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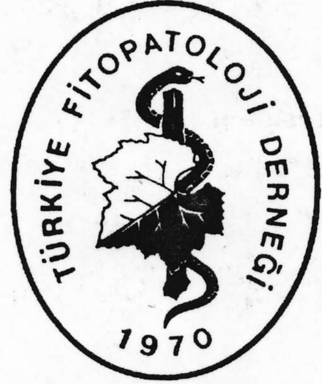

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Incidence of Chestnut Blight in Bursa Province and Reactions of Some Turkish Chestnut Cultivars Against it

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ABSTRACT

The present study was conducted on chestnut trees in Bursa Province and Atatürk Central Horticultural Research Institute in Yalova Province in Turkey during 1995-1996. At the end of the study, it was determined that the numbers of infected trees by the *Cryphonectria parasitica* Canker were 70%, 30% and 100 % in Cumalıkızık, Hamamlıkızık and Babasultan villages in Bursa Province, respectively. In addition, The cultivars Vakit and Dursun were more resistant to the pathogen than the other cultivars Firdula, Osmanoğlu, Hacı Ömer, Sarı Aşlama and Seyrek Diken.

INTRODUCTION

Cryphonectria parasitica (Murril) Barr (=*Endothia parasitica* (Murrill) P.J. Anderson & H.W. Anderson) the causal fungus of chestnut blight, was first reported in Turkey in 1967 and has virtually decreased the Turkish chestnut production (Akdoğan ve Erkman, 1968; Delen, 1975; Delen, 1979; Delen, 1980). According to the 1996 statistic, there were 2.454.000 chestnut trees in Turkey and annual production is approximately 84.900 tons (Anonymous, 1998). European chestnut which is native species of Turkey is grown widely on coast lines of Black Sea Region, around Marmara Sea, Western Anatolia Region until Antalya territory in the south (Erdem, 1951). Chestnut fruits as appetizers or sweets made out of them always have a good market in Bursa Province in Turkey. However, Since there is no definite method of preventing the disease, it is becoming a great problem for Turkey's chestnut production. For this reason, the objectives of this study were to determine the incidence of *Cryphonectria parasitica* Canker in Bursa Province and its pathogenicity to some Turkish chestnut cultivars.

MATERIALS and METHODS

Surveys were conducted in three locations including Cumalıkızık, Hamamlıkızık and Babasultan villages in 1995 and 1996 years. Each sampling strip was 10 m wide and the distance between each strip was 100 m. Because of the greatness of population, it was necessary to decrease the number of samples in 1:10 ratio (Delen, 1975; Torsello et al, 1994). Each tree was examined according to externel signs of the fungus and classified as infected and uninfected. The samples were taken from a maximum of 10 blighted trees with visible signs of the fungus and put into paper bags for transportation to the laboratory. Isolation of fungus was made on potato dextroz agar (Difco) medium and the identification of *C. parasitica* was based on culture morphology, including color. Isolates were transferred to PDA slants for long-term storage (Torsello et al, 1994).

After determining the most virulent isolate of the *C. parasitica* by the preliminary studies, pathogenicity of the isolate was determined against some Turkish chestnut cultivars according to the method of Huang et al. (1996) in Atatürk Central Horticulture Research Institute in Yalova Province in Turkey. However, we made some insignificant changes in the method such as diameter of cork borer increased to 5 mm and the number of replication was 5.

The most virulent isolate was isolated from a chestnut tree in Cumalıkızık Village in Bursa Province. Since canker widths and lenghts were closely correlated in the experiment, 8 weeks after inoculation, only canker lengthts were to be considered.

RESULTS

Survey of *C. parasitica* was conducted in Cumalıkızık, Hamamlıkızık and Babasultan villages in Bursa province because chestnut production is more important than the other locations.

Based on the surveys, the number of the infected trees were as 70%, 30% and 100% in Cumalıkızık, Hamamlıkızık and Babasultan villages, respectively (Table 1).

Table 1. Locations of surveyed area, number of trees examined, number of blighted trees and percentages of infected trees in Bursa Province in Turkey.

Locations	Number of Trees Examined	Number of Blighted Trees	Percentages of Blighted Trees
Cumalıkızık	30	21	70.00
Hamamlıkızık	10	3	30.00
Babasultan	60	60	100.00

As to be seen on table 1, the natural incidence of *C. parasitica* canker within chestnut trees in Bursa Province may be changed between 30% and 100%. The reactions of some Turkish chestnut cultivars against the pathogen were given on table 2.

Table 2. Mean canker lenghts of seven Turkish chestnut cultivars inoculated with a virulent isolates of *Cryphonectria parasitica* in 1996.

Code No of the Cultivar	Name of Cultivar	Mean canker lenghts (mm.)*
62309	Firdola	9.2
52214	Hacı Ömer	6.8
51111	Sarı Aşlama	4.6
63110	Seyrek Diken	3.0
52112	Vakit	0.0
51101	Osmanoğlu	11.0
61316	Dursun	0.0

* Each mean represents 5 observations

As to be seen on Table 2, significant differences in canker lenghts were found among the Turkish cultivars and average canker lenghts among Turkish chestnut varied from 0.0 mm on the cultivar Vakit and the cultivar Dursun to 11.0 mm on the cultivar Osmanoğlu.

DISCUSSION

The number of infected trees varied from 30% to 100% (Table 1). Hovewer, we observed many trees surviving despite repeated infections. In fact, Bursa Region is the end of chestnut horizon on the Black Sea, Coast and Karaca (1968) reported that incidences of *C. parasitica* on chestnut trees in Bursa and İnegöl were 10.8% and 8.3% respectively. In addition, Delen (1975) reported that 32.3% of Marmara Region was infected by the pathogen in 1975. Results of our survey showed that the canker is becoming more severe day by day in Bursa Province.

It was also a surprise that there were significant differences in canker lenghts among the Turkish chestnut cultivars tested (Table 2). Soylu et al. (1994) reported that vakit kestanesi (52112) and Firdula (62309) were less affected by the chestnut blight according to their field observations for 13 years. The result of present study was also the same with the cultivar Vakit esi (52112), but not with the cultivar Firdula (Table 2). This evidence might be weak to make a decision that the Turkish chestnut cultivars are resistant to chestnut blight, because they are originally European chestnut *(Castenea sativa)*. However, we suppose that dsRNA may be present within the *Cryphonectria*

populations in Turkey. Because there are many chestnut trees recovered from blight in Bursa Province. Like hyphovirulence was reported in Eurupean Countries and in U.S.A. (Dodds, 1980; Enebak et al., 1994; Heiniger and Rigling, 1994; Hillman el al, 1992; Sillick and McDonald, 1988). Furthermore, if populations are not at equilibrium, gene flow is likely to be overestimated because of the effects of gene flow in the past (Milgroom and Lipari, 1995, Stalkin, 1987).

According to the present study, we may say that some Turkish chestnut cultivars are more sensitive to the chestnut blight fungus *C. parasitica* (Murr.) Barr. than the others. As to be seen on Table 2., In spite of the fact that Osmanoğlu and Firdola cultivars were more sensitive, Vakit and Dursun cultivars were uneffected by the pathogen. Soylu et al. (1994) determined that Vakit Kestanesi 52112 was also found to be less affected by the other cultivars during the 13 years observations.

ÖZET

BURSA İLİ KESTANE AĞAÇLARINDA KESTANE KANSERİNİN YAYGINLIK ORANI VE BAZI TÜRK KESTANE ÇEŞİTLERİNİN HASTALIĞA KARŞI REAKSİYONLARI

Bu çalışma, Bursa ili kestane alanlarında ve Yalova ilindeki Atatürk Bitkileri Merkez Araştırma Enstitüsünde 1995-1996 yıllarında yapılmıştır. Çalışma sonunda *Cryphonectria parasitica* kanseri ile bulaşık ağaç sayısı Bursa'nın Cumalıkızık, Hamamlıkızık ve Babasultan köylerinde sırasıyle %70, %30 ve %100 olarak saptandı. Ayrıca, patojene karşı Vakit ve Dursun kestanelerinin denemede kullanılan diğer çeşitler olan Firdula, Osmanoğlu, Hacı Ömer, Sarı Aşlama ve Seyrek Diken çeşitlerinden daha dayanıklı oldukları belirlendi.

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First Report of Bacterial Diseases on kiwifruit (*Actinidia deliciosa*) in Turkey

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ABSTRACT

The causal organism of bacterial blossom blight of kiwifruits in Turkey was classified as *Pseudomonas syringae* pv. *syringae* on the basis of laboratory tests. Pathogenicity tests yielded severe blights and leaf spots in the kiwifruit plants and also on some herbaceous plants, peach and almond. The main symptoms observed in the field were blight of young canes, fresh tip die-back, bud rot, blossom blight and brown leaf spots without a halo. This is the report of *P. syringae pv. syringae* as causal agent of disease on several organs of kiwifruit in Turkey.

Another symptom was observed on kiwifruit plants in the Marmara region in Bursa province. The symptoms were darkening and rotting of cortical and woody tissues of stem near the ground level, general wilt and finally death of the plants. Results of morphological, physiological, biochemical tests and pathogenicity tests indicate that the isolated bacterium belongs to the pectolytic group of genus *Erwinia* and to the species *E. carotovora*. There is no record until now that *E. carotovora* or other pectolytic group of *Erwinia* is a pathogen on kiwifruit plants. Stem isolates could not be identified at subspecies level due to some different properties of them when compared to subsp. *atroseptica* or subsp. *carotovora*. Our analysis indicated that the kiwifruit pathogens are phenotypically different from the known subspecies in *E. carotovora*. We consider them to be a separate phenotypic group in *E. carotovora*. This is the first record of *E. carotovora* in kiwifruit plants (*Actinidia deliciosa*) in Turkey and possibly the first world record.

INTRODUCTION

The kiwifruit is a deciduous plant which yields well in the subtropical and temperate areas of the world. Turkey is one of the few countries that are suitable for kiwifruit production, due to its ecological conditions. Introduction to Turkey has only a 10-years past. East Black Sea Region is the most suitable area for kiwifruit cultivation and also if irrigation is practised properly, this crop can be grown readily in the north-

west and west coastal strip of Turkey (Yalçın *et al.*, 1998). About 50-60 thoùsands of kiwifruit vines are planted in one year, half of it was imported.

Several bacterial diseases among which were bacterial blight caused by *Pseudomonas viridiflava* (Young, 1988; Varvaro *et al.*, 1990; Conn *et al.*, 1993), bacterial blossom blight caused by *Pseudomonas syringae* pv. *syringae* (Pennycook and Triggs, 1992; Conn *et al.*, 1993;; Ieki, 1993; Mazarei and Mostofipour, 1994; Balestra and Varvaro, 1997) and bacterial canker caused by *Pseudomonas syringae* pv. *actinidia* (Takikawa et al., 1989; Ushiyama *et al.*, 1992; Scortichini and Simeoni, 1993; Koh *et al.*, 1996) were reported.

