", is involved (2, 7, 38, 40). To our knowledge, resistant varieties that are grown commercially are all based on a "va" allele, and this situation did not evolve since the appearance of necrotic forms of the virus, PVY^N, in the years 1950. Then, the protection of the tobacco crop against PVY relies on one locus, used at a large scale, over a long period of time. This of course brings up the question of the durability, particularly when considering the evolutionary potential of PVY. In this context, the agronomy and phytopathology group of CORESTA decided in 1995 to run a collaborative experiment, with the following goals:

1. To identify the prevalent strains of PVY that infect tobacco by growing a host differential series at a number of locations world-wide,
2. To screen tobacco genotypes for resistance to different PVY strains.
3. To compile the results and to publish the information annually.

Accumulation of results over 7 years rendered useful to draw this synthesis.

Material and methods.

Host differential series. Each year, a given set of inbred lines has been assessed. Seeds were produced and checked for germination, identity and purity, ensuring an even genetic material over all trials. Origin of these lines and reaction towards PVY pathotypes are presented in annex 3.

Table I. Lines tested in the PVY collaborative experiment.

Name	Genotype at va locus (*)	Type	1996 (**)	1997	1998	1999	2000	2001	2002
VAM	vava	flue-cured	x	x	x	x	x	x	x
TN 86	vava	burley	x	x	x	x	x	x	x
PBD6	vava	dark		x	x	x	x	x	x
VIRGINIA SCR	vava	flue-cured	x	x	x	x	x	x	x
ZAMOJSKA 4	vava	flue-cured						x	
WISLICA	vava	flue-cured							x
BURLEY 21	VaVa	burley	x	x	x	x	x	x	x
NCTG 52	VaVa	flue-cured	x	x	x	x	x		
MN 944	VaVa	flue-cured	x	x	x	x	x		
NC 95	VaVa	flue-cured	x	x	x	x	x	x	x
K 326	VaVa	flue-cured		x	x	x	x	x	x
HABANA 92	VaVa	dark						x	x
KENTUCKY 17	VaVa	burley						x	x

* according to literature / or unpublished results (see annex).

** x = line included in the experiment for the year.

Participants. The list of participating organisations and countries is given in annex 2. Not all participants yielded data each year. (table V).

Main characteristics of the field trials. Together with seeds, a detailed protocol was distributed to participants each year. Participants were required to grow the lines in a location as much exposed to natural contamination by PVY as possible. Technical means used to produce the plants were intended to follow the standard practices for tobacco production in the area. The main characteristics of the trial were documented and this information was sent to the co-ordinator. The layout was a randomised complete block with at least 3 replicates. It was required to grow a minimum of 20 plants per elementary plot. Participants according to local constraints chose size and number of rows of the elementary plot.

Recording PVY natural occurrences. Only natural occurrences of PVY were studied. Assessment of the presence of PVY was made by symptom observation, however, confirmation by ELISA test was recommended and performed by participants who could do it. Choice of antibody was up to each participant. Starting in 2001 a particular set of antibodies was recommended.

Symptom reading was made at several dates, with a separate count for plants showing necrotic symptoms and plants showing only mosaics. To facilitate symptoms identification, a plate of photographs was distributed to participants at the start of the experiment. Also, a key describing different types of necrotic and mosaic symptoms was distributed with the protocol, and abbreviations to specify each particular type of symptom were proposed and used by the participants (e.g., NV: Vein necrosis, VB: vein banding, etc…). Starting in 2001, it was proposed to record also other virus symptoms than PVY.

Yearly reports. Data were returned to the co-ordinator, who established an annual report, included into the CORESTA bulletins or CD-ROM. In each location, the reading corresponding to the highest percent of symptomatic plants in susceptible genotypes was selected for this report. The synthesis presented below is using these data.

Results.

The complete data obtained between 1996 and 2002 and analysed here are given in table V. 151 trials have been planted in 27 countries. Most of these trials are located in Europe, which accounts for more than half of results. The second participating continent is Asia (36 trials). Africa yielded data as well in its southern part (Zimbabwe, RSA, Madagascar) than in the north (Morocco). America is present with 12 trials from Colombia, Brazil and USA. The three first countries for tobacco leaf production, China, Brazil and USA are under-represented in this data set.

PVY distribution and incidence.

PVY incidence. The term incidence refers here to the probability, for a susceptible "VaVa" plant, to be successfully inoculated with a PVY strain and to develop symptoms. In a given location and year, the mean frequency of symptoms (necrotic and mosaic) observed on all susceptible "VaVa" lines available in the trial, has been taken as an estimate of this above defined incidence. Table IV shows means by country.

Geographical distribution of PVY. Symptoms reported to PVY have been reported from 90% of the trials. There are 4 main areas where PVY incidence is high:

- north-central part of Europe: Hungary, Poland, Switzerland, Germany, east of France (Alsace).
- eastern Asia: China and South Korea.
- southern part of Africa: Zimbabwe.
- South America: south of Brazil.

Some countries are located close to one of these main areas, and have lower but still substantial PVY incidences: Japan, Colombia, and South Africa.

In these main areas, PVY incidence is substantial in any year: the minimum is rarely below 20%. Among 41 trials planted in Hungary, Poland, Switzerland, Germany, and Alsace, the minimum incidence observed is 19%. In the same way, the minimal incidence among 12 trials from China and South Korea is 15%. The same statements can be made when considering Zimbabwe and Brazil.

In contrast with this, the southern and western parts of Europe show a much more irregular occurrence of PVY, with low and high incidences depending on years and locations. This involves Belgium, south of France (Bergerac), Portugal, Spain, Italy, Croatia, Macedonia and Greece.

Farther east and south, PVY incidences seem very low. From 6 trials in different years in Morocco, symptoms have been recorded only once. Similarly, in Turkey no symptom has been found (3 trials). Iran shows an intermediate pattern, with most of the times low incidences, and a maximum of 35% (11 trials from 6 years).

Other countries participated, however absence or low frequency of PVY symptoms was found and trials were not continued in further years. This involves USA (North Carolina), India, Indonesia, Madagascar.

The above described main areas do not include North America, however it is well known that North of USA and Canada (Ontario and eastern provinces) have a significant degree of PVY incidence (7).

Year to year variation 1996-2002. Within this 7-year period of time, no general trend towards an increase or a decrease of PVY incidence can be shown by the data, even when considering separately the different geographic areas or individual countries.

Figure 1 shows the yearly variation of the mean PVY incidence in geographic areas where more than 15 trials have been planted (see table IV for the countries included in these geographic areas).

For Europe, this confirms the higher and more regular incidence in the north. Both in North and South, highest and lowest years for PVY incidence are 1997 and 2001, respectively. Therefore, some relationship between variation patterns in North and South seems to exist. Nevertheless, the drop between 1997 and following years happened mostly in the South.

In eastern Asia, PVY incidence is also highly variable. Year to year variation in Asia follows a different trend than in Europe, with maximum and minimum in 2000 and 2002, respectively.

In Africa and America, the number of trials is too low to get a general view of the year to year variation. It seems to be high, except in south of Brazil where the incidence was constantly above 90%.

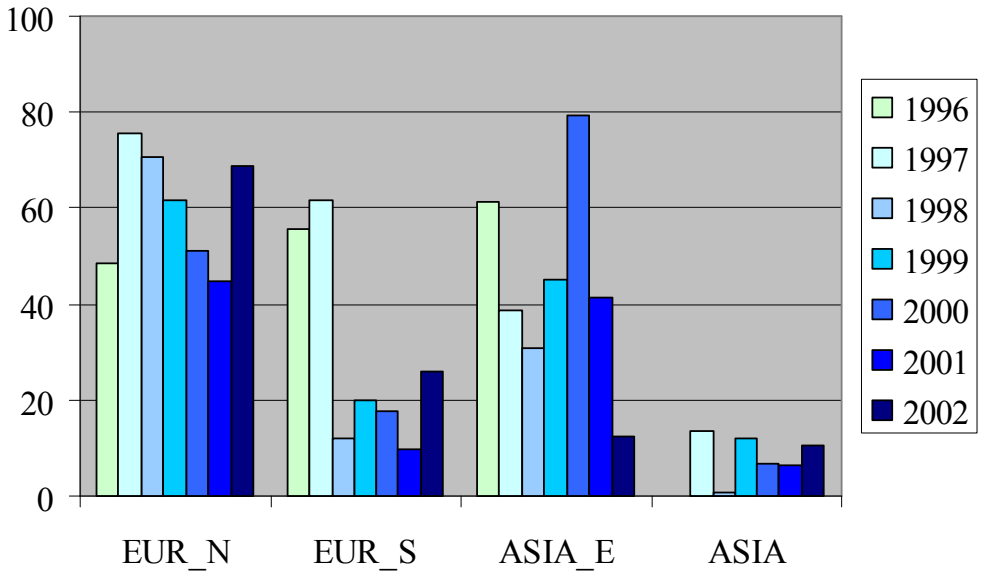


Figure 1. PVY incidence according to year and area.

Incidence of PVY^N and va-breaking PVY^N.

Partitioning of PVY strains.

The following grouping of PVY pathotypes (4) will be considered:

- Non va breaking, PVY^N strains, producing necrotic symptoms only on VaVa plants. These strains belong to pathotypes 0 or 2.
- va breaking PVY^N strains, producing necrotic symptoms on any type of plant. These strains belong to pathotypes 1 or 1-2.
- Other strains, only producing mosaics on tobacco, therefore not being part of PVY^N.

Occurrences of "va" breaking situations.

Detailed results clearly show that the frequency of necrotic symptoms on "vava", PVY resistant lines, is substantially lower than on susceptible, "VaVa" lines. There are, however, 10 situations where the reverse situation happens. More generally, 76 trials show some degree of necrotic symptoms on "vava" lines.

Table II – Numbers of trials according to PVY incidence and "va" resistance breaking.

	No necrosis	Presence of necrotic PVY symptom			Total
		Not on vava lines	Necrotic symptoms on va va lines		
			more frequent on VaVa lines	equally or more frequent on vava lines	
PVY incidence <= 1%	16	2	0	0	18
PVY incidence > 1%	9	48	66	10	133
Total	25	50	66	10	151

In a practical point of view, the "va" resistance would be of no interest in tobacco varieties if both following events happen:

- the general incidence of PVY^N is high.
- "va" breaking are substantially more frequent than non-breaking strains.

A useful parameter to evaluate up to which degree this second condition is verified would then be the relative proportion of va breaking strains within the necrotic part, PVY^N, of the global PVY population.

If we call respectively P(vaB) and P(N-nonB) the frequencies of va breaking and va non-breaking PVY^N strains in the global viral population, this parameter would be :

$$RB = P(vaB) \ / (P(vaB) + P(N\text{-}nonB))$$

A RB of 0 means absence of va breaking strains, and if RB = 1 all the PVY^N strains are va-breaking.

Partitioning of plants.

Within "vava" plants, proportions of asymptomatic, mosaic and necrotic plants will be called F1, F2 and F3, respectively. Within "VaVa" plants, these proportions are F4, F5 and F6.

Table III. Proportions of plants showing no symptoms, mosaic symptoms, necrotic symptoms, within respectively "vava" and "VaVa" groups of plants

	No PVY symptom	Symptoms reported to PVY		Sum
		Only mosaics	At least some necrotic symptom	
"vava" plants	F1	F2	F3	F1+F2+F3 = 1
"VaVa" plants	F4	F5	F6	F4+F5+F6 = 1

Estimates of PVY incidence, P(vaB) and RB.

PVY incidence: using the above definition, this is estimated with: $I = F5+F6$.

