and IAA, inhibit explant growth in cultured cells. Such growth inhibition is evident also at 2–4 D low concentrations (Figure 1) which cannot be increased for the toxicity of synthetic product. IAA inhibition of weight is nearly linear with the time as long as concentration of applied ethylene is maintained constant: the growth inhibition by low concentrations is smaller than high ones as reported in Figure 2. The reversal effect of ethylene-auxin inhibition is related to kinetin/auxin ratio[11]. As shown in the Table, the Student t-test on fresh weight of callus fragments grown in the presence of constantly applied ethylene and different hormonal conditions of kinetin and auxin is not significantly different from controls grown in the same experimental conditions but in presence of air. On the contrary, a significant difference exists when callus fragments are grown in presence of constantly applied ethylene and auxins.

Student t-test applied on fresh weight of callus fragments of *Nicotiana tabacum* cells grown in different hormonal conditions of 2–4 D (0.1 and 1 mg/l) and kinetin (0.1 and 1 mg/l) after 15 days of culture.

Media	Air	Ethylene
–	0.01	0.01
0.1 2–4 D	0.1	0.05
1 2–4 D	0.1	0.05
0.1 K	0.2	0.1
1 K	0.1	0.05
0.1 2–4 D 0.1 K	0.3	0.2
0.1 2–4 D 1 K	0.7	0.5
1 2–4 D 0.1 K	0.8	0.7
1 2–4 D 1 K	0.5	0.3

Riassunto. Si è studiato l'effetto dell'etilene su culture di cellule di *Nicotiana tabacum*, in vitro. I risultati ottunuti mostrano che le auxine in presenza di etilene inibiscono la crescita di frammenti di callus, e che tale inibizione viene rimossa dalla kinetina.

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Isolation and Classification of Water Leptospira Strains: Identification of Three New Serotypes

The authors have isolated from stagnant waters of the Friuli zone (Italy) 5 leptospira strains which they have first purified and then serologically compared with the leptospira serotypes so far described in the literature.

Materials and methods. Isolation. The isolation of the strains has been obtained by seminating the collected water samples in Zuelzer's medium and filtering the positive cultures through Millipore membranes at a porosity of 0.22 $\mu\mu$[1]. The filtrate was either seeded in KORTHOF's medium, or on COX's solid medium, obtaining in this way leptospira colonies. The cultures in fluid medium have been trcated according to BABUDIERI[2] to avoid the possibility of mixed cultures. The isolation and the first passage of the strains have been effected at environmental temperature.

Classification. The classification of the strains has been done by preparing the immune sera in rabbits and testing them with the reference strains of all serotypes belonging to the complex 'biflexa', and then setting the specific immune sera for the last ones, against the isolated strains. A first screening has been effected with cross-agglutination tests. In the cases in which an affinity was recognized, we have had recourse to the agglutinines cross absorption test, according to the methods suggested by the OMS Expert Committee on leptospirae (1967)[3].

Results. The 5 isolated strains have been given the following names: Friuli 8, Friuli 35, Friuli 37, Friuli 44, Friuli 48. One of these, Friuli 48, was unable to grow at a temperature of 30 °C or more, optimal for leptospirae. But from this strain, Friuli 48, after some passages, we have obtained a mutant able to grow at 30 °C. In Table I only the reference strains of the serotypes of the 'biflexa complex' which gave some positive cross-agglutination with the strains under study are reported. In some instances (Friuli 8 and 35 and Doberdò 1 and RPE) the well-known phenomenon of the unilateral agglutination was observed. The strains which, by the agglutination test, showed an antigenical affinity, were checked through the cross agglutinin absorbtion test. The results are reported in Table II.

According to the results of the cross absorption tests, 2 of our strains (Friuli 8 and 35) belong to the serotype 'San Giusto'; the 3 other strains, on the contrary, are antigenically independent and can be considered reference strains of 3 new serotypes. One of these, however (Friuli 37), shows some affinity with the Bulgarian serotype 'Maritza' and can be considered as belonging to the same serogroup Maritza. In Table III systematic of our 5 strains is reported.

Conclusions. Of the 5 new strains, 2 have shown their appurtenance to already known serotypes, while for the others we have observed only feeble agglutinations scattered with some strains isolated from the zone of Trieste and from Bulgaria. The existence of strains antigenically related such as Friuli 37 and Bulgaria 16, isolated in geographically distant zones, confirms once again the antigenic cosmopolitanism of the leptospirae. The finding of water leptospirae that do not multiply at 30 °C but at lower temperatures is a fact to be borne in mind, both from a practical and a theoretical point of view; it is ad-

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Table I. Results of the cross-agglutination tests reported in percentage values as regards the titer of each serum with the homologous strain

Serotypes	Strains	Immune sera and homologous agglutination titres															
		Friuli 8 100.000	Friuli 35 100.000	Friuli 37 50.000	Friuli 44 50.000	Friuli 48 50.000	Basovizza 1.000	S. Giusto 50.000	Doberdò 1 50.000	RPE 100.000	Farneti 100.000	S. Paulo 50.000	Veldrat 25.000	M. Fiascone 500.000	AM 13 100.000	Bulgaria 4 50.000	Bulgaria 16 100.000
	Friuli 8	100	100	0	0	0	100	100	100	100	0	0	0	0	0	0	0
	Friuli 35	100	100	0	0	0	100	100	100	1	0	0	0	0	0	0	0
	Friuli 37	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	50
	Friuli 44	0	0	0	100	0	0	0	0	0	0	0	0	0.2	0	0	0
	Friuli 48	0	0	0	0	100	0	0	1	0	0.1	0	0.4	0	0	0	0
Basovizza	Basovizza	10	5	0	0	0											
San Giusto	San Giusto	100	100	0	0	0											
Doberdò	Doberdò 1	0	0	0	0	0											
Rupino	RPE	0	0	0	0	0											
Farneti	Farneti	0	0	0	0	0											
Sao Paulo	S. Paulo	0	0	1	0	0											
Semaranga	Veldrat S 173	0	0	0	0	0											
Monfiascone	M. Fiascone 2	0	0	0	0	0											
Tredici	AM 13	0.1	0.1	0	2	0											
Thrancia	Bulgaria 4	0	0	0	0	10											
Maritza	Bulgaria 16	0	0	20	0	0											