In 1997, several kiwifruit vines cv. Hayward in the province of Fethiye (west Mediterranean) showed serious damage on buds and shoots. The symptoms on shoots were darkening and shrivelling along the bark and also fresh tip die-back. The floral buds were darkened and rotted. The internal tissues of such buds are found to be dark brown and completely rotten. Small brown spots without a halo were observed on the leaves.

In the same year, another disease of kiwifruit was found. Samples of infected kiwifruit plants were received in our laboratory from the area of Bursa province, in the Marmara region. Lower parts of stems of diseased plants showed darkening and rotting of cortical and woody tissues.

The objective of this research was to determine the causal agents of the symptoms.

MATERIALS and METHODS

Isolation of the pathogens

Isolations were made from buds, shoots, stems and necrotic lesions on leaves. Samples with symptoms were washed with sterile distilled water (SDW), rinsed for 30 seconds in 70% ethanol solution and then washed two times in SDW. Disinfected plant parts homogenized in SDW with a sterile mortar were streaked on plates of surface-dried nutrient agar with 5% sucrose (SNA) medium and King's B medium (King et al., 1954). Inoculated Petri dishes were incubated at 26±1°C. for 3 days to allow possible development of bacteria. Representative colonies were re- streaked to SNA to ensure purity. Pure cultures were maintained on NGA (0.8% nutrient broth, 2% glycerol, 1.8% agar) slants at 4±1°C.

Pathogenicity tests

In spring, female kiwifruit vines supplied from Olive Research Station located in Edremit were remowed to the laboratory. Inoculations were carried out on 2-year old pot-cultivated kiwifruit plants cv. Hayward in the growing room at 26±1 °C with 80-85% RH. Inoculum was prepared from 24 hour old NGA cultures suspended in SDW

and adjusted to about 10^8 cfu/ml. Bacterial suspensions of first isolate obtained from shoot, bud and leaf spots were sprayed on the leaves and also injected into shoots using a 26 swg needle and plants were then covered with plastic bags for 24 hours. Known strains of *P. s.* pv. *syringae* (bean, cherry and lemon strains), *P.s.*pv. *actinidia* (NCPPB 3739) and *P. viridiflava* (CFBP 1466) were used as control during the test.

Bacterial suspension of second isolate obtained from affected stem was injected into shoots and petioles by the same technique. Reference strains of *Erwinia carotovora* subsp. *carotovora* (CFBP 2046) and *E. c.* subsp. *atroseptica* (CFBP 1546) were used as control in these tests.

Control plants were treated SDW only. Re-isolations were made from all apparent symptoms.

Determination of host range of the kiwifruit isolates

Kiwifruit isolates obtained from shoots, buds and leaf spots and known strains mentioned above were inoculated on some plant species such as bean, tomato, pepper, peas, maize, sorghum, cucumber, watermelon, melon, peach and almond seedlings. Stem isolate of kiwifruit and reference strains of soft-rot *Erwinia* were tested on tomato, tobacco, pepper, bean and potato seedlings.

Identification of isolates

The identification of the bacterial isolates and their re-isolates was carried out according to the biochemical, physiological and nutritional tests described by Lelliott *et al.* (1966) and Dickey and Kelman (1988).

Gram stain, presence of fluorescent pigments on King's medium B (King *et al.*, *1954*), LOPAT tests (levan production, oxidase reaction, potato soft rot, arginine dihydrolase, tobacco hypersensitivity), nitrate reduction, gelatine liquefaction, aesculin hydrolysis, acid from sucrose, NaCl tolerance and maximum growth temperature were evaluated.

To characterise the strains isolated from affected kiwifruit stems the following tests were used. Gram stain, nitrate reduction, indole production from tryptophane, catalase reaction, reducing substances from sucrose, gelatine liquefaction, presence of arginine dihydrolase, starch hydrolysis, lecithinase activity, glucose metabolism, salt tolerance and pectolytic activity. The maximum temperature for growth was determined on YDA (yeast dextrose agar) medium, incubated in water baths at 35, 36, 37, 38, 39, 40 and 41 °C for 4 days. Ice nucleation activity (INA) was evaluated according to Lindow *et al.* (1978 a).

Nutritional tests were conducted on mineral salts medium (Ayers *et al.* 1919) solidified with 1.2% purified agar and with bromothymol blue indicator. All test com-

pounds were sterilised by Millipore filtration and added at a final concentration of 0.1% (w/v). Inoculated tubes incubated at 28°C for 14 days in the dark and examined daily for growth and acid production. Nutritional test similar to *Erwinia* spp. were also conducted. Also 2ml ethanol/250 ml of Ayers mineral salt medium was used for an ethanol utilisation test. Tubes without test compounds were used as control. Known strains which were given previously were used as control during the tests.

In addition, erythromycin sensitivity test and growth on Logan's differential medium (Logan, 1966) used for to distinguish *E. c.* subsp. *atroseptica* from subsp. *carotovora* were also conducted for Erwinia strains.

RESULTS

Symptoms

In 1997, the symptoms appeared in about 10% of the kiwifruit plants grown in 0.5 da orchard in the area of Fethiye in west Mediterranean region and included darkening and shrivelling along the bark of the shoots (Fig.1), bud rotting, fresh tip die-back (Fig. 2) and brown necrotic leaf spots without a halo (Fig. 3).

The symptoms of diseased samples received in our laboratory from the area of Bursa included discolouration and rotting of cortical and woody tissues of stem near the ground level, general wilt and finally death of the plants. It was observed that affected plants continued to produce new shoot from stem near ground level when the stem cut off under the infected area. In last observation, these plants seemed healthy.

Characterisation of isolates

The following group of bacterial strains were consistently obtained from infected plant tissues.

1. Gram negative aerobic bacteria with polar flagellea. In LOPAT tests for the identification of *Pseudomonas* sp. these strains were found to be negative for oxidase, potato rot and arginine dihydrolase reaction, and positive for levan production and tobacco hypersensitive reaction. On King's medium B, the colonies were whitish-grey, raised and produced a bright yellowish green diffusible fluorescent pigment. Isolates did not grow at 37 °C and induced ice nuclei at -8 °C. These strains were characterised as *Pseudomonas syringae* according to the determinative schemes proposed by Lelliott *et al.* (1966). Physiological and biochemical properties of strains are summarised in Table 1.

2. Isolates obtained from affected stems produced a positive hypersensitive reaction on tobacco leaves and caused rot of potato slices. Colonies were greyish-white to creamy-white, smooth, glistening and slightly raised. All strains tested were Gram negative, motile rods with peritrichous flagellae, catalase positive and oxidase negative,

showed fermentative metabolism of glucose, hydrolyzed pectin and gelatin, produced H_2S, reduced nitrate and formed reducing subtances from sucrose. They grew at 39 °C but not at 40 °C. The aesculin hydrolysis was positive, but the anaerobic breakdown of arginine, indole production and starch hydrolysis were negative. Acid was produced from ethanol, maltose, rhamnose, lactose, cellobiose, mannitol, mannose and threhalose but not from erythritol, arabitol, dulcitol, adonitol, sorbitol and xylose. Isolates streaked on Logan's medium reduced the tetrazolium to insoluble red formazon and colonies (about 2 mm diameter) developed a red to purple center after 24 hours. On the basis of these results, kiwifruit strains belonged to the genus *Erwinia*. The results of tests are presented in Table 2.

Table 1. Tests to compare strains of *Pseudomonas* sp. isolated from kiwifruit with *P. syringae* pv. *syringae* and *P. s.* pv. *actinidia*

Comparative test	Kiwifruit isolates (3 strains)	*P.s.* pv. *syringae* (two strains)	*P.s.* pv. *actinidia* (NCPPB 3739)[a]
Levan	+	+	+
Oxidase	-	-	-
Potato rot	-	-	-
Arginine dihydrolase	-	-	-
Tobacco hypersensitivity	+	+	+
Nitrate reduction	-	-	-
Fluorescent pigment	+	+	+
Gelatine liquefaction	+	+	-
Aesculin hydrolysis	+	+	-
Acid from sucrose	+	+	+
Utilization of:			
Sucrose	+	+	+
D(+) xylose	+	+	+
D(+) mannose	+	+	+
D-raffinose	+	+	-
D-mannitol	+	+	+
D-sorbitol	+	+	+
m-inositol	+	+	+
L-arabitol	-	-	-
i-erythritol	+	+	-
L(+) tartrate	-	-	-
Ethanol	-	-	-
Growth at 37°C	+	+	+
Growth in 5% NaCl	-	-	+[w]
Almond seedling infection	+	+	-

[a] NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, UK.

+, positive; -, negative; w, weak

Table 2. Tests to compare kiwifruit isolates with strains of *Erwinia carotovora* subsp. *carotovora E. carotovora* subsp. *atroseptica* and *E. chrysanthemi*

Tests	Kiwifruit isolates	Ecc (CFBP2046)	Eca (CFBP1546)	*E. chrysanthemi* [a]
Potato soft rot	+	+	+	+
Tobacco HR	+	+	+	+
Nitrate reduction	+	+	+	+
Gelatin liquefaction	+	+	+	+
O/F	F	F	F	F
Starch hydrolysis	-	-	-	-
Production of indole	-	-	-	-
Reducing substance from sucrose	+	-	+	-
Lecithinase	-	-	-	nt
Phosphatase	-	-	-	+
Arginine dihydrolase	-	-	-	+
Growth at 37 ^oC	+	+	-	+
Growth at 39 ^oC	+	-	-	+
Acid from:				
lactose	+	+	+	+
maltose	$+^w$	-	+	-
L(+) rhamnose	+	+	+	-
D(+)cellobiose	+	+	+	nt
threhalose	+	+	+	-
dulcitol	-	-	-	+
adonitol	-	-	-	nt
i-erythritol	-	-	-	nt
D(-)sorbitol	-	-	-	nt
D(+)xylose	-	-	+	nt
L(-)arabinose	+	+	+	nt
ethanole	+	+	-	nt
Growth in 5% NaCl	+	+	$+^w$	-
Erythromycin sensitivity	R	R	R	S
Blue pigment on GYCA	-	-	-	+
Potato plants infection	+	+	+	+

+, positive; -, negative; w, weak; nt, not tested; R, resistent; S, sensitive
[a] Reactions are from Malathrakis and Goumas (1987)
CFBP, Collection Française de Bactéries Phytopathogénes, Angers, France.