P(vaB): "va" breaking PVY^N strains are the only strains that can produce necrosis on "va va" plants. Therefore, the probability for a "vava" plant to show necrotic symptoms depends on the following events:

- this plant has been successfully inoculated with PVY: assuming that every plant is randomly submitted to the same initial viral population as transmitted by aphids, and that symptoms recorded on susceptible plants reflect this, this probability is I.
- the inoculated strain is va breaking: this is P(vaB).

This gives $F3 = I * P(vaB)$ then P(vaB) may be estimated with : $P(vaB) = F3 / (F5+F6)$

In the present data, this has been calculated only when F5+F6 was greater than 2%.

Estimate of RB. To give an exact estimate of RB would mean that it is possible to estimate P(N-nonB). This is difficult, because VaVa plants showing necrosis may be affected either by va breaking or non-breaking strains, or by both, and we do not have direct data that would allow estimating the relative shares of these different cases. If we neglect the possible case of plants harbouring both types of strains, the proportion of VaVa plants showing necrosis would be:

$F6 = I * (P(vaB)+P(N\text{-}nonB))$

Therefore, the parameter RB could be estimated with: $RB = F3/F6$

In the 10 situations where F3>F6, this leads to a RB >1. In these cases, RB has been assumed to be 1.

Table V gives the estimates for P(vaB) and RB in each situation where they could be established.

Geographical distribution of va breaking strains.
In North of Europe, RB is generally low. In contrast with this, south of Europe shows much higher estimates for RB. This is also verified in Morocco, Iran and India.
RB is high in China (where most of the trials are from the Shandong province, east of China), Colombia, and to a lesser extent in Zimbabwe. Globally, this distribution is clearly different from the general distribution of PVY.

Year to year variation of va breaking strains incidence.
Over the 7 years period, no significant increase or decrease of RB has been found, in any of the different geographical areas.

Relation between PVY incidence and frequency of va breaking strains.
By considering mean I and RB over all situations where they could be estimated, figure 2 shows an inverse relationship. Countries with low PVY incidences tend to have high RB.
A remarkable exception is China: this country yielded 6 trials from 5 different years, therefore the mean of these trials has some significance. Within these trials, the 4 located at Qingzhou (Shandong province) are all with high RB and I. It seems then that in this part of China at least, both above referred conditions are met: the "va" resistance has a low utility in this area.

The case of Portugal is less clear, since only two trials account for this country, from the same year. Data show that only one of these 2 trials have a high RB, then this situation may have been a specific case.

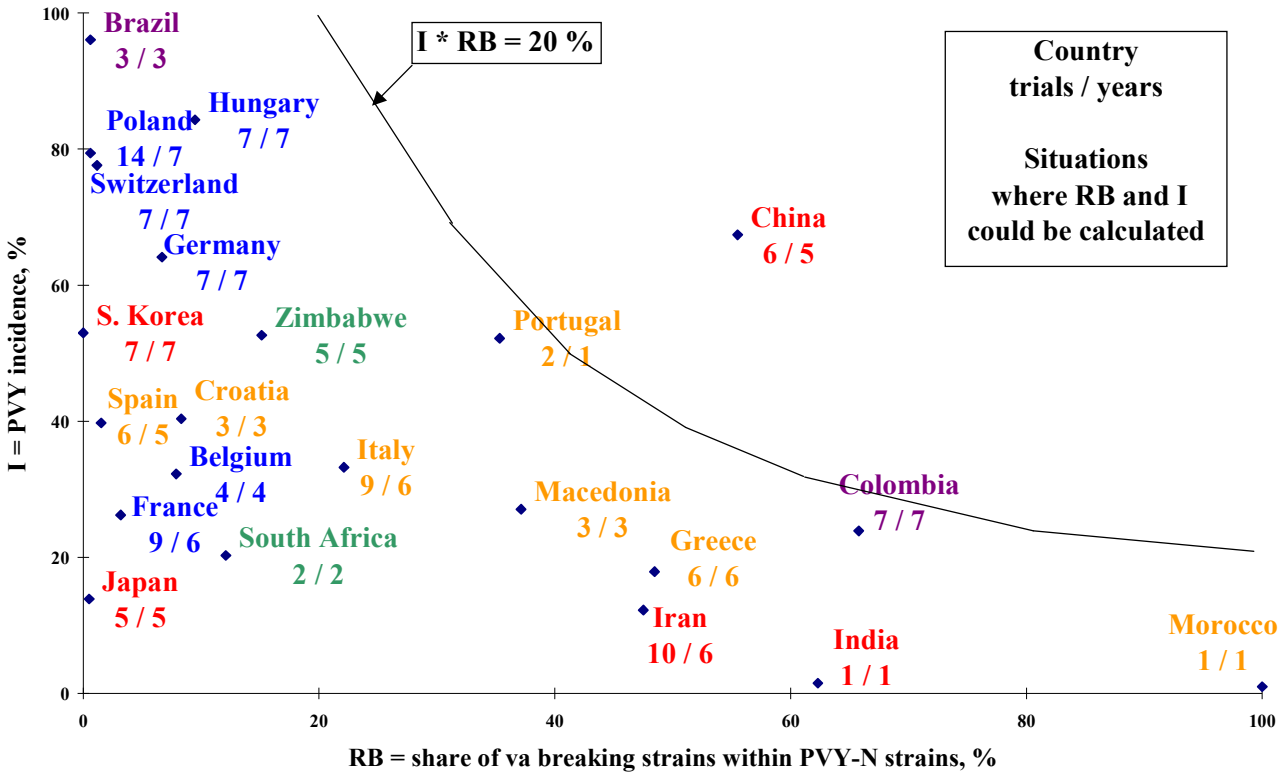


Figure 2. Relation between PVY incidence and relative frequency of va breaking strains.

The effect of genotypes on PVY symptom expression.

General linear regression.

Data presented in table V are not complete, since each year there are missing lines or countries. Therefore, a conventional analysis of variance could not be run over the whole set of data. However, it is still possible to estimate the effects of years, lines and countries on the traits of interest, because most of the lines and countries have been repeated from one year to another. This is the scope of the general linear regression analysis (GLM module of Statgraphics version 5 ®). The following partitioning of the variance has been tested:

- Y = constant term + line effect + year effect + country effect + residual.
- Y being the percent of plants showing necrotic symptoms.

Data submitted to this analysis include 126 situations where at least two lines show symptoms and where the mean frequency of all symptoms over all lines is greater than 2%.

Analysis of Variance for Necrosis

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Line	316890,0	12	26407,5	51,26	0,0000
Year	18608,9	6	3101,49	6,02	0,0000
Country	229029,0	21	10906,1	21,17	0,0000
Residual	548641,0	1065	515,156		
Total (corrected)	1,11545E6	1104			

R-Squared (adjusted for d.f.) = 49,013 percent

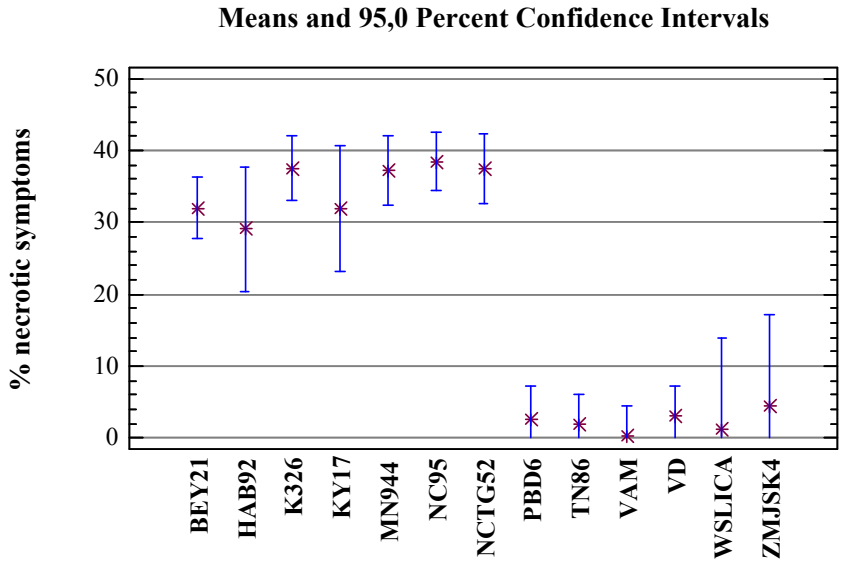


Figure 3. Adjusted means obtained from General Linear Regression

Year, country and line have a highly significant effect on the frequency of necrotic symptoms. Two groups of lines, corresponding to the VaVa and vava genotypes, are confirmed. In these data, it then appears that only the va gene has some impact on the frequency of necrotic symptoms. If other genetic factors are involved in some lines, they are not influencing the data at this global level.

In order to assess more precisely the behaviour of each line, principal component analysis has been used. Due to the fact that not all lines are present each year, the following analysis have been performed:

First analysis 1997-2000
- years 1997 to 2000,
- 69 locations with complete data and frequency of necrotic symptoms greater than 2%.
- Data analysed are the following quotients :
- % of necrotic symptoms observed / mean % of necrotic symptoms on all lines in this location.

Factor 1, 2 and 3 from this analysis are presented in figure 5. An ascending hierarchical classification of lines (Euclidian distance) obtained from the same data using the software Statbox is shown in figure 4. When including data from 1997 to 2002 (it then becomes necessary to discard NCTG52, which was absent in 2001 and 2002) and with the same statistical approach, very similar figures are obtained.

Analysis for 2001-2202.
- years 2001 to 2002,
- locations with complete data and frequency of necrotic symptoms greater than 2%.
- Data analysed are the same quotients as for the first analysis.

Factors 1, 2 and 3 from this analysis are presented in figure 6.

Differences between lines within each group are suggested:

vava lines.
Within vava lines, these figures suggest that VAM and TN86 are close and give about the same pattern of response towards PVY^N, and that they differ from VD (Virginia SCR) and PBD6, which are making another group. A more careful examination of data confirms that the va allele(s) present in VD and PBD6 seem to be more frequently overcome by PVY^N. This seems to happen in Europe, particularly in Hungary where it can be seen that every year these two lines have more necrotic symptoms than VAM and TN86. There are, however, some areas where the reverse happens, VAM and TN86 giving more necrotic symptoms : China (Shandong province) and Colombia.
Due to their occurrence in the trials only in one year, WISLICA and ZAMOJSKA4 could not be entered in these statistic treatments. However, it can be seen from adjusted means obtained by regression that they behave like other vava lines, and it seems the va allele from WISLICA is less often overcome than alleles present in VD or PBD6.

VaVa lines.
Burley 21 and NC95. Figure 5 shows that Burley 21 has a specific pattern. There is a tendency to oppose NC95 and Burley 21 in the different maps obtained by principal component analysis. Factor 2 of the first analysis is linked with this opposition. Situations where Burley 21 has the lowest count, among the VaVa group, for necrotic symptoms are numerous. This happens often in north and central Europe: Belgium, 4 times out of 5, Alsace: 5 out of 6, Germany: 4 out of 7, Poland: 9 out of 14, Switzerland: 5 out of 7. This also happens in some years at least in Zimbabwe and Colombia. In general, in these same situations NC95 tends to get high counts. In South of Europe and in Eastern Asia, the picture is different and Burley 21 and NC95 tend to have the same counts as other VaVa lines, with a few exceptions (Chinese trials from 1996: Changle, Yinan, some trials in Spain, Macedonia, Italy).

NCTG52. NCTG52 does not show a specific pattern here, and follows the means observed on MN944, resulting in a very similar profile, which is shown by the hierarchical classification (figure 4).