Table II. Results of the cross absorption tests expressed for each strain in percentage as regards the titer in the non-absorbed serum

Serum anti Friuli 8	post-absorption titer	
Strains	Abs. with San Giusto	Abs. with Friuli 35
Friuli 8	<1	<1
Friuli 35	<1	<1
S. Giusto	<1	<1
Serum anti Friuli 35		
Strains	post-absorbtion titer Abs. with Friuli 8	
Friuli 8	<1	
Friuli 35	<1	
S. Giusto	<1	
Serum anti S. Giusto		
Strains	post-absorbtion titer Abs. with Friuli 8	
Friuli 8	<1	
Friuli 35	<1	
S. Giusto	<1	
Serum anti Bulgaria 16		
Strains	post-absorbtion titer Abs. with Friuli 37	
Bulgaria 16	40	
Friuli 37	<1	
Serum anti Friuli 37		
Strains	post-absorbtion titer Abs. with Bulgaria 16	
Friuli 37	25	
Bulgaria 16	<1	
Serum anti Doberdò 1		
Strains	post-absorbtion titer Abs. with Doberdò 1	
Friuli 8	60	
Friuli 35	80	
Doberdò 1	<1	

visable, in fact, to maintain the filtered materials, and the first passages in culture obtained from them, at temperatures lower than 30 °C, during the isolation. It is also interesting to remark that serum anti Doberdò 1, absorbed with the homologous strain, is still able to agglutinate the strains Friuli 8 and 35.

Table III

Serogroup	Serotypes	Strains
Basovizza	San Giusto	Friuli 8
Basovizza	San Giusto	Friuli 35
Maritza	Valderio	Friuli 37
Orvenco	Orvenco	Friuli 44
Udine	Udine	Friuli 48

Résumé. 5 souches de léptospires saprophytes ont été isolées et classifiées, avec l'identification de 3 nouveaux sérotypes (Orvenco, Udine, Valderio) et de 2 nouveaux sérogroupes (Orvenco, Udine). Une des souches isolées fut incapable de se développer à la temperature de 20 °C ou au dessus.

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Herbicidal Pollution. Pollen Damage by the Herbicide Vapours

The herbicides, because of their specific weed killing activities, have become assets but they may prove harmful also. The lethal effects of herbicides to the pollen grains have not been investigated. While the field estimates are under investigation, a possible effect of 2,4-D on pollen grains of brinjals (*Solanum melongena* Willd.) has been described here.

Method. 75 ml of 2,4-D in concentrations of 100, 250, 500 and 1000 ppm was kept in beakers. The mouth of the beaker was covered by a muslin cloth and pollens were sprinkled on the cloth. The beakers were kept at room temperature (32 ± 1.6 °C) for 4 days. At the end of each day, pollens were taken from the muslin surface and were kept on the extracts of stigmatic surfaces (of brinjal flowers) so as to ensure germination. Average amount of herbicide solution evaporated per day from beakers was 13 ± 2.3 ml. Viability of pollen grains was expressed by its germination.

Results and discussion. The treated pollens appeared to be damaged by the herbicidal vapours. Up to a certain extent, even 100 and 250 ppm concentration had damaging effects which increased with time, while 500 and 1000 ppm of 2,4-D starts its effect right from the first day, of course with a further increase in the effect in later days. It was very clear that a treatment for 4 days with a higher dose of herbicide, i.e. 1000 ppm, can reduce the percentage of the viable pollens to 31%, a decrease of 56% with respect to the untreated ones. Results thus point (Table) to the possible pollen losses in fields after the herbicidal trials because of the subsequent evaporation of the herbicides. In fact the number of the pollens required for fertilization against the number of pollen grains produced is so small that we may not be able to feel a difference in the yields in the earlier stages. But a constant use of the herbicides will definitely increase the amount of herbicidal vapours in the air around the fields. This will certainly cause an air pollution, having definite impacts on pollen viability and the germination.

The study thus reveals the possible role of the herbicides in polluting the air. Among the various works (MIDDLETON[1], HILTON et al.[2], RICH[3], KING[4], MORELAND[5], HECK[6], STERN[7], DUGGER and TING[8]) described, this aspect was never covered.

Zusammenfassung. Herbiziddämpfe reduzieren die Pollenfertilität von *Solanum melongena* Willd.

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Effect of herbicidal vapours on viability of pollen grains

Conc. of 2,4-D (ppm)	Pollen grains germinated (%) (treatment in days)			
	1	2	3	4[a]
100	81 ± 2.6	76 ± 5.5	79 ± 2.8	73 ± 3.6
250	81 ± 2.6	76 ± 5.5	70 ± 6.5	64 ± 3.6
500	74 ± 3.4	66 ± 2	59 ± 3	51 ± 3
1000	74 ± 3.6	53 ± 1.7	44 ± 2	31 ± 4.3
Untreated	87 ± 4.3	87 ± 4.3	88 ± 4.3	87 ± 4.3

[a] Fertility significant at 5% level.

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