As shown in Table 2, differences in maltose (+ and -) and reducing subtances from sucrose (+ and -) were determined by comparing kiwifruit isolates and control strain *(E. carotovora* subsp. *carotovora)* CFBP 2046, respectively. Some differences also were determined between kiwifruit stem isolates and control strain (*E. carotovora* subsp. *atroseptica*) CFBP 1546. Differences in ethanol (+ and -) and growth at 39 ^oC (+ and -) can be seen in the same table. Colonies of kiwifruit isolates were more similar to Eca than Ecc on King B or SNA medium. But on Logan's differential medium (Logan,

1966), kiwifruit isolates showed similar colony morphology and growth properties with control strain (*E. carotovora* subsp. *carotovora*) CFBP 2046 and they were very different from control strain (*E. carotovora* subsp. *atroseptica*) CFBP 1546.

Isolates of kiwifruit were not identcal with *E. chysanthemi* because they have some different characters when compared to *E. chysanthemi* in some distinctive tests (Table 2.)

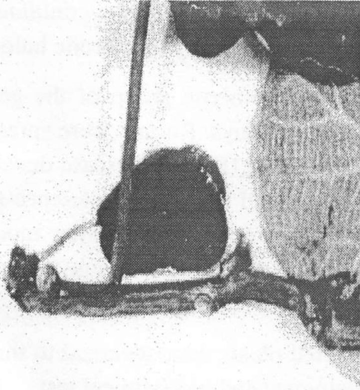

Fig 1. Natural symptoms on shoots of kiwifruit plants caused by *Pseudomonas syringae* pv. *syringae*

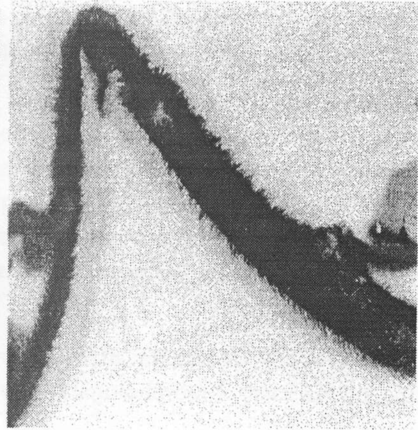

Fig 2. Fresh tip die-back causedy by *P.s.*pv. *syringae*

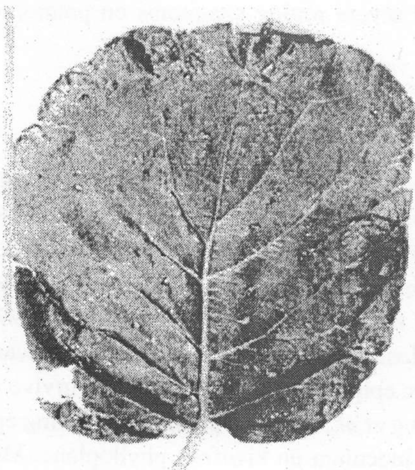

Fig 3. Brown necrotic leaf spots of bacterial blight disease of kiwifruit

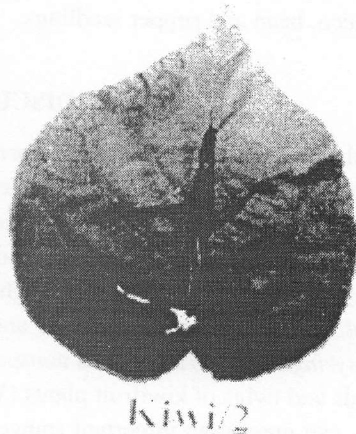

Fig 4. Rotted main veins of kiwifruit leaves artifically inoculated with *Erwinia carotovora*

Pathogenicity tests

Kiwifruit isolates classified as *P. syringae* pv. *syringae* caused shoot blight symptoms four days after shoot inoculation of young kiwifruit plants. Inoculated shoots were completely died in 10 days. When kiwifruit leaves were sprayed with a bacterial suspension of the same strains brown spots without a halo appeared in 6 to 8 days. Young kiwifruit plants inoculated with sterile distilled water or with the bean, cherry and lemon strains of *P. syringae* pv. *syringae* did not show any symptoms and grew normally. Inoculations made with the reference strain of *P. syringae* pv. *actinidiae* caused tiny cankers at the inoculation sites and leaf spots surrounded by a chlorotic haloes.

Strains from Bursa and characterised as the pectolytic group of the genus *Erwinia* caused severe rot of the shoot and petioles in 2-3 days. Rotting were spread along the main veins of the leaves and affected leaves dropped (Fig. 4). Disease development was very rapid under conditions of high temperature and humidity. Reference strain of *E. carotovora* subsp. *atroseptica* only showed slight rotting 11 days after inoculation. No symptoms appeared on the plants inoculated with *E. carotovora* subsp. *carotovora* or sterile distilled water.

Isolates obtained from the artificially infected plants were identical to those used as inocula, on the basis of morphological, physiological and biochemical tests.

Host range tests

Isolates characterised as *P. syringae* pv. *syringae* caused blight symptoms on bean, peas, tomato, pepper, watermelon, sorghum, maize, peach and almond seedlings. *P. syringae* pv. *actinidiae* did not cause any symptom on above mentioned plant species.

Isolates obtained from stem rot showed severe rotting symptoms on potato, tomato, tobacco, bean and pepper seedlings.

DISCUSSION

In the present research, *P. s.* pv. *syringae* that causes shoot and bud blight symptoms of kiwifruit was isolated and characterized. This bacterial species are well known pathogenic bacterium which can cause disease on different organs (leaves, buds, blossoms, twigs) of many herbaceous and arboraceous plant species (Bradbury, 1986) and have been reported to cause blossom blight of kiwifruit (Pennycook and Triggs, 1992; Conn *et al.*, 1993; Ieki, 1993; Mazarei and Mostofipour, 1994; Balestra and Varvaro, 1997). *P. syringae* pv. *syringae* is a nonspecific epiphytic bacterium and can survive on leaves, buds and twigs of kiwifruit plants (Young *et al.*, 1988). Pathogen, surviving epiphytically, can present an important source of inoculum on kiwifruit phylloplane. Mild temperatures, frequent rainfalls and high RH values facilitate their spread (Balestra and Varvaro, 1995). On kiwifruit plants, *P. syringae* pv. *syringae* was reported to be effective

INA agents (Young, 1987), it appears to be dangerous on kiwifruit buds, especially with sudden decreases of temperature by its higher INA (Lindow *et al.* 1978 b; Varvaro et al., 1990). It may be possible to say that the kiwifruit isolates of *P. syringae* pv. *syringae* have some difference from the others according to their pathogenicity test results, although on the basis of biochemical tests they could not be differentiated from isolates of this pathovar from other hosts. As known, other isolates obtained from bean, lemon and cherry of this pathovar did not cause any symptom on young kiwifruit plants in the pathogenicity tests. However, it was not determined any specificity between isolates and host plant. This is the first report of *P. syringae* pv. *syringae* as causal agent of disease on floral buds and twigs of kiwifruit plants cv. Hayward in Turkey.

Another part of this study was aimed to determine causal agent of stem rotting symptoms on kiwifruit plants. Results indicate that the isolated bacterium belongs to the pectolytic group of genus *Erwinia* and to the species *E. carotovora.* There is no record until now that *E. carotovora* or other pectolytic group of *Erwinia* is a pathogen on kiwifruit plants. Stem isolates could not be identified at subspecies level due to different properties of them when compared to subsp. *atroseptica* or subsp. *carotovora.* Our analysis indicated that the kiwifruit pathogens are phenotypically different from the known subspecies in *E. carotovora.* We consider them to be a seperate phenotypic group in *E. carotovora.* Verdonck *et al.* (1987) seperated several phenons within *E. carotovora* at a high level of similarity. Consecutively adequate differentiation between some soft rot groups was sometimes difficult. There are more than 40 serogroups of *E. carotovora* and five of *E. c. atroseptica* based on surface antigens (De Boer *et. al.,* 1979, 1987). It may be possible to say that the kiwifruit isolates were a new subspeies which were specialize to kiwifruit, but it was determined that these isolates caused very rapid rotting symptoms on some other inoculated plant species. Soft rot erwinias are known to survive in the rhizosphere of many crops (Burr and Schroth, 1977). However, it is difficult to establish the source of primary inoculum and to explain the rapid death of the kiwifruit plants. This is the first record of *E. carotovora* in kiwifruit plants *(Actinidia deliciosa)* in Turkey and possibly the first world record.

Considering the increasing economic importance of this fruit in Turkey, attention should be given to these pathogens to avoid losses. Further research will be planned.

ÖZET

TÜRKİYE'DE KİWİLERDE (*Actinidia deliciosa*) GÖRÜLEN BAKTERİYEL HASTALIKLARIN İLK RAPORU

Türkiye'de, kiwi bitkilerinde çiçek yanıklığına neden olan organizma, laboratuvar testlerinin sonucuna göre *Pseudomonas syringae* pv. *syringae* olarak tanılanmıştır. Kiwi bitkilerinde yürütülen patojenisite testlerinde, ciddi yanıklıklar ve yaprak lekeleri meydana

geldi. Benzer belirtiler bazı otsu bitkilerde, şeftali ve badem fidelerinde de elde edilmiştir. Genç sürgünlerde yanıklık, genç uçlarda geriye ölüm, tomurcuklarda çürüme, çiçek yanıklığı ve çevresi hale ile çevrili olmayan kahverengi yaprak lekeleri arazide gözlenen ana belirtilerdi. Bu çalışma, *P. syringae* pv. *syringae*'nin Türkiye'de kiwilerde çiçek tomurcuklarında ve sürgünlerde hastalık nedeni olduğunu ortaya koyan ilk rapordur.

Bursa ilinde bulunan bir bahçede kiwi bitkilerinde bir başka belirti daha gözlenmiştir. Belirtiler; gövdenin toprak yüzeyine yakın olan kısımlarında kabuk ve odun dokularının kararması ve çürümesi, genel solgunluk ve sonunda bitkilerin ölümü şeklindeydi. Morfolojik, fizyolojik ve biyokimyasal testlerin ve patojenisite testlerinin sonuçları, izole edilen bakterinin *Erwinia* sınıfının pektolitik grubuna ve *E. carotovora* türüne ait olduğunu ortaya koymaktadır. Bugüne kadar *E. carotovora* veya diğer pektolitik grup erwinialarin kiwi bitkilerinde patojen olduğunu belirten bir kayıt yoktur. Gövde izolatları, subspecies *carotovora* veya subspecies *atroseptica* ile karşılaştırıldığında, bazı farklı özellikler taşımaları nedeniyle alt tür düzeyinde tanılanamamıştır. Bulgularımız, kiwi patojeninin *E. carotovora*'nın bilinen alt türlerinden fenotipik olarak farklı olduğunu göstermiştir. Biz, kiwi patojeninin *E. carotovora* türü içinde ayrı bir penotip olduğu düşüncesindeyiz. Bu çalışma, kiwi bitkilerinde *E. carotovora*'nın varlığının Türkiye'de ve muhtemelen dünyada ilk kaydıdır.