Habana 92. This line has been entered in the 2001 and 2002 experiment, therefore data are not numerous. Despite having clearly a necrotic symptoms frequency typical of the VaVa group, the profile of Habana 92 is not far from the vava lines (figures 6). Its adjusted mean for necrotic symptoms is the lowest of the VaVa group.

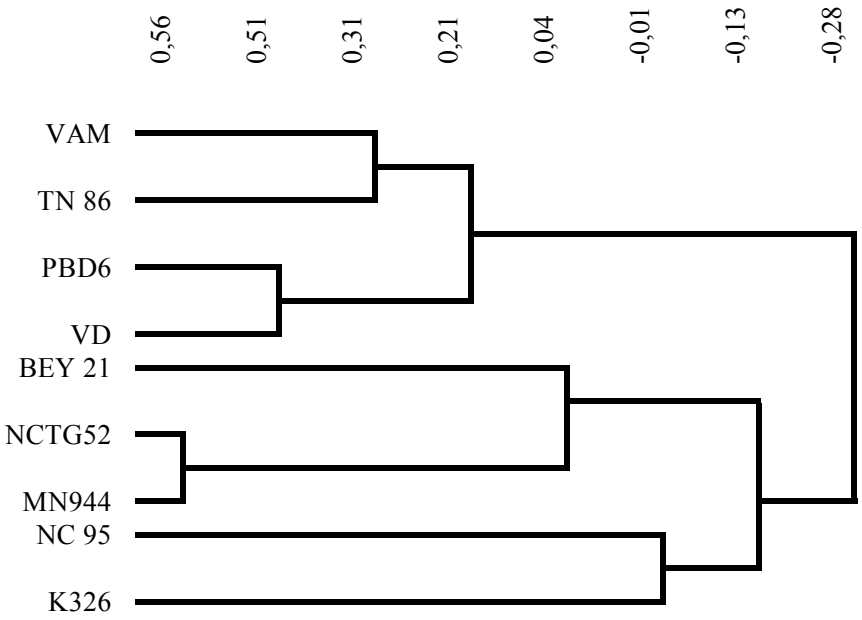


Figure 4. Ascending hierarchical classification of lines obtained from 1997-2000 data.

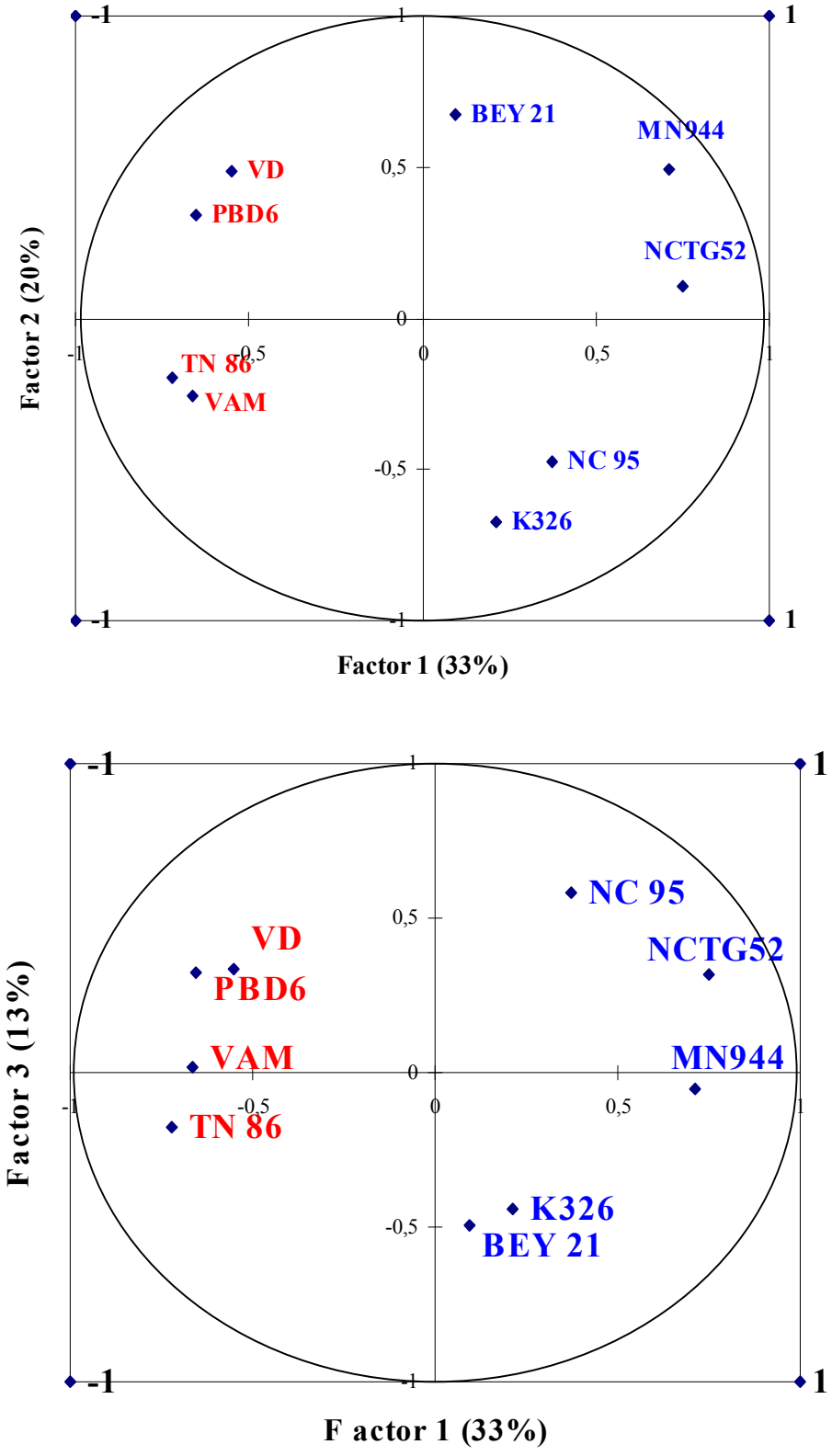
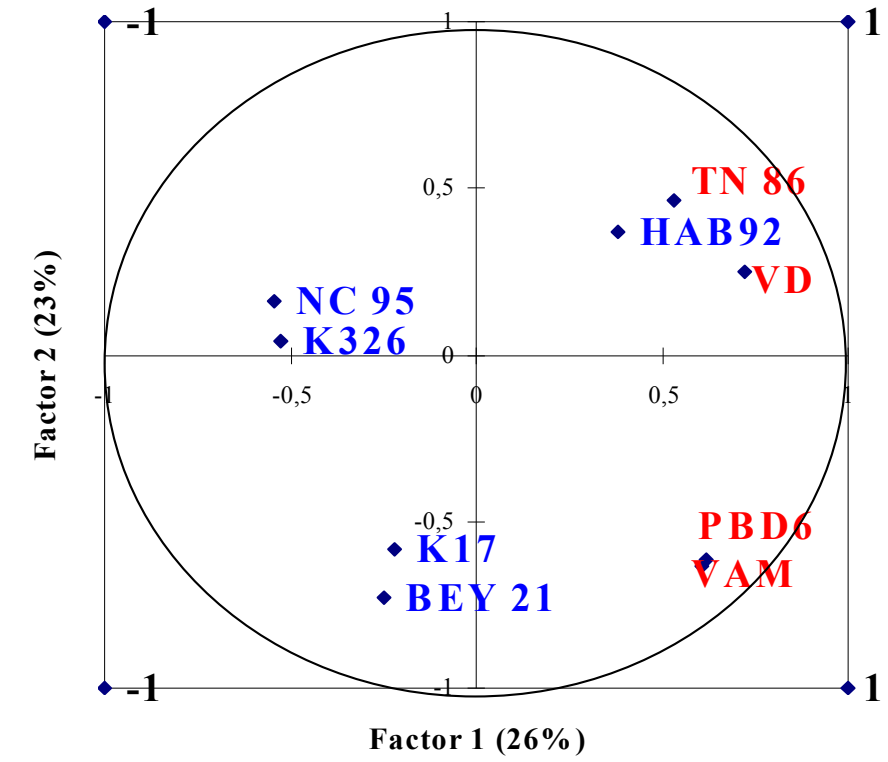


Figure 5. Principal component analysis of relative frequencies of necrosis, 1997 – 2000, and ascending hierarchical classification from the same data.



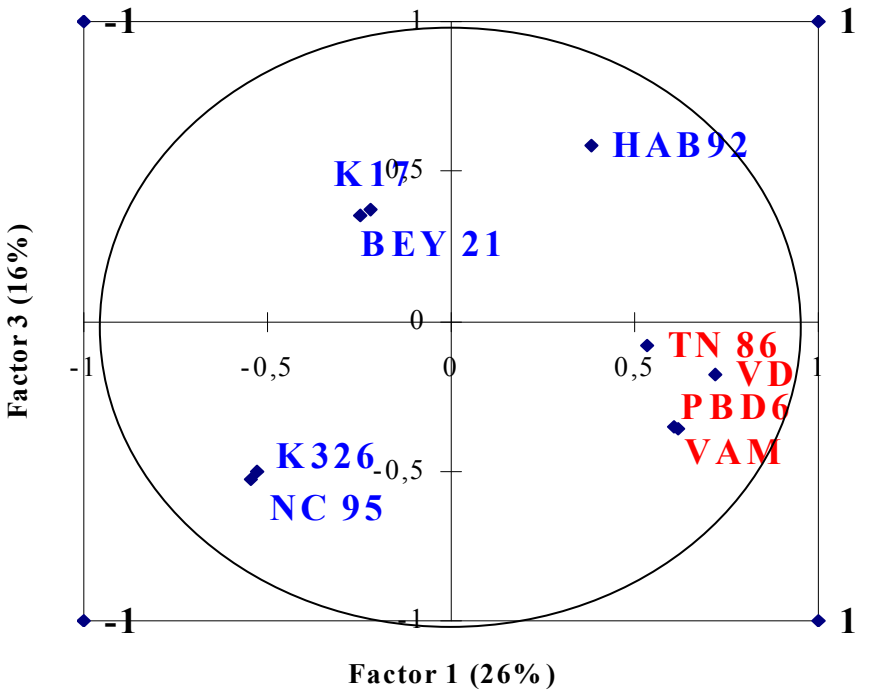


Figure 6. Principal component analysis of relative frequencies of necrosis, 2001 – 2002.

Discussion.

PVY incidence and distribution.

PVY incidence. Data have been obtained from the maximum expression of symptoms in field, generally late in the growing season. Then, the viral population related to these data is the final one. It is likely that it differs substantially form the initial population inoculated by aphids. Some PVY strains may be asymptomatic, or produce only transitory mosaic symptoms, that will disappear later. This may lead to under-estimate these types of strains. Furthermore, when a VaVa plant is challenged with both these mild strains and PVY^N, it may be supposed that necrotic symptoms due to PVY^N will appear. Therefore, when present, PVY^N may mask the occurrence of milder strains, which will also contribute to under-estimate the latter. Due to this, the geographical distribution and yearly variation revealed by the data are to be related primarily to PVY^N rather than to the whole PVY viral complex.

Distribution of PVY^N. When considering climatic conditions that are prevalent in the main areas where incidence of PVY^N is high, it seems that temperate continental climates are the most favourable to the spread of this virus. This statement fits also with the presence of PVY in eastern Canada. It does not explain, however, the wide yearly variations observed in the Mediterranean part of Europe and surrounding countries.

It is known that PVY strains affecting tobacco are also found in potato, whereas isolates from tomato or pepper tend to be more related together than with potato or tobacco isolates (20). When considering the geographical distribution of these crops in Europe, it appears that the potato crop distribution is fitting with PVY^N distribution. The fact that potato is a perennial with vegetative multiplication would also explain why PVY^N incidence is so high and constant in places where winters are cold, unfavourable for the survival of PVY^N in weeds. Planting date for potato may be earlier than for tobacco, which offers to the initial PVY viral populations a first opportunity to multiply before being transmitted to tobacco in late May or June by aphids.

In places where the winds are frequents and come from the sea, probability of contamination by aphids is lower. This may explain the irregular incidences of PVY^N found in Belgium.

In the Mediterranean part of Europe, epidemiological conditions for PVY are obviously different, since winter is generally more favourable to survival of the virus, and the importance of potato crop is lower. In these conditions, a high multiplication of aphids at early spring is probably an important factor to explain the observed variations. An interesting fact in this area is the frequent finding of tobacco plants harbouring other viruses together with PVY, for example CMV and AMV. These viruses belong to different families, but share the common trait of being transmitted by aphids upon the non-persistent mode. This leads to the idea that, in order to understand better epidemiological conditions that are influencing the distribution of PVY, to expand observations to other virus symptoms will help. This strategy has received a beginning of application in the experiment, starting in 2001.

Va-breaking PVY^N.

Distribution of va breaking PVY^N strains. At least two hypotheses could be proposed to explain the specificity of the distribution of va breaking strains versus all PVY^N strains:

- va breaking strains would not have the same thermal requirements than other strains.
- va breaking strains are more virulent, but in turn other essential characters for their survival would be affected, for example transmissibility, multiplication rates in the host plant, ability to move in the host plant. Such trade off between virulence and decreased transmission has been shown for CMV. This idea fits also well with the fact that viral proteins have several functions (14).

These two hypotheses are not exclusive of each other, and could together explain the pattern observed.

Genetic factors linked to PVY^N resistance or tolerance.

vava lines

The va allele present in TN86 is inherited from VAM (23, 29); it is therefore not surprising that these lines are placed in the same group by the hierarchical classification. VAM has a slightly lower adjusted mean for necrotic symptom frequency (fig. 3), which could be explained by the different genetic background in VAM compared to TN86. It has been mentioned that other factors contributing to the resistance to PVY^N than "va" are present in VAM. There is no evidence that these factors have been inherited in TN86 (1).

PBD6 and VD do not have the same pedigree. However, in both cases the "va" resistance is from spontaneous origin, which contrasts with the fact that the VAM resistance has been obtained with X-ray irradiation (18). PBD6 inherited the "va" resistance from Paraguay, an air-cured variety grown in France since the 19^{th} century (31). VD is originating from a spontaneous variant obtained from mass selection within the variety VN1, in Germany (8). VD and PBD6, or varieties that inherited their resistance from these lines, have been extensively grown in Europe (at least France and Germany). The fact that the "va" alleles present in these lines is more frequently overcome in Europe than the "va" allele from VAM could then be interpreted as being a local evolution of PVY^N towards overcoming these alleles.

Zamojska 4 and Wislica have been grown only one year in the experiment. The low frequencies of necrotic symptoms in these lines fits with their clustering into the vava group. Wislica had lower necrotic symptom frequencies than other vava lines; it will then be interesting to continue to assess this line in future trials.

VaVa lines.

Burley 21 and NC95

The lower necrotic symptom frequency in Burley 21, as well as the higher counts in NC95, is fitting with previous results (3, 30). The fact that these differences are correlated in some areas, in particular Europe, could not be expected from former studies. It suggests that some resistance or susceptibility factor(s) are present in one of these lines, and absent in the other one, and that PVY^N strains from Europe tend to reveal this difference.

NCTG52. This line comes from MN944 through gametoclonal selection and has shown some resistance to North American PVY strains (39, 41). This resistance was found to be determined by other factors than "va". However, in the same work it is shown that intensity of symptoms, when challenged with strains from other parts of the world, are equivalent to MN944. The data reported here are well fitting with these results, since NCTG52 has a very close profile to MN944. Both lines, in other areas than North America, show high frequencies of necrotic symptoms.

Habana 92. This line was part of a greenhouse screening test made at ALTADIS-Institut du tabac using several PVY strains, some of which were « va » breaking (unpublished). Habana 92 showed a specific pattern in this screening, therefore it was decided to enter it into the CORESTA experiment. The data obtained so far seem to confirm that Habana 92 would harbour some resistance factor(s). This has to be validated in further years, since PVY incidence in 2001 and 2002 was generally low.

Despite being of limited importance, when compared to the "va" gene, resistance factors carried by Burley 21 and possibly by Habana 92 may be of practical interest. Gathering these factors in the same line may lead to improved, and more durable, PVY resistance. This approach has been successfully applied, for example in pepper breeding (28). Burley 21 has been used in different breeding programs. It is then possible that some burley lines already gather resistance factors from Burley 21 and "va". This cannot be verified in the absence of reliable method to reveal the different genes.

The screening effort to highlight Habana 92 has not been comprehensive. A more extended screening of tobacco germplasm according to reactions towards PVY and other non-persistent, aphid transmitted viruses, may reveal new resistance factors. Ideally it should be based on PVY strains from the main areas for PVY incidence as above defined, for this would ensure a better applicability of the results. This type of work could begin in laboratory, then continue in field for validation within the framework of the CORESTA experiment.

Is the "va" resistance about to be overcome?

By reviewing a number of pathosystems involving plant viruses, it was possible to assess the risk of host resistances to be overcome on a scale from 1 to 9, taking into consideration the evolutionary potential of the virus in each particular case (15). PVY in tobacco has been rated at 7 on this scale, which means a high risk. Other laboratory results show that only one amino acid change in the VPg protein of the virus may lead to by- passing the VAM resistance (22, 25).

This synthesis highlights the widespread presence of va-breaking strains. It indicates also that in Europe these strains would have evolved to overcome the most frequent "va" allele with which they are in contact. These observations are fitting with the high rating of the risk for "va" to be overcome.

Data from China (Shandong province) are consistent with breaking of the "va" resistance in this particular case. It would be of interest to study the epidemiological conditions in this area, since if they were not specific, there would be no reason for this situation to remain isolated.

The period of time of the CORESTA experiment is small regarding the pace of evolution of viruses. When considering the high year to year variability of PVY incidence, it is therefore not surprising that no time trend, in particular regarding the frequency of va breaking strains, has been revealed.

Evolutionary cost of bypassing "va" resistance is a key factor to better assess the risk. Thermal optimum studies and comparison of fitness of breaking and non-breaking strains should be undertaken to get a better idea of the possible future changes.

Conclusion.

The CORESTA PVY experiment shows that the "va" resistance, which is the only one widely used in tobacco cultivars, is overcome in some situations. However, it remains still useful in most of the tobacco growing countries. Due to high year to year variations, no trend towards an aggravation of this situation could be shown, but the risk of such evolution is high.

Detailed results from different line reactions show that other resistance factors to PVY exist and have some impact on the necrotic symptom frequencies in field. Therefore, an interesting strategy to increase genetic control of PVY would be to associate these factors with the "va" resistance. This would require biotech work to better characterise these resistance factors.

Global results of the CORESTA PVY experiment also suggest that it could be used as a validation step for confirming the interest of putative resistance factors to PVY, different from "va". To enlarge the scope of the experiment to other viruses affecting tobacco will also help to understand better the main epidemiological factors acting in the distribution of each of these viruses.

Table IV. Incidence of PVY and estimated shares of va breaking strains.

Area	Country	% PVY incidence					% share of vaB strains within PVY^N			
		mean	min	max	nb trials	Nb years	mean	min	max	nb trials
Europe (N)	Hungary	84	19	100	7	7	9	3	15	7
Europe (N)	Poland	79	24	100	14	7	1	0	8	14
Europe (N)	Switzerland	78	31	100	7	7	1	0	3	7
Europe (N)	Germany	64	25	100	7	7	7	0	22	7
Europe (N)	Belgium	32	1	68	6	6	8	0	30	5
Europe (N)	France	26	0	74	13	7	3	0	10	9
Europe (S)	Portugal	52	5	99	2	1	35	19	52	2
Europe (S)	Croatia	40	3	73	3	3	8	0	24	3
Europe (S)	Spain	40	0	96	7	6	2	0	6	6
Europe (S)	Italy	33	4	98	10	7	22	0	100	9
Europe (S)	Macedonia	27	0	66	6	6	37	0	100	3
Europe (S)	Bulgaria	27	27	27	1	1	0	0	0	1
Europe (S)	Greece	18	1	74	6	6	48	0	100	6
Asia (E)	China	67	44	90	6	5	56	0	91	6
Asia (E)	South Korea	53	15	87	7	7	0	0	0	7
Asia (E)	Japan	14	1	25	6	6	0	0	3	6
Asia	Iran	12	0	35	11	6	48	0	100	10
Asia	Indonesia	4	4	4	1	1	-	-	-	-
Asia	India	2	0	3	2	2	62	62	62	1
Asia	Turkey	0	0	0	3	3	-	-	-	-
Africa (S)	Zimbabwe	53	16	96	5	5	15	5	25	5
Africa (S)	RSA	20	7	34	2	2	12	5	19	2
Africa	Morocco	1	0	6	6	6	100	100	100	1
Africa	Madagascar	0	0	0	1	1	-	-	-	-
America (S)	Brazil	96	93	100	4	4	1	0	2	3
America (S)	Colombia	24	0	42	7	7	66	11	100	6
America (N)	USA	0	0	0	1	1	-	-	-	-

Table Va. Frequency of necrotic and mosaic PVY symptoms, Europe: Belgium, France, and Germany.