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Rolle von Avenaluminen bei der Besiedlung der Getreide durch ff. sp. von *Erysiphe graminis*

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ZUSAMMENFASSUNG

Es solte untersucht werden, ob die Avenalumine an der Resistenz von Haferblättern gegen ff. sp. von *Erysiphe graminis* entscheidend beteiligt sind, und wenn dies zutrifft, ob auf Blätter aufgesprühte Lösungen reiner Avenalumine die Pathogenese nachhaltig beeinflussen können. Versuche zur Klärung dieser Frage wurden sowohl mit abgetrennten Blättern als auch an intakten Haferpflanzen von cv. "Erbgraf" durchgeführt. An abgetrennten Blättern bewirkten Endkonzentrationen von 70-130 µg/g TG an Avenalumin 1 zum Zeitpunkt der protektiven Sprühapplikationen eine beträchtliche und signifikante Befallsreduktion 8 Tage p.i.. Bei den mit hohen Konzentrationen von 700-800 µg/g TG behandelten intakten Pflanzen wurde eine starke Reduktion der Entwicklung der f. sp. *avenae* festgestellt. Postinfektionelle Anwendungen 1 und 3 Tage p.i. mit Avenalumin 1 und 2 bewirkten in allen Varianten eine gleichartige, signifikante Reduktion des Befalls mit der f. sp. *avenae,* der allerdings deutlich geringer war als bei protektiver Anwendung. Eine protektive Sprühapplikation von Avenalumin 1 und 2 an Weizen und Gerste führte zu einer signifikanten Reduktion der entsprechenden kompatiblen f. sp. *tritici* oder *hordei.*

EINLEITUNG

Unter den Abwehrfaktoren von Pflanzen gegen Pilzbefall scheint den Phytoalexinen eine besondere Rolle zuzukommen (BAILEY & MANSFIELD, 1982). Die genaue Wirkungsweise von Phytoalexinen ist in vielen Fällen noch unklar. Meist reagiert das toxische Prinzip mit Membranen und führt zu einer Störung ihrer Funktionen (OSSWALD & ELSTNER, 1984). Dadurch kann das Pilzwachstum während oder nach erfolgter Penetration begrenzt werden.

Ziel dieser Arbeit ist es, die Akkumulation preinfektionell angewandter und postinfektionell gebildeter Phytoalexine zu bestimmen und ihre Bedeutung für die Abwehr gegenüber formae speciales von *Erysiphe graminis* zu untersuchen. Können auf

Blätter applizierte Lösungen von reinem Avenalumin 1 und 2 die Entwicklung von ff. sp. *Erysiphe graminis* beeinflussen?

MATERIAL UND METHODEN

Vorbereitung der Avenaluminlösungen: Synthetisch hergestellte Avenalumine wurden in Dimethylformamid vorgelöst und in sterilisiertes Leitungswasser (max. 0.1% DMF) oder unmittelbar in sterilisiertes Leitungswasser mit einer Endkonzentration von 200 µg/ml zugegeben.

Anwendung der Avenaluminlösungen

Abgetrennte Blätter: Die Primärblätter in Schalen gewachsener Haferpflanzen wurden 6 cm lang abgeschnitten und je Petrischale 8 Blätter auf einen gerade erstarrenden Benzimidazolagar ausgelegt. Anschließend wurde die Blätter mit der vorbereiteten Avenaluminlösung mittels eines Handsprühers vorsichtig und weitgehend oberflächendeckend besprüht. Die Schalen wurden für 24 Stunden ohne Deckel im Abzug zur Lufttrocknung belassen. Am nächsten Tag wurden die Avenalumibehandelten und-unbehandelten Blätter mit 80 mg Konidien im Impfturm inokuliert und 8 Tage im Klimaschrank inkubiert. Die Proben für eine Avenaluminanalyse wurden 2, 4, 6 und 8 Tage p.i. entnommen und eingefroren. Pusteln wurden 8 Tage nach der Inokulation gezählt.

Intakte Pflanzen: Die Hafer- cv. "Erbgraf" sowie die Weizen- cv. "Kanzler" und Gerstenpflanzen cv. "Aura" wurden ausgerichtet, mit Avenaluminlösung mittels einer Airbrush mit einem Druck von 3 bar besprüht und für 24 Stunden im Klimaschrank bei 20 °C zur Luftrocknung belassen. Am nächsten Tag wurden die Pflanzen inokuliert, inkubiert und zur Analyse vorbereitet.

ERGEBNISSE

Mehltaubefall an abgetrennten Haferblättern (gesprüht mit Avenaluminlösungen)

Avenalumin 1 in Wasser (EWAvIA) bewirkte, protektiv appliziert, eine Befallsverminderung der f. sp. *avenae* um 97% (Abb. 1), in DMF vorgelöst und in Wasser verdünnt (EDAvIA) um 85%. Interessanterweise waren bei Wasser allein (EWA) bzw. 0.01% wässriger DMF-Lösung (EDA) ebenfalls eine Befallsreduktion zu beobachten, die allerdings geringer war als die durch Avenalumin 1. Während dieser Versuche wurden alle 2 Tage Proben genommen und auf ihren Gehalt an Avenalumin 1 analysiert.

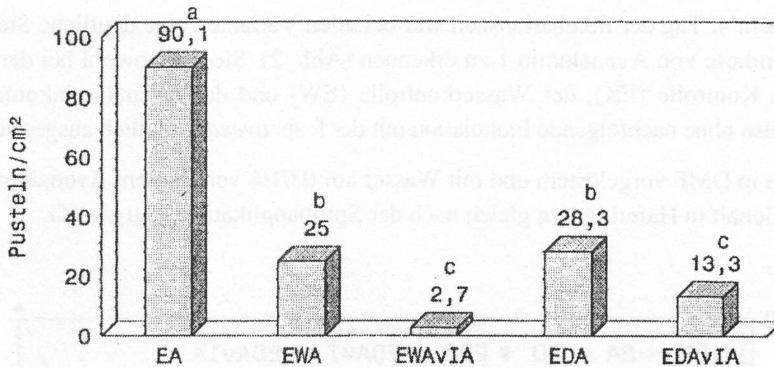

Abb. 1. Wirkung einer Sprühapplikation von Avenalumin 1 auf auf den Mehltaubefall an Blättern cv. "Erbgraf" 8 Tage p.i.

EA : f.sp. *avenae* ohne Vorbehandlung

EWA: f.sp. *avenae* nach Vorbehandlung mit Wasser

EWAvIA : f.sp. *avenae* nach Vorbehandlung mit Avenalumin 1 in Wasser

EDA : f.sp. *avenae* nach Vorbehandlung mit wässriger DMF (max. 0.01%)

EDAvIA : f.sp. *avenae* nach Vorbehandlung mit Avenalumin 1 in DMF (max. 0.01%)

Bei direkt in Wasser gelöstem Avenalumin 1 betrug der Gehalt der gleich nach der Sprühapplikation genommenen Proben 132 µg/g TG und lag damit in einem Bereich, der in Haferblättern induziert werden kann.

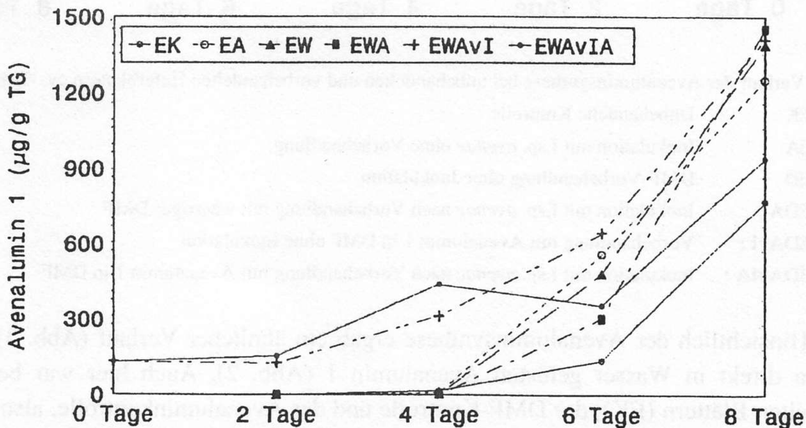

Abb. 2. Verlauf der Avenaluminsynthese bei unbehandelten und vorbehandelten Haferblättern cv. "Erbgraf"

EK : Unbehandelte Kontrolle

EA: Inokulation mit f. sp. *avenae* ohne Vorbehandlung

EW : Wasservorbehandlung ohne Inokulation

EWA : Inokulation mit f.sp. *avenae* nach Vorbehandlung mit Wasser

EWAvI : Vorbehandlung mit Avenalumin 1 in Wasser ohne Inokulation

EWAvIA : Inokulation mit f.sp. *avenae* nach Vorbehandlung mit Avenalumin 1 in Wasser

Ab dem 4. Tag der Inkubationszeit war bei allen Varianten eine deutliche Steigerung der Synthese von Avenalumin 1 zu erkennen (Abb. 2). Sie war sowohl bei der unbehandelten Kontrolle (EK), der Wasserkontrolle (EW) und der Avenaluminkontrolle (EWAv1), also ohne nachfolgende Inokulation mit der f. sp. *avenae*, deutlich ausgeprägt.

Beim in DMF vorgelöstem und mit Wasser auf 0.01% verdünntem Avenalumin 1 betrug der Gehalt in Haferblättern gleich nach der Sprühapplikation 70 µg/g TG.