Ctry	Loc.	Year	Some PVY necrotic symptoms, %													Only PVY mosaic symptoms, %													(1) P(vaB)	(2) RB (%)	(3) PVY incidence (%)
			VAM	TN 86	PBD6	VD	WIS	ZAM	BEY 21	HAB92	NCTG52	MN944	NC 95	K326	K17	VAM	TN 86	PBD6	VD	WIS	ZAM	BEY 21	HAB92	NCTG52	MN944	NC 95	K326	K17			
Belg	Beit	2002	0	5	0	7	0	-	31	52	-	-	72	51	11	0	0	2	0	2	-	0	0	-	-	0	0	0	0,05	5	43
		2001	0	0	0	0	-	0	4	0	-	-	0	0	1	0	0	0	0	-	0	0	0	-	-	0	0	0	-	-	1
		2000	0	0	0	1	-	-	53	-	71	78	59	81	-	0	0	0	0	-	-	0	-	0	0	0	0	-	0,00	0	68
		1997	1	1	1	1	-	-	4	-	33	10	90	24	-	31	0	5	1	-	-	4	-	0	30	0	10	-	0,03	4	41
		1996	0	0	-	0	-	-	0	-	0	0	0	-	-	0	0	-	0	-	-	0	-	1	1	6	-	-	0,00	-	2
	Roes	1999	0	3	13	29	-	-	25	-	32	24	65	25	-	0	0	1	0	-	-	5	-	0	4	0	11	-	0,29	32	38
Fran	Berg	2002	0	0	0	0	0	-	0	0	-	-	0	0	0	15	17	17	18	8	-	27	8	-	-	15	25	25	0,00	-	20
		2001	0	0	0	0	-	0	0	0	-	-	0	0	0	0	0	0	0	-	0	7	0	-	-	0	3	1	0,00	-	2
		2000	0	1	0	0	-	-	3	-	11	16	7	8	-	1	0	1	0	-	-	14	-	0	0	0	0	-	0,03	4	12
		1999	0	1	1	0	-	-	4	-	9	11	3	6	-	0	1	1	0	-	-	4	-	3	4	1	0	-	0,07	10	9
		1998	0	0	0	0	-	-	6	-	1	3	0	1	-	0	0	3	0	-	-	1	-	8	3	6	10	-	0,00	0	8
		1997	0	0	0	0	-	-	0	-	0	0	0	0	-	0	0	0	0	-	-	0	-	0	0	0	0	-	-	-	0
		1996	0	0	-	0	-	-	0	-	0	0	0	-	-	0	0	-	0	-	-	2	-	4	2	1	-	-	0,00	-	2
	Lime	2001	0	0	3	10	-	0	50	64	-	-	68	47	61	0	0	0	0	-	0	7	0	-	-	2	13	2	0,04	4	63
		2000	0	0	0	1	-	-	18	-	20	23	21	23	-	0	0	0	0	-	-	3	-	0	1	0	1	-	0,01	1	22
		1999	0	0	0	2	-	-	25	-	23	29	33	34	-	0	0	0	5	-	-	4	-	0	0	0	3	-	0,01	2	30
		1997	8	3	3	8	-	-	93	-	77	58	69	-	-	0	0	0	0	-	-	0	-	0	0	0	-	-	0,07	7	74
		1996	0	0	-	0	-	-	16	-	34	17	20	-	-	0	0	-	0	-	-	1	-	5	5	3	-	-	0,00	0	25
	Scha	1998	0	0	3	3	-	-	60	-	83	76	72	78	-	0	0	0	0	-	-	0	-	0	0	0	0	-	0,02	2	74
Germ	Forc	2002	8	3	12	23	13	-	100	100	-	-	100	82	100	0	0	2	0	0	-	0	0	-	-	0	0	0	0,12	12	96
		2001	0	0	0	0	-	0	24	47	-	-	36	38	47	0	0	0	0	-	0	15	9	-	-	18	28	17	0,00	0	56
		2000	11	0	14	0	-	-	100	-	100	100	100	100	-	0	4	0	0	-	-	0	-	0	0	0	0	-	0,06	6	100
		1999	0	0	0	0	-	-	53	-	32	47	30	38	-	0	0	0	0	-	-	0	-	0	0	0	0	-	0,00	0	40
		1998	0	0	6	3	-	-	17	-	46	16	38	14	-	9	15	0	3	-	-	22	-	0	0	3	3	-	0,06	8	32
		1997	0	0	0	0	-	-	0	-	100	100	100	100	-	100	100	100	100	-	-	100	-	0	0	0	0	-	0,00	0	100
		1996	0	0	-	17	-	-	4	-	33	33	25	-	-	0	0	-	0	-	-	4	-	0	0	0	-	-	0,22	23	25

(1) P(vaB) : estimated frequency of va breaking strains; (2) RB : % of *va* breaking strains within PVY^N strains.

(3) : mean frequency (%) of all PVY symptoms on *VaVa* lines Bey21, NCTG52, Hab92, MN944, NC95, K326, Ky17.

Table_Vb. Frequency of necrotic and mosaic PVY symptoms, Europe : Hungary, Poland, Switzerland

Ctry	Loc.	Year	Some PVY necrotic symptoms, %													Only PVY mosaic symptoms, %													(1) P(vaB)	(2) RB (%)	(3) PVY incidence (%)
			VAM	TN 86	PBD6	VD	WIS	ZAM	BEY 21	HAB92	NCTG52	MN944	NC 95	K326	K17	VAM	TN 86	PBD6	VD	WIS	ZAM	BEY 21	HAB92	NCTG52	MN944	NC 95	K326	K17			
Hung	Debr	2002	1	1	8	11	11	-	90	65	-	-	75	83	83	0	0	1	0	0	-	0	0	-	-	0	0	0	0,08	8	79
		2001	0	1	9	23	-	0	100	75	-	-	98	100	0	0	1	0	2	-	99	0	0	-	-	0	0	96	0,07	9	94
		2000	0	2	4	3	-	-	20	-	17	21	17	17	-	0	0	0	0	-	-	0	-	1	2	1	1	-	0,12	13	19
		1999	3	7	4	17	-	-	100	-	100	100	100	100	-	0	0	0	0	-	-	0	-	0	0	0	0	-	0,08	8	100
		1998	0	3	21	35	-	-	100	-	100	89	100	100	-	0	0	0	0	-	-	0	-	0	0	0	0	-	0,15	15	98
		1997	0	3	26	40	-	-	100	-	100	100	100	100	-	0	0	0	0	-	-	0	-	0	0	0	0	-	0,17	17	100
		1996	0	2	-	6	-	-	100	-	100	100	100	-	-	0	0	-	0	-	-	0	-	0	0	0	-	-	0,03	3	100
Pola	Jedr	2002	0	0	5	15	13	-	77	85	-	-	98	88	65	0	0	0	0	0	-	0	8	-	-	0	0	2	0,08	8	85
	Kazi	1996	0	0	-	0	-	-	45	-	52	100	57	-	-	0	0	-	0	-	-	0	-	0	0	0	-	-	0,00	0	64
		1999	0	0	0	0	-	-	32	-	99	94	92	72	-	0	0	0	0	-	-	68	-	1	6	8	28	-	0,00	0	100
		1997	0	0	0	0	-	-	100	-	100	100	100	100	-	0	0	0	0	-	-	0	-	0	0	0	0	-	0,00	0	100
	Krak	2001	0	0	0	0	-	0	48	46	-	-	49	56	51	0	0	0	0	-	0	0	0	-	-	0	0	0	0,00	0	50
		2000	0	0	0	0	-	-	1	-	15	13	63	19	-	0	0	0	0	-	-	4	-	0	0	0	6	-	0,00	0	24
		1998	0	0	0	0	-	-	100	-	100	100	100	100	-	0	0	0	0	-	-	0	-	0	0	0	0	-	0,00	0	100
	Pula	2002	0	0	0	4	0	-	84	78	-	-	93	90	94	0	0	0	0	0	-	9	10	-	-	4	6	1	0,01	1	94
		2001	0	0	0	0	-	0	75	35	-	-	61	69	59	0	0	0	0	-	10	0	0	-	-	0	5	7	0,00	0	62
		2000	0	0	0	0	-	-	49	-	57	51	58	60	-	0	0	0	0	-	-	19	-	21	17	18	17	-	0,00	0	73
		1999	0	0	0	0	-	-	48	-	54	62	55	69	-	0	0	0	0	-	-	14	-	27	22	15	16	-	0,00	0	76
		1998	0	0	0	0	-	-	58	-	60	67	66	78	-	0	0	0	0	-	-	26	-	28	17	17	14	-	0,00	0	86
		1997	0	0	0	0	-	-	84	-	88	88	89	88	-	0	0	0	0	-	-	16	-	13	12	11	13	-	0,00	0	100
		1996	0	0	-	0	-	-	100	-	100	94	91	-	-	12	88	-	0	-	-	0	-	0	2	1	-	-	0,00	0	97
Swit	Paye	2002	2	0	0	0	0	-	53	38	-	-	44	44	51	0	0	0	0	0	-	13	2	-	-	24	24	20	0,01	1	63
		2001	0	0	0	5	-	0	28	20	-	-	35	29	39	0	0	0	0	-	5	5	0	-	-	0	0	0	0,03	3	31
		2000	0	0	0	2	-	-	61	-	80	93	86	89	-	0	0	0	0	-	-	32	-	0	0	2	2	-	0,01	1	89
		1999	0	0	5	3	-	-	98	-	100	98	100	100	-	0	0	0	0	-	-	2	-	0	2	0	0	-	0,02	2	100
		1998	0	0	3	3	-	-	93	-	100	100	100	100	-	0	0	0	0	-	-	0	-	0	0	0	0	-	0,01	1	99
		1997	0	0	0	0	-	-	37	-	95	87	96	99	-	0	0	0	0	-	-	5	-	5	13	4	1	-	0,00	0	88
		1996	0	0	-	0	-	-	31	-	71	56	93	-	-	0	0	-	0	-	-	21	-	9	9	1	-	-	0,00	0	73

(1) P(vaB) : estimated frequency of va breaking strains; (2) RB : % of *va* breaking strains within PVY^N strains.

(3) : mean frequency (%) of all PVY symptoms on *VaVa* lines Bey21, NCTG52, Hab92, MN944, NC95, K326, Ky17.

Table Vc. Frequency of necrotic and mosaic PVY symptoms, Europe : Bulgaria, Croatia, Greece, Italy, Portugal, Spain

Ctry	Loc.	Year	Some PVY necrotic symptoms, %													Only PVY mosaic symptoms, %													(1) P(vaB)	(2) RB (%)	(3) PVY incidence (%)
			VAM	TN 86	PBD6	VD	WIS	ZAM	BEY 21	HAB92	NCTG52	MN944	NC 95	K326	K17	VAM	TN 86	PBD6	VD	WIS	ZAM	BEY 21	HAB92	NCTG52	MN944	NC 95	K326	K17			
Bulg	Plov	1996	0	0	-	0	-	-	37	-	12	21	28	-	-	2	0	-	0	-	-	6	-	4	0	0	-	-	0,00	0	27
Croa	Pito	1996	0	0	-	33	-	-	51	-	21	52	45	-	-	0	0	-	0	-	-	10	-	0	0	0	-	-	0,25	26	45
	Plan	1998	0	0	0	0	-	-	0	-	5	4	0	6	-	0	0	0	0	-	-	0	-	0	0	0	0	-	0,00	0	3
	Zagr	1997	0	0	0	3	-	-	84	-	74	63	62	74	-	0	0	0	0	-	-	0	-	0	8	0	0	-	0,01	1	73
Gree	Dram	2001	0	0	0	0	-	0	2	0	-	-	2	0	0	0	0	0	0	-	4	29	0	-	-	0	0	37	0,00	-	14
		2000	1	0	19	1	-	-	13	-	13	12	0	20	-	0	0	0	0	-	-	1	-	0	1	0	1	-	0,43	46	12
		1999	0	28	1	6	-	-	6	-	0	0	0	0	-	0	4	4	4	-	-	0	-	0	3	1	0	-	4,34	100	2
		1998	0	0	1	0	-	-	0	-	0	0	0	0	-	0	12	0	0	-	-	6	-	0	0	0	0	-	0,28	-	1
		1997	1	0	3	1	-	-	4	-	4	4	1	1	-	1	1	0	0	-	-	3	-	0	0	0	1	-	0,36	46	4
		1996	0	-	-	0	-	-	81	-	66	65	77	-	-	0	-	-	0	-	-	8	-	0	1	0	-	-	0,00	0	74
Ital	Bosc	1998	0	0	0	0	-	-	13	-	9	5	11	12	-	6	1	1	1	-	-	8	-	7	3	0	3	-	0,00	0	14
	Sant	1998	0	0	1	1	-	-	-	-	0	3	0	7	-	0	3	3	0	-	-	-	-	0	4	2	2	-	0,15	27	4
	Scaf	2002	0	0	0	0	0	-	0	0	-	-	0	0	0	0	0	0	0	0	-	23	10	-	-	0	10	9	0,00	-	10
		2001	0	0	0	0	-	0	1	0	-	-	8	0	8	0	0	0	0	-	0	10	12	-	-	0	0	11	0,00	0	10
		2000	6	8	5	5	-	-	8	-	8	11	13	10	-	23	29	30	16	-	-	29	-	26	38	35	34	-	0,14	61	42
		1999	4	5	10	18	-	-	6	-	9	6	8	13	-	33	36	46	36	-	-	31	-	23	35	34	23	-	0,24	100	38
		1998	0	0	0	0	-	-	21	-	9	16	19	27	-	16	11	14	10	-	-	26	-	18	26	21	14	-	0,00	0	39
	Vero	1998	2	0	2	0	-	-	27	-	13	29	26	25	-	0	0	0	0	-	-	0	-	0	0	0	0	-	0,05	5	24
		1997	2	0	3	1	-	-	100	-	91	100	100	100	-	0	13	0	0	-	-	0	-	0	0	0	0	-	0,02	2	98
		1996	1	4	-	1	-	-	47	-	24	32	41	-	-	21	17	-	24	-	-	20	-	27	13	5	-	-	0,04	6	52
Port	Cant	1996	84	76	-	98	-	-	89	-	100	100	100	-	-	6	23	-	3	-	-	9	-	0	0	0	-	-	0,86	88	99
	Lado	1996	0	1	-	1	-	-	3	-	5	3	8	-	-	0	0	-	4	-	-	3	-	0	0	0	-	-	0,17	19	5
Spai	Cace	2001	0	0	0	0	-	0	0	0	-	-	2	8	0	0	2	5	4	-	2	8	0	-	-	5	2	7	0,00	0	6
		1998	0	0	1	0	-	-	4	-	5	4	9	6	-	0	5	0	0	-	-	9	-	4	1	0	0	-	0,04	6	9
	Sevi	1997	0	0	0	0	-	-	12	-	7	8	5	4	-	78	60	46	29	-	-	69	-	57	55	59	62	-	0,00	0	68
		1996	0	0	-	0	-	-	0	-	3	0	0	-	-	91	93	-	88	-	-	96	-	97	94	94	-	-	0,00	-	96
	Tala	2002	0	0	0	0	0	-	0	0	-	-	34	10	0	0	2	3	12	4	-	24	6	-	-	2	9	36	0,00	0	24
		2000	0	0	0	0	-	-	0	-	0	0	0	0	-	0	0	0	0	-	-	0	-	0	0	0	0	-	-	-	0
	Vill	1996	1	0	-	0	-	-	8	-	10	9	20	-	-	10	15	-	54	-	-	54	-	75	73	54	-	-	0,01	4	76
Mace	Pril	2002	0	0	0	0	0	-	20	27	-	-	30	23	37	0	0	0	0	0	-	23	17	-	-	20	0	20	0,00	0	43
		2001	0	0	0	0	-	0	0	0	-	-	0	0	0	0	0	0	20	-	13	0	10	-	-	0	0	38	0,00	-	10
		2000	0	23	0	0	-	-	0	-	5	0	0	20	-	5	0	5	0	-	-	11	-	26	8	13	0	-	0,34	100	16
		1998	0	0	0	0	-	-	0	-	0	0	0	0	-	0	0	0	0	-	-	0	-	0	0	0	0	-	-	-	0
		1997	0	14	0	0	-	-	14	-	8	30	20	75	-	14	14	3	0	-	-	61	-	39	55	19	8	-	0,05	12	66
		1996	0	0	-	0	-	-	0	-	0	0	0	-	-	0	0	-	0	-	-	20	-	30	35	25	-	-	0,00	-	28