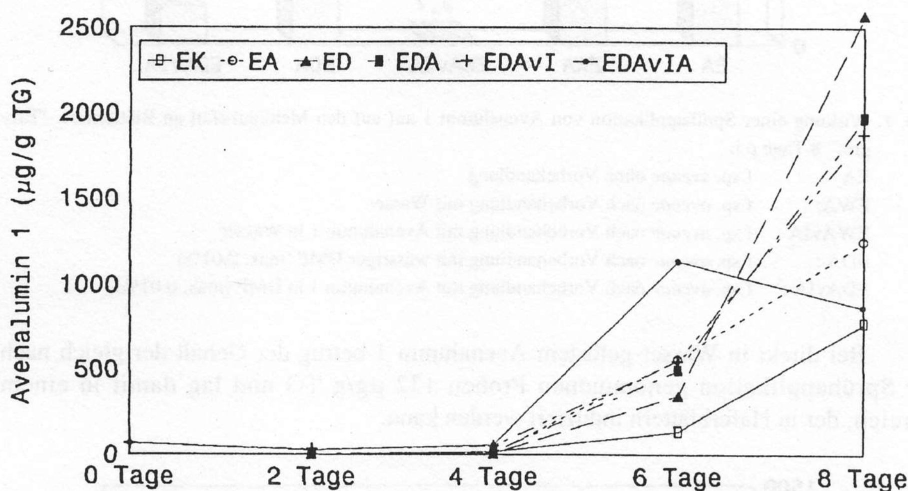

Abb. 3. Verlauf der Avenaluminsynthese bei unbehandelten und vorbehandelten Haferblättern cv. "Erbgraf"

EK :	Unbehandelte Kontrolle
EA:	Inokulation mit f.sp. *avenae* ohne Vorbehandlung
ED :	DMF-Vorbehandlung ohne Inokulation
EDA :	Inokulation mit f.sp. *avenae* nach Vorbehandlung mit wässriger DMF
EDAvI :	Vorbehandlung mit Avenalumin 1 in DMF ohne Inokulation
EDAvIA :	Inokulation mit f.sp. *avenae* nach Vorbehandlung mit Avenalumin 1 in DMF

Hinsichtlich der Avenaluminsynthese ergab ein ähnlicher Verlauf (Abb. 3), wie bei dem direkt in Wasser gelösten Avenalumin 1 (Abb. 2). Auch hier war bei unbehandelten Blättern (EK), der DMF-Kontrolle und der Avenaluminkontrolle, also ohne Einwirkung der phytopathogenen Pilze, ab dem 4. Tag der Inkubationszeit eine deutliche Steigerung der Synthese von Avenalumin 1 zu messen. Die Avenalumingehalte für die Wasser-und DMF-kontrollen waren allerdings um etwa 50% geringer als in den Varianten mit nachfolgender Inokulation durch die f. sp. *avenae* von *E. graminis*.

In einem weiteren Versuch mit abgetrennten Blättern wurde eine höher konzentrierte wässrige Lösung von Avenalumin 1 appliziert. Gleich nach dem Sprühen

betrug der Gehalt 850 µg/g TG. Auch dieses Mal wurde der Mehltaubefall um 97% reduziert (Abb. 4). Durch Wasser allein wurde der Avenalumingehalt nicht gesteigert.

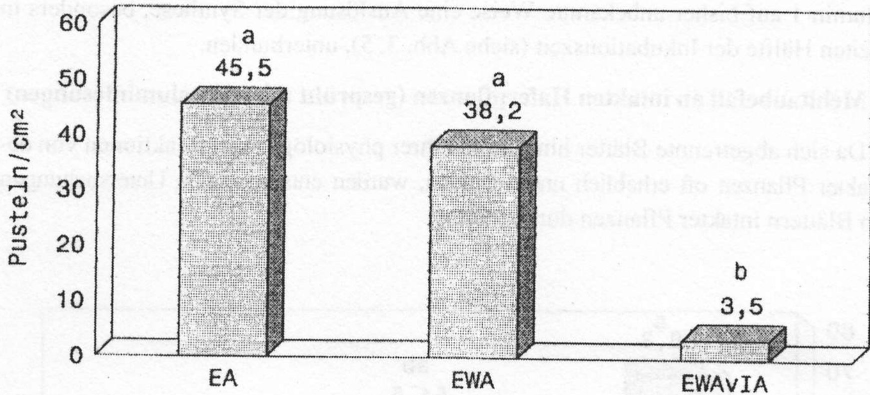

Abb. 4. Wirkung einer Sprühapplikation von Avenalumin 1 auf auf den Mehltaubefall an Blättern cv. "Erbgraf" 8 Tage p.i.

EA : Inokulation mit f. sp. *avenae* ohne Vorbehandlung

EWA: Inokulation mit f.sp. *avenae* nach Vorbehandlung mit Wasser

EWAvIA : Inokulation mit f.sp. *avenae* nach Vorbehandlung mit Avenalumin 1 in Wasser

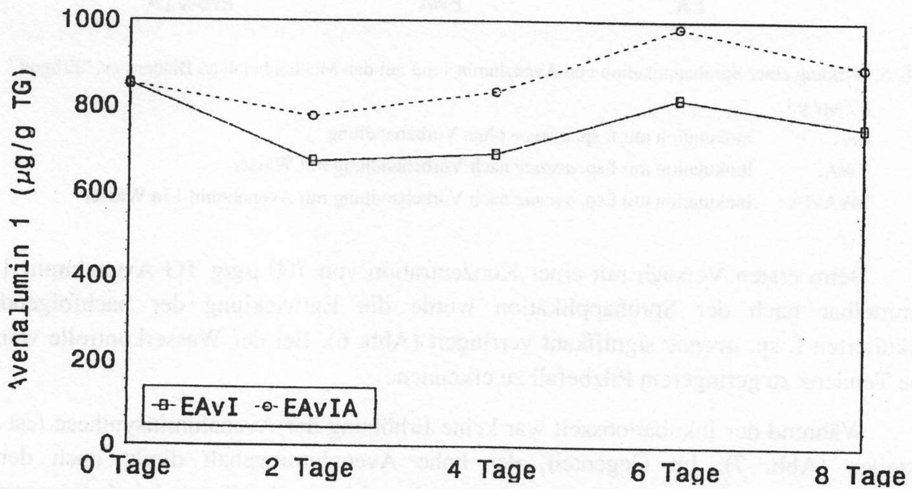

Abb. 5. Verlauf der Avenaluminsynthese bei unbehandelten und vorbehandelten Haferblättern cv. "Erbgraf"

EAvI : Vorbehandlung mit Avenalumin 1 in Wasser ohne Inokulation

EAvIA : Inokulation mit f.sp. *avenae* nach Vorbehandlung mit Avenalumin 1 in Wasser

Bei der höheren Aufwandmenge war praktisch keine *de novo* Synthese von Avenalumin vorhanden (Abb. 5). Der Gehalt lag über die ganze Inkubationszeit auf etwa gleichem Niveau. Es sieht also so aus als hätte die hohe aufgesprühte Menge an Avenalumin 1 auf bisher unbekannte Weise eine Auslösung der Synthese, besonders in der zweiten Hälfte der Inkubationszeit (siehe Abb. 3, 5), unterbunden.

Mehltaubefall an intakten Haferpflanzen (gesprüht mit Avenaluminlösungen)

Da sich abgetrennte Blätter hinsichtlich ihrer physiologischen Reaktionen von denen intakter Pflanzen oft erheblich unterscheiden, wurden entsprechende Untersuchungen auch an Blättern intakter Pflanzen durchgeführt.

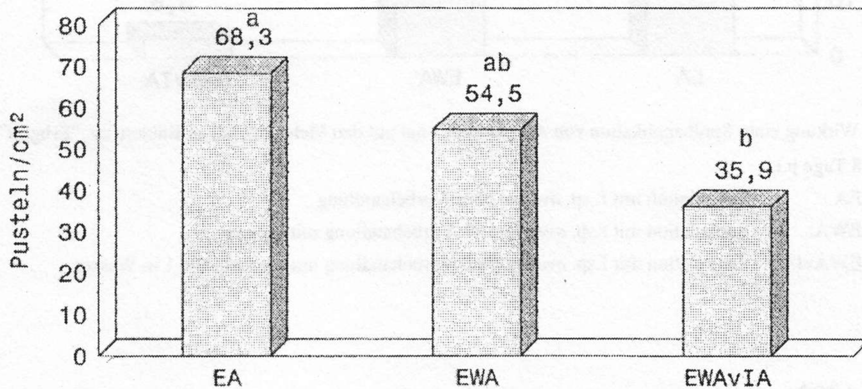

Abb. 6. Wirkung einer Sprühapplikation von Avenalumin 1 auf auf den Mehltaubefall an Blättern cv. "Erbgraf" 8 Tage p.i.

EA : Inokulation mit f. sp. *avenae* ohne Vorbehandlung

EWA: Inokulation mit f.sp. *avenae* nach Vorbehandlung mit Wasser

EWAvIA : Inokulation mit f.sp. *avenae* nach Vorbehandlung mit Avenalumin 1 in Wasser

Beim ersten Versuch mit einer Konzentration von 700 µg/g TG Avenalumin 1 unmittelbar nach der Sprühapplikation wurde die Entwicklung der nachfolgend inokulierten f. sp. *avenae* signifikant verringert (Abb. 6). Bei der Wasserkontrolle war eine Tendenz zu geringerem Pilzbefall zu erkennen.

Während der Inkubationszeit war keine Erhöhung der Avenaluminsynthese festzustellen (Abb. 7). Im Gegenteil, der hohe Avenalumingehalt direkt nach der Sprühapplikation verringerte sich kontiniunierlich und lag nach 8 Tagen Inkubationszeit nur noch bei 50% der Ausgangskonzentration. Bei den unbehandelten Pflanzen und den Wasserkontrollen war der Gehalt nach 8 Tagen 4 bzw. 107 µg/g TG. Demnach hatte auch hier keine *de novo* Synthese von Avenaluminen stattgefunden.

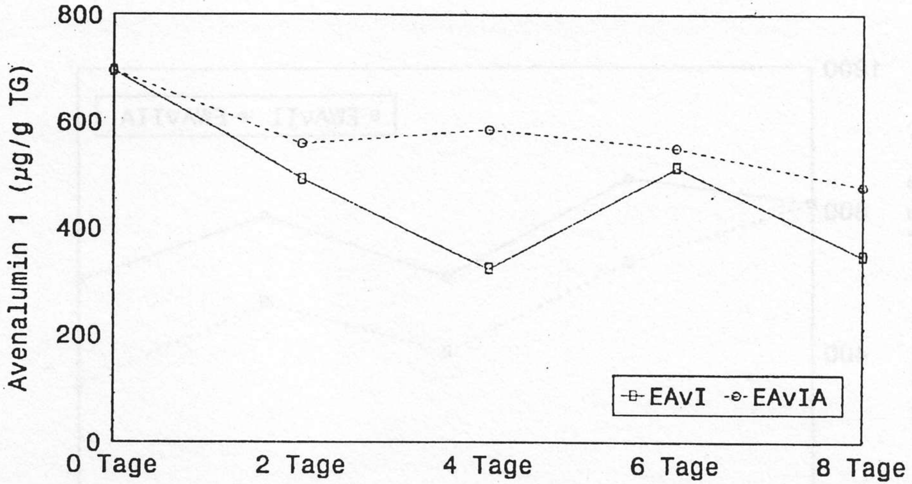

Abb. 7. Verlauf der Avenaluminsynthese bei unbehandelten und vorbehandelten Haferblättern cv. "Erbgraf"

EAvI : Vorbehandlung mit Avenalumin 1 in Wasser ohne Inokulation

EAvIA : Inokulation mit f.sp. *avenae* nach Vorbehandlung mit Avenalumin 1 in Wasser

In einem weiteren Versuch wurde geprüft, ob von Avenalumin 2 eine ähnliche Wirkung ausgeht wie von Avenalumin 1. Nach der Sprühapplikation betrug der Gehalt 815 µg/g TG. Bis 8 Tage p.i. war die Entwicklung der f. sp. *avenae* an den Blättern von cv. "Erbgraf" fast völlig unterdrückt (Abb. 8). Während der Inokulationsperiode sank der Avenalumingehalt kontinuierlich ab (Abb. 9), lag aber bei der inokulierten Variante auf einem insgesamt höheren Niveau.

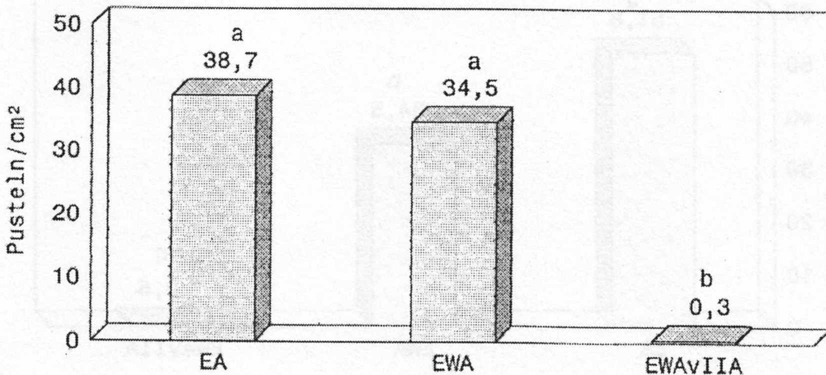

Abb. 8. Wirkung einer Avenalumin 2-Lösung auf den Mehltaubefall an Haferpflanzen

EA : Inokulation mit f. sp. *avenae* ohne Vorbehandlung

EWA: Inokulation mit f.sp. *avenae* nach Vorbehandlung mit Wasser

EWAvIIA : Inokulation mit f.sp. *avenae* nach Vorbehandlung mit Avenalumin 2 in Wasser

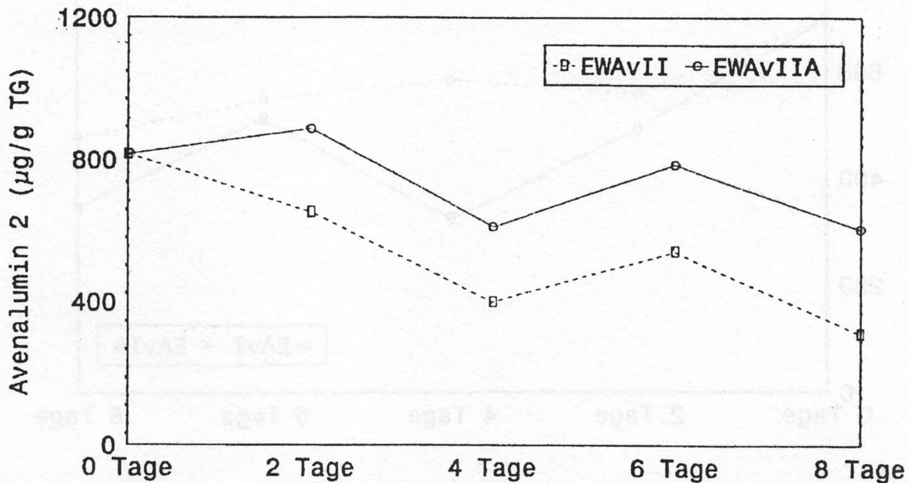

Abb. 8. Verlauf der Avenaluminsynthese bei unbehandelten und vorbehandelten Haferblättern
EAvII: Vorbehandlung mit Avenalumin 2 in Wasser ohne Inokulation
EAvIIA : Inokulation mit f.sp. *avenae* nach Vorbehandlung mit Avenalumin 2 in Wasser

Der Gehalt unbehandelter Blätter und der Wasserkontrolle betrug zu Versuchsende nach 8 Tagen 10 bzw. 34 µg/g TG. Es war demnach keine Neusynthese von Avenalumin 2 erfolgt. In einer Wiederholung dieser Versuche mit einem Gehalt von 800 Avenalumin 2 µg/g TG unmittelbar nach der Sprühapplikation wurde ein vergleichbares Resultat erhalten (Abb. 10).

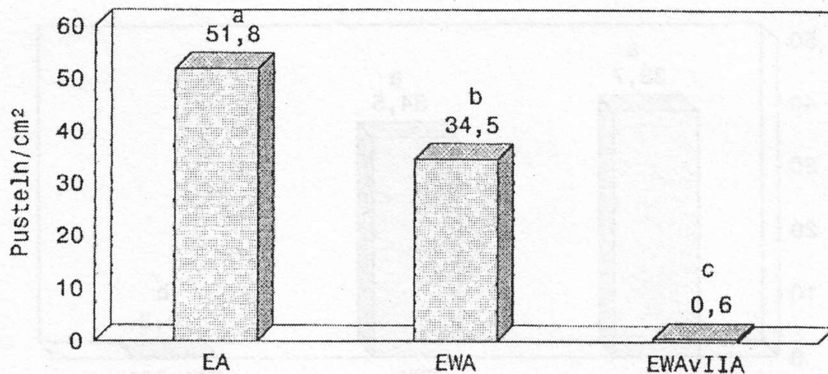

Abb. 10. Wirkung einer Avenalumin 2-Lösung auf den Mehltaubefall an Haferpflanzen
EA : Inokulation mit f. sp. *avenae* ohne Vorbehandlung
EWA: Inokulation mit f.sp. *avenae* nach Vorbehandlung mit Wasser
EWAvIIA : Inokulation mit f.sp. *avenae* nach Vorbehandlung mit Avenalumin 2 in Wasser

Hier war bei der Wasserkontrolle ein signifikant geringerer Befall vorhanden, der allerdings noch deutlich höher war, als bei der Avenaluminbehandlung mit 99% Wirkungsgrad. Auch in diesem Versuch sank die hohe Ausgangskonzentration von 800 µg/g TG kontinuierlich ab und betrug am Versuchsende nur noch 393 µg/g TG. Der Gehalt an Avenalumin 2 von Blättern unbehandelter Pflanzen und denen der Wasserkontrolle lag bei Versuchsende nach 8 Tagen bei 10 bzw. 82.5 µg/g TG.

Bisher waren alle Behandlungen protektiv erfolgt. Es wurde daher untersucht, ob und in welchem Maße postinfektionelle Sprühapplikationen von Avenalumin 1 und 2 eine antimykotische Wirkung haben. Dazu wurden 1 bzw. 3 Tage p.i. Lösungen appliziert, die jeweils zu einem Gehalt an Avenalumin 1 und 2 von 200 µg/g TG unmittelbar nach der Behandlung führten.

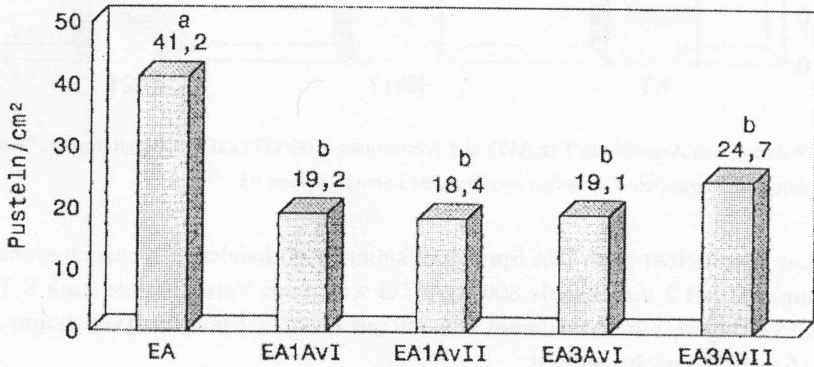

Abb. 11. Mehltaubefall mit Avenalumin 1 und 2 nachbehandelten Haferpflanzen

EA :	Inokulation mit f. sp. *avenae* ohne Nachbehandlung
EA1AvI :	Avenalumin 1 in Wasser 1 Tag nach einer Inokulation mit f. sp. *avenae*
EA1AvII :	Avenalumin 2 in Wasser 1 Tag nach einer Inokulation mit f. sp. *avenae*
EA3AvI :	Avenalumin 1 in Wasser 3 Tage nach einer Inokulation mit f. sp. *avenae*
EA3AvII :	Avenalumin 2 in Wasser 3 Tage nach einer Inokulation mit f. sp. *avenae*

In allen Fällen führten die postinfektionellen Behandlungen zu einem signifikant geringeren Befall (Abb. 11). Die Reduktionen waren allerdings deutlich geringer als bei protektiver Applikation.

Mehltaubefall an intakten Weizen- und Gerstenpflanzen (gesprüht mit Avenaluminlösungen)

Wenn die Avenalumine eine Besiedlung von Haferblättern durch die f. sp. *tritici* und f. sp. *hordei* verhindern, dann sollten sie auf Blätter von Weizen- bzw. Gerstenpflanzen appliziert die Entwicklung der an diesen Pflanzen kompatiblen Pathogene weitgehend unterbinden können. In Versuchen mit cv. "Kanzler" und cv. "Aura" wurde diese Hypothese bestätigt.

An Weizen bewirkten Avenalumin 1 und 2 eine signifikante und gleich hohe Reduktion der Befallsentwicklung der kompatiblen f. sp. *tritici* (Abb. 12).