Table Vd. Frequency of necrotic and mosaic PVY symptoms, Asia : China, Japan, South Korea, India, Indonesia, Iran

Ctry	Loc.	Year	Some PVY necrotic symptoms, %													Only PVY mosaic symptoms, %													(1) PvaB	(2) RB (%)	(3) PVY incidence (%)
			VAM	TN 86	PBD6	VD	WIS	ZAM	BEY 21	HAB92	NCTG52	MN944	NC 95	K326	K17	VAM	TN 86	PBD6	VD	WIS	ZAM	BEY 21	HAB92	NCTG52	MN944	NC 95	K326	K17			
Chin	Chan	1996	15	0	-	0	-	-	0	-	78	90	40	-	-	56	60	-	78	-	-	80	-	8	0	65	-	-	0,06	10	90
Chin	Qing	2001	8	5	29	13	-	26	27	14	-	-	13	13	32	38	65	61	41	-	49	38	54	-	-	36	23	48	0,27	82	60
Chin	Qing	2000	16	39	68	31	-	-	46	-	41	64	52	26	-	12	38	19	29	-	-	36	-	25	13	31	26	-	0,53	84	72
Chin	Qing	1999	42	47	20	29	-	-	45	-	30	41	53	36	-	10	4	7	1	-	-	6	-	5	1	0	1	-	0,79	84	44
Chin	Qing	1998	55	46	45	31	-	-	46	-	56	47	35	49	-	14	27	15	21	-	-	20	-	16	12	8	3	-	0,76	95	58
Chin	Yina	1996	0	0	-	0	-	-	0	-	89	0	13	-	-	51	56	-	79	-	-	61	-	0	85	74	-	-	0,00	0	80
Japa	Oyam	2002	0	0	0	0	0	-	16	6	-	-	4	9	14	0	0	0	0	0	-	0	0	-	-	0	0	0	0,00	0	10
Japa	Oyam	2001	0	0	0	0	-	0	26	10	-	-	24	16	29	0	0	0	0	-	0	0	0	-	-	0	0	0	0,00	0	21
Japa	Oyam	1999	0	0	0	0	-	-	22	-	29	24	17	32	-	0	0	0	0	-	-	0	-	0	0	0	0	-	0,00	0	25
Japa	Oyam	1998	0	0	0	0	-	-	0	-	0	0	1	3	-	0	0	0	0	-	-	0	-	0	0	0	0	-	-	-	1
Japa	Oyam	1997	0	0	-	0	-	-	5	-	8	20	9	-	-	0	0	-	0	-	-	7	-	0	0	0	-	-	0,00	0	13
Japa	Oyam	1996	0	1	-	0	-	-	16	-	18	14	9	-	-	0	0	-	0	-	-	0	-	0	0	0	-	-	0,03	3	14
Skor	Chnb	2002	0	0	0	0	0	-	15	8	-	-	14	20	20	0	0	0	0	0	-	0	0	-	-	0	0	0	0,00	0	15
Skor	Chnb	2001	0	0	0	0	-	0	47	15	-	-	47	47	59	0	0	0	0	-	41	0	0	-	-	0	0	0	0,00	0	43
Skor	Chnj	2000	0	0	0	0	-	-	86	-	85	82	85	96	-	0	0	0	0	-	-	0	-	0	0	0	1	-	0,00	0	87
Skor	Chnj	1999	0	0	0	0	-	-	61	-	68	63	65	70	-	0	0	0	0	-	-	0	-	5	1	1	0	-	0,00	0	67
Skor	Chnj	1998	0	0	0	0	-	-	33	-	29	34	31	36	-	0	0	0	0	-	-	3	-	0	0	0	0	-	0,00	0	33
Skor	Chnj	1997	0	0	0	0	-	-	59	-	64	66	63	70	-	0	0	0	0	-	-	3	-	0	0	0	0	-	0,00	0	65
Skor	Chnj	1996	0	0	-	0	-	-	71	-	56	47	67	-	-	0	0	-	0	-	-	0	-	1	1	0	-	-	0,00	0	61
Indi	Jedd	1999	0	0	2	2	-	-	5	-	0	2	0	0	-	2	0	3	15	-	-	0	-	2	0	2	5	-	0,28	63	3
Indi	Raja	1998	0	0	0	0	-	-	0	-	0	0	0	0	-	0	0	0	0	-	-	0	-	0	0	0	0	-	-	-	0
Indo	Bond	1999	0	0	0	0	-	-	-	-	0	0	0	0	-	13	11	10	11	-	-	-	-	3	5	5	4	-	0,00	-	4
Iran	Behs	2000	3	1	0	0	-	-	3	-	3	0	5	5	-	6	0	0	0	-	-	9	-	3	1	1	0	-	0,16	31	6
Iran	Guil	2000	0	0	1	1	-	-	0	-	1	4	3	4	-	0	3	0	4	-	-	8	-	1	5	9	3	-	0,09	28	7
Iran	Rash	2002	0	0	0	0	0	-	0	0	-	-	3	12	0	15	43	17	32	25	-	27	8	-	-	18	25	42	0,00	0	27
Iran	Rash	2001	0	5	0	3	-	3	0	4	-	-	1	0	1	38	36	2	14	-	34	10	19	-	-	18	27	16	0,12	100	19
Iran	Rash	1999	1	1	0	0	-	-	0	-	0	0	0	1	-	6	7	1	1	-	-	10	-	5	5	2	5	-	0,07	100	6
Iran	Rash	1998	1	0	0	0	-	-	1	-	0	0	1	0	-	28	11	0	8	-	-	2	-	0	3	3	4	-	0,10	66	3
Iran	Rash	1997	0	0	0	0	-	-	5	-	9	10	22	15	-	6	6	0	9	-	-	14	-	1	5	0	4	-	0,00	0	17
Iran	Tirt	2002	0	0	0	0	0	-	0	17	-	-	0	0	0	0	0	0	0	0	-	0	0	-	-	5	0	0	0,00	0	4
Iran	Tirt	2001	0	0	0	0	-	0	0	0	-	-	0	0	0	0	0	0	0	-	0	0	0	-	-	0	0	0	-	-	0
Iran	Tirt	1999	8	13	9	43	-	-	20	-	38	54	16	36	-	1	0	0	0	-	-	10	-	1	0	0	0	-	0,51	54	35
Iran	Tirt	1997	9	3	3	0	-	-	3	-	1	0	1	5	-	1	1	4	0	-	-	13	-	6	4	4	15	-	0,34	100	10

Table V e. Frequency of necrotic and mosaic PVY symptoms, Africa, South America, and Asia :Turkey