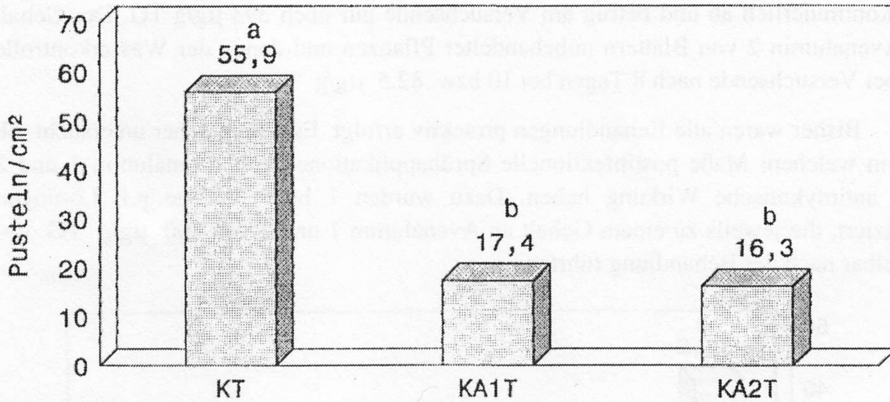

Abb. 12. Wirkung von Avenalumin 1 (KA1T) und Avenalumin 2 (KA2T) auf den Befall von cv. "Kanzler" durch die kompatible f. sp. *tritici* von *Erysiphe graminis* 8 Tage p.i.

Die unmittelbar nach den Sprühapplikationen vorhandenen Konzentrationen an Avenalumin 1 und 2 von jeweils 800 µg/g TG waren am Versuchsende nach 8 Tagen erheblich verringert. Für Avenalumin 1 waren nur noch 12.7 und für Avenalumin 2 nur noch 70.6 µg/g TG nachzuweisen.

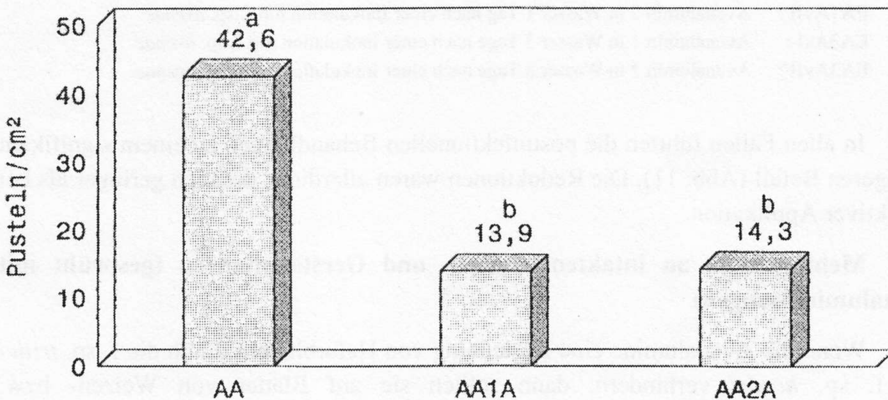

Abb. 12. Wirkung von Avenalumin 1 (AA1H) und Avenalumin 2 (AA2H) auf den Befall von cv. "Aura" durch die kompatible f. sp. *hordei* von *Erysiphe graminis* 8 Tage p.i.

An Gerste bewirkten die Avenalumine 1 und 2 eine gleich hohe Reduktion des Befalls mit der kompatiblen f. sp. *hordei* (Abb. 13).

Die unmittelbar nach den Sprühapplikationen vorhandenen Konzentrationen von 800 µg/g TG waren am Versuchsende nach 8 Tagen erheblich verringert. Für Avenalumin 1 waren nur noch 25.9 und für Avenalumin 2 nur 96.3 µg/g TG machzuweisen.

DISKUSSION

In dieser Arbeit, konnte nachgewiesen werden, daß Avenalumine an der Resistenz (Getreidepflanzen) gegen *E. graminis* eine entscheidende Rolle übernehmen.

An abgetrennten Haferblättern bewirkten Endkonzentrationen von 70-130 µg/g TG an Avenalumin 1 zum Zeitpunkt der protektiven Sprühapplikationen eine beträchtliche und signifikante Befallsreduktion 8 Tage p.i.. Bei der Avenaluminanalyse 2, 4, 6 und 8 Tage nach Anwendung ergab sich ein interessantes Phänomen. Ab dem 4. Tag war bei der unbehandelten Kontrolle, der Wasserkontrolle und der Avenaluminapplikation -ohne nachfolgende Inokulation mit der f. sp. *avenae*- ein deutlicher Anstieg der Avenaluminsynthese festzustellen. Dies ist mit großer Wahrscheinlichkeit auf eine zunehmende Seneszenz der abgetrennten Blätter zurückzuführen, worauf auch schon JERSCH (1986) hingewiesen hatte. Wurden statt 10 µg/g TG höhere Mengen von Avenalumin 1 von 800 µg/g TG appliziert, dann war die Entwicklung der f. sp. *avenae* erwartungsgemäß dratisch reduziert. Es erfolgte aber kaum eine *de novo* Synthese von Avenalumin. Dies könnte bedeuten, daß die höhere Avenaluminkonzentration auf bisher unbekannte Weise eine Induktion der Synthese bzw. den Syntheseablauf hemmen kann.

Wegen der Seneszenzeffekte abgetrennter Blätter wurden Avenalumin 1 und 2 auch auf Blätter intakter Pflanzen gesprüht. Die hohen Konzentrationen von 700-800 µg/g TG bewirkten eine starke Reduktion der Entwicklung der f. sp. *avenae,* wobei Avenalumin 2 eine deutlich bessere Wirkung hatte als Avenalumin 1. Auch hier erfolgte keine *de novo* Synthese von Avenaluminen.

Neben einer protektiven Sprühapplikation wurde auch die Wirkung einer postinfektionellen Anwendung 1 und 3 Tage p.i. geprüft. Avenalumin 1 und 2 bewirkten in allen Varianten eine gleichartige, signifikante Reduktion des Befalls mit der f. sp. *avenae,* der allerdings deutlich geringer war als bei protektiver Anwendung.

Von besonderer Bedeutung sind die Ergebnisse mit dem Winterweizen cv. "Kanzler" und der Sommergerste cv. "Aura". Hier führte eine protektive Sprühapplikation von Avenalumin 1 und 2 zu einer signifikanten Reduktion der entsprechenden kompatiblen f.sp. *tritici* oder *hordei.*

Die Ergebnisse komplementieren die Befunde zur induzierten Resistenz und bestätigen noch einmal die Rolle der Avenalumine an der Getreidepflanzen bei der Abwehr von ff. sp. von *graminis.*

ÖZET

TAHILLARIN *Erysiphe graminis*'e KARŞI DAYANIKLILIĞINDA AVENALUMINLERIN ROLLERI

Bu çalışmada Avenaluminlerin yulaf, buğday ve arpa yapraklarının *E. graminis*, küllemeye karşı dayanıklılığındaki rolü araştırıldı. Saf Avenaluminler Erbgraf yulaf çeşidinde kesilmiş yapraklara veya intakt bitkiler üzerine püskürtüldükten sonra hastalık gelişimi izlendi. Kesilmiş yapraklarda koruyucu olarak Avenalumin 70-130 µg/g KA (kuru ağırlık) konsantrasyonunda uygulanması sonucu hastalık şiddetinde infeksiyondan 8 gün sonra kolaylıkla farkedilen ve istatistiki olarak da çıkan bir azalma gözlenmiştir. Intakt bitki ile çalışıldığında uygulanan yüksek dozlar (700-800 µg/g KA) f. sp. *avenae* infeksiyonunu çok yüksek oranda azaltmıştır. Postinfeksiyonel (1-3 günlük) uygulamalar koruyucu uygulamalara göre daha düşük etkide bulunmuştur. Kanzler (buğday) ve Aura (arpa) çeşitlerine yapılan muamelenin de hastalık şiddetini azalttığı bulunmuştur.

Bu sonuçlara dayanılarak, Avenaluminlerin tahıl çeşitlerinin külleme hastalığına karşı dayanıklılığın uyarılmasında önemli bir rol üstlendikleri kanısına varılmıştır.

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Untersuchungen über die induzierten Resistenz an Hafer gegen *Erysiphe graminis* durch Vorinokulation mit inkompatiblen ff. sp. des Erregers

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ZUSAMMENFASSUNG

Welche Rolle spielen die induzierbaren Phytoalexine in Blättern von Haferpflanzen bei der Abwehr von ff. sp. von *Erysiphe graminis?* Zur Klärung dieser Frage wurde versucht, mittels avirulenter Vorinokulationen mit inkompatiblen Stämmen vor einer möglich nachfolgenden Inokulationen mit der f. sp. *avenae,* die Entwicklung der Pathogenese zu beeinflussen. Es wurden folgende Ergebnisse erhalten;

- Eine Vorinokulation von Blättern cv. "Erbgraf" mit Konidien der inkompatiblen ff. sp. *tritici* und *hordei* bewirkte in den meisten Fällen signifikante Befallsreduktion der 24 Stunden später nachinokulierten f. sp. *avenae.*

- Bei signifikanten Befallsunterschieden war immer eine negative Korrelationen zwischen Pustelzahl/cm^2 und dem Avenalumingehalt ($r = \geq - 0.70$) vorhanden. Damit konnte der induzierten Resistenz eine chemisch definierte Basis gegeben werden.

Mit diesen Ergebnissen wird die entscheidende Rolle der Avenalumine in Haferblättern bei der Abwehr der ff. sp. von *Erysiphe graminis* demonstriert.

EINLEITUNG

Über Resistenzinduktion durch Vorinokulation mit inkompatiblen Pilzarten oderstämmen wurde bereits berichtet (SCHÖNBECK et al. 1980, 1993). Unter anderem wurde dieses Phänomen auch bei Gerstenmehltau gefunden (SMEDEGARD-PETERSEN et al., 1992; THORDAL-CHRISTENSEN & SMEDEGARD-PETERSEN, 1988a) WILLEMS et al. (1994) gelang es, eine Befallsverminderung durch Vorinokulationen mit inkompatiblen ff. sp. von *E. graminis* zu erreichen und eine Beziehung zwischen Avenalumingehalt und dem Grad der Resistenzausprägung für das Wirt-Parasit-System *Avena sativa*-Hafermehltau herzustellen. Bei Hafer, Weizen und Gerste kann durch eine Vorinokulation mit inkompatiblen ff. sp. von *E. graminis* eine Resistenz gegen eine nachfolgende Inokulation mit der jeweiligen kompatiblen f. sp. induziert werden. Der Avenalumin 1-Gehalt und die Pustelzahl sind im System *Avena sativa*-Hafermehltau eng negativ korreliert (WILLEMS et al. 1994).

MATERIAL UND METHODEN

Saatgut: Für die Versuche an Haferpflanzen und -blättern dienten die Sorten Erbgraf sowie Barra und Ascot, (Saatzuchtanstalt Weibullsholm, Landskrona/Schweden). Weiterhin wurden die Gerstensorte Aura und die Weizensorte Kanzler verwendet.

Pilze: *Erysiphe graminis* f. sp. *avenae,* f. sp. *tritici* und f. sp. *hordei* wurden ursprünglich von Freilandpopulationen gewonnen und auf den entsprechenden Wirtspflanzen erhalten.