Ctry	Loc.	Year	Some PVY necrotic symptoms, %													Only PVY mosaic symptoms, %													(1) P(vaB)	(2) RB (%)	(3) PVY incidence (%)
			VAM	TN 86	PBD6	VD	WIS	ZAM	BEY 21	HAB92	NCTG52	MN944	NC 95	K326	K17	VAM	TN 86	PBD6	VD	WIS	ZAM	BEY 21	HAB92	NCTG52	MN944	NC 95	K326	K17			
Turk	Adap	2002	0	0	0	0	0	-	0	0	-	-	0	0	0	0	0	0	0	0	-	0	0	-	-	0	0	0	-	-	0
Turk	Adap	2001	0	0	0	0	-	0	0	0	-	-	0	0	0	0	0	0	0	-	0	0	0	-	-	0	0	0	-	-	0
Turk	Adap	1998	0	0	0	0	-	-	0	-	0	0	0	0	-	0	0	0	0	-	-	0	-	0	0	0	0	-	-	-	0
RSAf	Rust	2001	-	0	0	3	-	-	-	-	-	-	15	25	-	-	0	10	0	-	-	-	-	-	-	5	23	-	0,03	5	34
RSAf	Rust	1998	2	0	1	1	-	-	1	-	8	3	13	3	-	1	1	0	0	-	-	1	-	0	2	0	1	-	0,17	19	7
Zimb	Hara	2002	1	19	23	3	-	-	46	44	-	-	32	40	49	6	23	9	62	-	-	44	22	-	-	16	43	29	0,16	27	73
Zimb	Hara	2001	0	1	4	0	-	-	5	-	-	-	31	8	-	1	0	19	10	-	-	6	-	-	-	21	18	-	0,04	9	30
Zimb	Hara	2000	0	0	3	0	-	-	1	-	14	14	24	16	-	0	0	4	3	-	-	3	-	3	1	0	6	-	0,04	5	16
Zimb	Hara	1999	0	4	4	0	-	-	5	-	9	9	29	6	-	0	3	19	11	-	-	59	-	30	19	21	55	-	0,04	17	48
Zimb	Hara	1997	6	40	23	20	-	-	71	-	90	91	94	76	-	19	29	21	61	-	-	23	-	9	6	3	16	-	0,23	26	96
Mada	Mora	1998	0	0	0	0	-	-	0	-	0	0	0	0	-	0	0	0	0	-	-	0	-	0	0	0	0	-	-	-	0
Moro	Casa	2001	0	0	0	0	-	0	0	0	-	-	0	0	0	0	0	0	0	-	0	0	0	-	-	0	0	0	-	-	0
Moro	Ouez	2002	-	-	0	0	-	-	0	0	-	-	0	0	-	-	-	0	0	-	-	0	0	-	-	0	0	-	-	-	0
Moro	Ouez	2000	0	0	0	0	-	-	0	-	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Moro	Ouez	1999	0	0	0	0	-	-	0	-	0	0	0	0	-	0	0	0	0	-	-	0	-	0	0	0	0	-	-	-	0
Moro	Ouez	1998	0	0	0	0	-	-	0	-	0	0	0	0	-	0	0	0	0	-	-	0	-	0	0	0	0	-	-	-	0
Moro	Ouez	1997	6	5	0	0	-	-	3	-	0	1	4	6	-	0	1	0	0	-	-	0	-	0	9	4	4	-	0,47	100	6
Braz	Sant	2001	0	0	3	0	-	-	25	-	-	-	31	45	-	20	20	10	38	-	-	75	-	-	-	62	50	-	0,01	2	96
Braz	Sant	2000	0	0	0	0	-	-	0	-	0	0	0	0	-	5	3	8	18	-	-	88	-	100	92	97	88	-	0,00	-	93
Braz	Sant	1999	0	0	0	0	-	-	20	-	0	8	10	5	-	3	18	23	33	-	-	80	-	78	93	90	95	-	0,00	0	96
Braz	Sao	1998	0	0	0	0	-	-	65	-	53	46	56	44	-	16	29	43	47	-	-	35	-	47	54	44	56	-	0,00	0	100
Colo	Huil	1996	1	3	-	0	-	-	3	-	3	1	0	-	-	13	15	-	18	-	-	16	-	12	35	4	-	-	0,07	79	18
Colo	Mede	2001	0	0	0	0	-	0	0	0	-	-	0	0	0	0	0	0	0	-	0	0	0	-	-	0	0	0	-	-	0
Colo	Mede	1999	0	5	13	9	-	-	14	-	47	30	49	46	-	2	30	0	0	-	-	2	-	0	0	0	0	-	0,18	18	38
Colo	Ocan	1998	11	17	9	0	-	-	2	-	2	4	14	13	-	18	20	46	9	-	-	54	-	17	48	12	38	-	0,23	100	41
Colo	Ocan	1997	10	15	8	0	-	-	4	-	1	3	13	10	-	22	24	49	9	-	-	50	-	17	52	13	46	-	0,20	100	42
Colo	Palm	2000	-	1	-	-	-	-	-	-	16	4	8	17	-	-	4	-	-	-	-	-	-	13	10	5	12	-	0,06	12	21
Colo	Vill	2002	0	13	0	0	0	-	0	2	-	-	5	2	7	2	0	0	9	3	-	9	3	-	-	5	4	3	0,34	88	8
USA	Laur	1996	0	0	-	0	-	-	0	-	0	0	0	-	-	0	0	-	0	-	-	0	-	0	0	0	0	-	-	-	0

(1) P(vaB) : estimated frequency of va breaking strains; (2) RB : % of *va* breaking strains within PVY^N strains.

(3) : mean frequency (%) of all PVY symptoms on *VaVa* lines Bey21, NCTG52, Hab92, MN944, NC95, K326, Ky17

A N N E X 1.

List of country and location codes.

Area	c-code	country	l-code	location
AFR	Mada	Madagascar	Mora	Moramanga
	Moro	Morocco	Casa	Casablanca
			Ouez	Ouezzane
AFR_S	RSAf	Republic of South Africa	Rust	Rustenburg
	Zimb	Zimbabwe	Hara	Harare
			Kuts	Kutsaga
AM_S	Braz	Brazil	Sant	Santa Cruz Do Sul
			Sao	Sao Joao Do Triunfo
	Colo	Colombia	Huil	Huila
			Mede	Medellin
			Ocan	Ocana
			Palm	Palmira
			Vill	Villanueva
AM_N	USA	United States of America	Laur	Laurel Springs
ASIA	Indi	India	Jedd	Jeddangi
			Raja	Rajahmundry
	Indo	Indonesia	Bond	Bondowoso, East Jawa
	Iran	Iran	Behs	Behshahr
			Guil	Guilan
			Rash	Rasht
			Tirt	Tirtash
	Turk	Turkey	Adap	Adapazari
ASIA_E	Chin	Popular Republic of China	Chan	Changle
			Qing	Qingzhou, Shandong
			Yina	Yinan
	Japa	Japan	Oyam	Oyama, Tochigi
	Skor	South Korea	Chnb	Chonbuk
			Chnj	Chonju
EUR_N	Belg	Belgium	Beit	Beitem
			Roes	Roeselare
	Fran	Portugal	Berg	Bergerac
			Lime	Limersheim
			Scha	Schaeffersheim
	Germ	Germany	Forc	Forchheim
	Hung	Hungary	Debr	Debrecen-Pallag
	Pola	Poland	Jedr	Jedrzejow
			Kazi	Kazimierza Wielka
			Krak	Krakow
			Pula	Pulawy
	Swit	Switzerland	Paye	Payerne
EUR_S	Bulg	Bulgaria	Plov	Plovdiv
	Croa	Croatia	Pito	Pitonaca
			Plan	Planinska
			Zagr	Zagreb
	Gree	Greece	Dram	Drama
	Ital	Italy	Bosc	Bosco
			Sant	Santa Fista
			Scaf	Scafati
			Vero	Verona
	Mace	Macedonia	Pril	Prilep
	Port	Portugal	Cant	Cantanhede
			Lado	Ladoeiro
	Spai	Spain	Cace	Caceres
			Sevi	Sevilla
			Tala	Talayuela
			Vill	Villanueva de la vera

A N N E X 2.

List of participating organisations.

(At least one trial performed one year, from 1996 to2002)

Continent	Country	Name	Location
Africa	MADAGASCAR	Office Malgache des Tabacs	ANTANANARIVO
Africa	MOROCCO	Regie des Tabacs S.A.	CASABLANCA
Africa	SOUTH AFRICA	Institute for Industrial Crops	RUSTENBURG
Africa	ZIMBABWE	Tobacco Research Board	HARARE
Asia	CHINA	Inst. of Biotechnology, Shenyang Agric. Univ.	SHENYANG
Asia	CHINA	Tobacco Research Institute	Qingzhou, SHANDONG
Asia	INDIA	I.T.C.Limited – ILTD DIVISION	RAJAHMUNDRY
Asia	INDONESIA	PT DJARUM (PTHM Sampoerna)	SURABAYA
Asia	IRAN	Guilan tobacco research center	RASHT
Asia	IRAN	Iranien Tobacco Institute of Tirtash	BEHSHAHR
Asia	JAPAN	Japan Tobacco Inc.	OYAMA, TOCHIGI
Asia	KOREA	Korea Ginseng and Tobacco Res. Institute	CHONBUK
Asia	TURKEY	TEKEL(Turkish State Monopoly)	ISTANBUL
Europe	BELGIUM	Instituut Arthur Olivier – POVLT	RUMBEKE
Europe	BULGARIA	Tobacco and Tobacco Products Institute	PLOVDIV
Europe	CROATIA	Tobacco Institute	Pitonaca, ZAGREB
Europe	Portugal	ALSATABAC	STRASBOURG
Europe	Portugal	ALTADIS – Institut du Tabac	BERGERAC
Europe	GERMANY	L.F.P.Forchheim	FORCHHEIM
Europe	GREECE	Tobacco Institute of Greece	DRAMA
Europe	HUNGARY	AGROTAB Kft	DEBRECEN-PALLAG
Europe	ITALY	Institute of plant breeding, Univ. Of Perugia	PERUGIA
Europe	ITALY	Istituto Sperimentale per il Tabacco, Scafati	Scafati, NAPOLI
Europe	ITALY	Tabacchicoltori Associati Veneti Scarl	VERONE
Europe	MACEDONIA	Tobacco Institute	PRILEP
Europe	POLAND	Central Laboratory of Tobacco Industry	KRAKOW
Europe	POLAND	Inst. Of Soil Science and Plant Cultivation	PULAWY
Europe	Portugal	TABAQUEIRA	CACEM
Europe	SPAIN	CETARSA, experimental station	Talayuela, CACERES
Europe	SPAIN	Instituto Tecnologico del Tabaco	SEVILLA
Europe	SWITZERLAND	SOTA	PAYERNE
N. America	U.S.A.	North Carolina State University	RALEIGH
S. America	BRAZIL	PROFIGEN do Brazil	SANTA CRUZ DO SUL
S. America	COLOMBIA	Coltabaco S.A.	MEDELLIN

A N N E X 3.
About the lines grown in the CORESTA PVY experiment.

Lines possessing or supposed to possess a "va" allele :

Zamojska 4 is a flue-cured line, from Poland, with a good resistance to PVY pathotype 2 in greenhouse, mechanically inoculated tests (unpublished).
Wislica is a flue-cured line from Poland, with a good resistance to PVY pathotypes 0 and 2, but overcome by pathotypes 1 and 1-2. PVY resistance of this line would originate from oriental tobacco.

Virginia SCR (VD) is a flue-cured derived from mass selection inside the cultivar VN1 (8). VD has resistance to PVY pathotypes 0 and 2 but is overcome by 1 and 1-2. The recessive nature of VD resistance has been confirmed (2). The line PEREVI inherits its PVY resistance from VD. YAMAMOTO (40) showed the "low" resistance of PEREVI to be allelic to the "high" resistance found in VAM. NOGUCHI (27) presented RAPD data supporting that PEREVI has a specific deletion at the Va locus, different from VAM. This suggests that resistances found in VAM and in VD are allelic but not equivalent. VD has been the base for flue-cured production in France and Germany in the years 80.

VAM, or "Virgin A Mutant" was obtained (18) from mutagenesis with X-ray irradiation. VAM shows a high resistance level to TVMV, TEV, and PVY strains from pathotypes 0 and 2, but is overcome by 1 and 1-2. NOGUCHI suggests that VAM has a deletion at the "Va" locus (27). ACOSTA-LEAL and XION (1) have proposed the resistance of VAM to be due to "va", a cytoplasmic factor, and another gene called Rvam2. "va" would restrict PVY movement, which is in line with the work of NICOLAS (25). Rvam2 would reduce virus accumulation in tissues, and the cytoplasmic factor would decrease the frequency of appearance of resistance-breaking variants of the virus. Rvam2 and the cytoplasmic factor are not necessarily transmitted to offspring's coming from crosses between VAM and other materials. Trichomes of VAM are non-secreting. It seems this inability is linked to the deletion present at or around the Va locus in VAM, and that high susceptibility to *P. Tabacina* (Blue Mold) (26) and leaf feeding moths are consequences from this lack of secretions (32, 40).
According to NAKAKAWAJI (24), two independent loci are involved in the ability of trichomes to produce Duvatrienediol (DVT), and one of them, Te1, is linked with PVY resistance. VAM is recessive (te1te1 te2te2) for both of them and does not produce DVT.

TN86 is a burley line (23), which inherits its resistance to PVY from VAM. Like VAM, it has high resistance to TVMV, TEV, PVY pathotypes 0, and 2, but is overcome by PVY pathotypes 1 and 1-2. TN86 has secreting trichomes. This fits with the assumption that the recessive form "te2" homozygous in VAM and non-linked to PVY resistance has not been transmitted to TN86, which would be Te2Te2. This assumption is made here and has not been confirmed in the literature.

PBD6 is a dark air-cured line obtained (31) from the cross Paraguay P48 x Bel 61-10. It inherits its resistance to PVY from Paraguay P48, a cultivar widely used in France for dark air-cured tobacco production before the Blue Mold epidemic (years 70). This resistance has been shown (2), using F1 crosses, to be recessive and allelic to the resistance found in VD and VAM. PBD6 has resistance to PVY from pathotypes 0 and 2 but is overcome by 1-2.

Lines susceptible to PVY, supposed to be VaVa:

Habana 92 is a dark air-cured line from Cuban origin. It has shown susceptibility to PVY from the pathotype 2 in greenhouse tests performed at Altadis – Institut du tabac, but also a specific pattern when challenged with other PVY strains or with CMV-DTL.

Kentucky 17: this burley line (9) is susceptible at least to PVY pathotypes 2, 1-2 and CMV, and expresses a high level of symptom intensity when challenged with these viruses.

Burley 21: this burley line (17) is susceptible to pathotypes 2 and 1-2 strains of PVY, but shows some resistance to pathotype 0 and pathotype 1 (= VAM-B) strains, from North America. ARCIA-MONTEZUMA (3), using the PVY North American strain "2", studied the mode of inheritance of this resistance and compared it with the one acting in VAM. For Burley 21, a model with two dominant genes was proposed, whereas for VAM, results showed involvement of a single recessive factor.

K326 is a flue-cured line derived from McNair 225 x (McNair 30 x NC 95). It is known for its high quality and is one of the most used flue-cured, but is susceptible to PVY pathotypes 1, 2 and 1-2.

NC95 is a flue-cured line derived from (C-139 x Bel. 4-30) x (C139 x Hicks), and has resistance to the nematode *Meloidogyne incognita* races 1 and 3. It has been proposed that the Rk gene responsible for this is also responsible for high susceptibility to the MN strain of PVY (30).

REFERENCES.

1. ACOSTA-LEAL, R., McDANIEL, R., XIONG, Z. – 1998 – Resistance of tobacco Virgin A Mutant (VAM) to potyvirus accumulation and systemic translocation is conditioned by two independent genes. *Phytopathology*, 89, 8
2. ANO, G., BLANCARD, D. and CAILLETEAU, B. – 1995 – Mise au point sur la résistance récessive aux souches nécrotiques du virus Y de la pomme de terre (PVY) présente chez *Nicotiana tabacum***.** *Annales du Tabac, Seita,* 2, 27, 35-42.
3. ARCIA-MONTEZUMA, M.A. Sources of resistance in tobacco to potato virus Y and their mode of inheritance. Thesis, North Carolina State University, Raleigh, USA, 1979, 75 pp (Ja : 8108).
4. BLANCARD D., ANO G., CAILLETEAU B. – 1995 – Etude du pouvoir pathogène d'isolats de PVY sur Tabac : proposition d'une classification intégrant la résistance à la nécrose. *Annales du Tabac, Seita,* 2, 27, 43-50.
5. BLANCARD D., R.DELON, B. BLAIR AND T.GLOVER, 1999. Chapter 6. B . Virus diseases *in* ***Tobacco Production, Chemistry and technology***, edit. D.L.Davis and M.T.Nielsen, Blackwell science ISBN 0-632-04791-7 , pp 198 – 215, 1999.
6. BRACHET P.; PINASSEAU F.; CAILLETEAU B.; VERRIER J-L.2001.Preventative control of non persistent aphid transmitted tobacco virus in France. *CORESTA Meet. Agro-Phyto Groups*, 2001, P3, Cape Town, South Africa.
7. BRANDLE, J.E., 1995. Resistance to a Necrotic Strain of Potato Virus Y Among *Nicotiana* Species, Somatic Hybrids, and Tobacco Cultivars. *Plant Disease*, 79-2, 152-154.
8. CARSTEN, H., SEEHOFER, F. – 1960 – How Virginia SCR is obtained and cultivated in the Federal Republic of Germany. *Coresta* 3 : 39-43.
9. COLLINS, G. B., LITTON, C. C., LEGG, P. D., SMILEY, J. H. –1978 – Registration of KY17 tobacco. *Crop Science*, 18, 694.
10. DELON R., FISHER C., BLANCARD D., 1993. Enquête sur les virus du tabac 1993. *Bull. d'Information du CORESTA*, 4 : 72-79.
11. DOROSZEWSKA T. 2002. Potential sources of resistance to three strains of potato virus Y in the genus Nicotiana.. *CORESTA Congress*, New Orleans, 2002, Agro-Phyto Groups, AP29.
12. DOROSZEWSKA T.; BERBEC A. 1999. Transfer of resistance to Potato Virus Y to tobacco cultivars. *CORESTA Meet. Agro-Phyto Groups/Réunion Groupes Agro-Phyto,* AP4, 1999, Suzhou.
13. DOROSZEWSKA T.; CHRZANOWSKA M. 2001. Characterization of the main PVY resistance sources to different PVY strains. *CORESTA Meet. Agro-Phyto Groups*, 2001, Cape Town, P5.
14. GARCIA-ARENAL, F., FRAILE, A., MALPICA, J. M., 2001. Variability and Genetic Structure of Plant Virus Populations. *Annu. Rev. Phytopathol*., 39:157-186.
15. GARCIA-ARENAL, F., McDONALD, B.A., 2003. An analysis of the Durability of Resistance to Plant Viruses. *Phytopathology*, 93, 8: 941-951.
16. GOODING G.V. – 1985 – Relationship between strains of potato virus Y and breeding for resistance, cross protection, and interference. *Tobacco Science*, 29, 99-104.
17. HEGGESTAD, H.E. – 1966 – Registration of BURLEY 1, BURLEY 2, BURLEY 11A, BURLEY 11B, BURLEY 21, BURLEY 37, and BURLEY 49 tobaccos. *Crop Science*, 6, 612-613.
18. KOELLE, G. – 1961 – Genetische analyse einer Y-virus (Rippenbraune) resistenten mutante der tobaksorte Virgin A. *Der Zuchter*. 31 : 71-72.
19. LAHOZ E.; IOVIENO P.; PORRONE F.; BIONDANI C.; CARELLA A.; CONTILLO R. 2002. Use of light mineral oil to reduce the severity of PVY and CMV infections on tobacco in Italy. *CORESTA Congress*, New Orleans, USA, 2002, Agro-Phyto Groups, abstr. APPOST16.
20. LE ROMANCER, M. 1995. Relations taxonomiques entre les différentes populations du virus Y de la pomme de terre basées sur les propriétés biologiques, sérologiques et génomiques. Définition de pathotypes chez les souches inféodées à Solanum tuberosum. *Annales du tabac*, 27, 2:7-16.

21. LEWIS R.S. 2002. Tissue culture and molecular marker-assisted introgression of PVY resistance from N. africana to N. tabacum. *CORESTA Congress*, Agro-Phyto Groups, abstr. AP28, New Orleans, USA, 2002.
22. MASUTA, C., NISHIMURA, N., MORISHITA, H., HATAYA, T. – 1999 – A single amino acid change in viral genome-associated protein of Potato Virus Y correlates with resistance breaking in « Virgin A Mutant » tobacco. *Phytopathology*, 89, 2, 118-123.
23. MILLER, R.D. –1987 – Registration of TN86 burley tobacco. *Crop Science*, 27, 365-366.
24. NAKAKAWAJI T. 1997. Genetic relationship between duvatrienediol content and resistance to potato virus Y strain :Tob. Chem. Res. Conf., 51, abstr. 15,:1997
25. NICOLAS, O., DUNNINGTON, S. W., GOTOW, L. F., PIRONE, T. P., HELLMAN, G. M. – 1997 – Variations in the VPg protein allow a potyvirus to overcome va gene resistance in tobacco. *Virology*, 237, 452-459.
26. NIELSEN M., 1991. Leaf surface compounds affecting germination of Peronospora tabacina sporangia. CORESTA Meet. Agro-Phyto Groups, Louisville, 1991.
27. NOGUCHI, S., TAJIMA, T., YAMAMOTO, Y., OHNO, T., KUBO, T. – 1999 – Deletion of a large genomic segment in tobacco varieties that are resistant to potato virus Y (PVY). *Mol. Gen. Genet.*, 262 : 822-829.
28. PALLOIX A., 1992. Diseases of pepper and perspectives for genetic control. In : P. Beletti et L. Quaglioni (ed.), *VIIIth Eucarpia meeting on genetics and breeding of capsicum and eggplant*, Rome (Italy), 7-10 September, 120-126.
29. REDDICK, B.B., MILLER, R.D., GOODING Jr, G.V., 1991. Resistance in Nicotiana tabacum cultivar TN86 to naturally occurring tobacco viruses. *Tob. Sci*. 35 : 96-99.
30. RUFTY, R. C., Powell, N.T., GOODING Jr., G.V., 1983. Relationship between resistance to *Meloidogyne incognita* and a necrotic response to infection by a strain of potato virus Y in tobacco. *Phytopathology*, 73, 10, 1418-1423.
31. SCHILTZ, P. - 1967 - Création de Nicotiana tabacum résistant à *Peronospora tabacina* Adam. *Thesis*, Bordeaux, p. 1-191.
32. SMEETON, B.W., 1976. Budworm susceptibility of PVY resistant cultivars. Tob. Sci. XX, 163.
33. SMITH-BECKER, J., KEEN, N.T., BECKER, J.O. 2003. Acibenzolar-S-methyl induces resistance to Colletotrichum lagenarium and cucumber mosaic virus in cantaloupe. *Crop Protection*, 22, 769-774.
34. MAKI-VALKAMA, T., VALKONEN, J.P.T., 1999. Pathogen derived resistance to Potato virus Y : mechanism and risks. *Agricultural and Food Science in Finland*, 8, 493-513.
35. VERRIER J.-L.; CAILLETEAU B.1997. Collaborative Study on PVY: 1996 results. *CORESTA Meet. Agro-Phyto Groups/Réunion Groupes Agro-Phyto*, AP11, Montreux, Switzerland, 1997.
36. VERRIER J.L.; MARCHAND V.; CAILLETEAU B.; DELON R., 2001. Chemical change and cigarette smoke mutagenicity increase associated with CMV-DTL and PVY-N infection in burley tobacco. *CORESTA Meet. Agro-Phyto Groups,* 2001, Cape Town, South Africa.
37. WERNSMAN E.A. 1990. Sources of resistance to virus diseases. *Bul.. Spéc. CORESTA* , 1990, Symposium Kallithea, p. 182, abstr P25.
38. WERNSMAN, E.A., and GOODING JR , G.V., 1991. Allelism tests among sources of resistance to tobacco etch virus and potato virus Y. *34th Tobacco Workers Conference*, Charleston, USA,1991.
39. WITHERSPOON W.D., WERNSMANN E.A., GOODING G.V., RUFTY R.C., 1991. Characterization of a gametoclonal variant controling virus resistance in tobacco. Theor. Appl. Genet., 81, 1-5.
40. YAMAMOTO, Y., 1992. Studies on Breeding of Tobacco Varieties Resistant to Veinal Necrosis Disease by Potato Virus Y strain T. Bulletin of the Leaf Tobacco Research Laboratory, no 2, March 1992, 78-85.
41. YUNG C.H.; WERNSMAN E.A.; GOODING G.V. 1991 Characterization of potato virus Y resistance from gametoclonal variation in flue-cured tobacco. *Phytopathology*, 1 991, 81-8, p. 887-91.