Gewinnung von Inokulum: Als Inokulum für *Erysiphe graminis* wurden befallene Pflanzen einen Tag vor der Inokulumgewinnung gründlich geschüttelt, um alte Konidien zu entfernen. Am nächsten Tag standen dann neu gebildete, infektionstüchtige Konidien zur Verfugung.

Inokulationsversuche mit Hafermehltau: Es wurden in viereckigen Töpfen (9x9 cm) an einem Rand ausgesäte 10 Tage alte Haferpflanzen verwendet und die Oberfläche ihrer Primärblätter für die Mehltauinokulation durch Fixieren mit Hilfe von Gummiringen auf Pappe oder durchsichtigen Plexiglasplatten (7x24 cm) ausgerichtet.

Mit frisch gebildeten und infektionstüchtigen Konidien wurden ausgerichtete Pflanzen in einem großen Impfturm nach der Methode von KILIAN (1990) inokuliert. Die Inokulumdichte/cm^2 wurde mit einer Fuchs-Rosenthal-Zählkammer bestimmt. Nach der Inokulation wurden die Pflanzen in mit Wasser besprühten Cellophanbeuteln eingetütet und für 24 Stunden bei 20 °C im Klimaschrank inkubiert, um eine optimale Keimung der Konidien zu gewährleisten.

Vorinokulationsversuche: Haferpflanzen wurden 2, 4, 6, 8 und 10 Tage vor einer Inokulation mit der kompatiblen *E. graminis* f. sp. *avenae* mit inkompatiblen ff. sp. vorinokuliert. Die Vorinokulationen mit *E. graminis* f. sp. *tritici* und *E. graminis* f. sp. *hordei* wurden jeweils mit 80 mg Konidieneinwage durchgeführt. Nach 24-stündigem Eintüten mit Cellophanbeuteln wurden die Pflanzen mit 20 mg Konidien von *Erysiphe graminis* f. sp. *avenae* nachinokuliert. Dieser Vorgang wurde im Abstand von 2, 4, 6, 8 und 10 Tagen nach der Vorinokulation durchgeführt. Frisch inokulierte Pflanzen wurden immer für die ersten 24 Stunden zur Keimung in Cellophanbeuteln eingetütet und insgesamt 8 Tage im Klimaschrank inkubiert. Die Pusteln wurden 8 Tage nach der Inokulation mit der f. sp. *avenae* gezählt, zum gleichen Zeitpunkt wurden die Proben für die Avenaluminanalyes entnommen und in Schnappdeckel-gläsern eingefroren.

HPLC-Bedingungen: Die Anlage bestand aus einem Gradientenformer 250 B, Hochdruckpumpe 300 CS, und UV-Detektoren SP4, SP6. Stationäre Phase hat als Merck Lichrospher 100, RP 18, Partikelgröße 5 µm, 250x4 mm mit Vorsäulenkartusche 4x4 mm und Mobile Phase als 24-40% Acetonitril (mit gradienten Verlauf) stattgefunden.

ERGEBNISSE

Vorinokulation mit inkompatiblen ff. sp. von *Erysiphe graminis:* Bei allen Vorinokulationsversuchen mit inkompatiblen ff. sp. wurden 24 Stunden später die Inokulationen mit der kompatiblen f. sp. *avenae* durchgeführt. Im ersten Versuch wurde bei mit f. sp. *tritici* vorinokulierten Varianten eine Befallsverminderung in Höhe von 76% ermittelt. Die Pustelzahlen der mit f. sp. *tritici* vorinokulierten und der nicht vorinokulierten Haferpflanzen waren signifikant verschieden (Abb. 1). Vorinokulation mit f. sp. *hordei* hatte keine befallsvermindernde Wirkung.

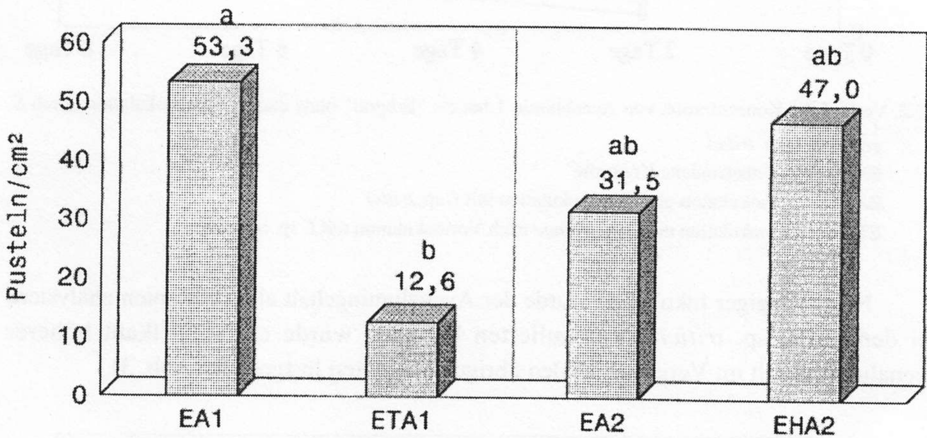

Abb.1. Mehtaubbefall von cv. "Erbgraf" durch *E. graminis* f. sp. *avenae* in Abhängigkeit von einer Vorinokulation mit inkompatiblen ff. sp. von *E. graminis* (Gleiche Buchstaben bedeuten nicht signifikant verschiedene Mittelwerte)

EA1 : Inokulation ohne Vorinokulation mit f. sp. *tritici*
ETA1 : Inokulation mit f. sp. *avenae* nach Vorinokulation mit f. sp. *tritici*
EA2 : Inokulation ohne Vorinokulation mit f. sp. *hordei*
EHA2 : Inokulation mit f. sp. *avenae* nach Vorinokulation mit f. sp. *hordei*
(1 und 2 bedeuten unabhängige Versuche)

Bei allen Varianten lag der Avenalumingehalt am 2. Tag p.i. im Bereich von 5-10 µg/g TG. Der Avenalumingehalt blieb bei den unbehandelten Kontrollpflanzen (EK) während des Untersuchungsintervalls konstant. Nach Inokulation mit f. sp. *avenae* (EA1) stieg der Avenalumingehalt bis zum 8. Tag bis auf ca. 30 µg/g TG an. Bei den mif f. sp. *tritici* vorinokulierten Haferpflanzen (ETA1) lag die Syntheserate deutlich höher, der Gehalt betrug am 8. Tag annähernd 70 µg Av. 1/g TG (Abb. 2).

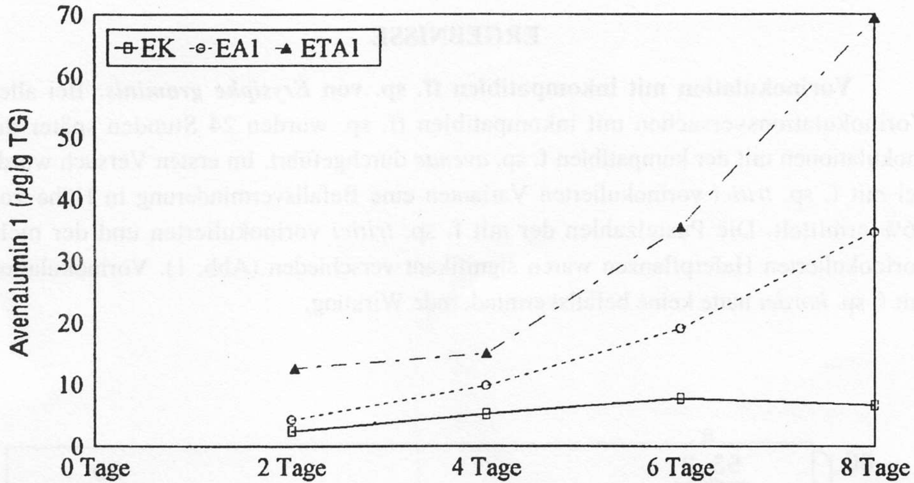

Abb.2. Verlauf der Konzentration von Avenalumin 1 bei cv. "Erbgraf" ohne und mit Vorinokulation durch *E. graminis* f. sp. *tritici*

EK : Unbehandelte Kontrolle
EA1 : Inokulation ohne Vorinokulation mit f. sp. *tritici*
ETA1 : Inokulation mit f. sp. *avenae* nach Vorinokulation mit f. sp. *tritici*

Nach 8-tägiger Inkubation wurde der Avenalumingehalt aller Varianten analysiert. Bei den mit f. sp. *tritici* vorinokulierten Pflanzen wurde ein signifikant höherer Avenalumingehalt im Vergleich zu den übrigen Varianten festgestellt (Abb. 3).

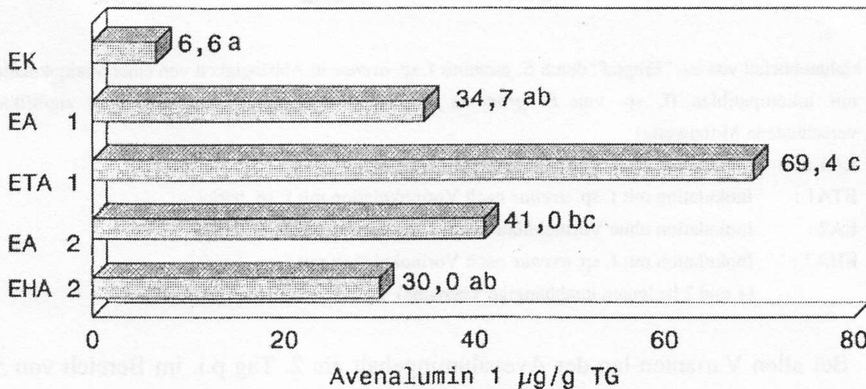

Abb.3. Avenalumingehalt in Blättern der Inokulationsvarianten von cv. "Erbgraf" nach 8-tägiger Inkubation

EA1 : Inokulation ohne Vorinokulation mit f. sp. *tritici*
ETA1 : Inokulation mit f. sp. *avenae* nach Vorinokulation mit f. sp. *tritici*
EA2 : Inokulation ohne Vorinokulation mit f. sp. *hordei*
EHA2 : Inokulation mit f. sp. *avenae* nach Vorinokulation mit f. sp. *hordei*

Zwischen Pustelzahlen/cm² und dem Avenalumingehalt der mit f. sp. *tritici* vorinokulierten und mit f. sp. *avenae* nachinokulierten Pflanzen wurde eine negative Korrelation mit r= -0.93 ermittelt. Je höher der Avenalumingehalt im Gewebe, desto weniger Pusteln von f. sp. *avenae* wurden gebildet (Abb. 3).

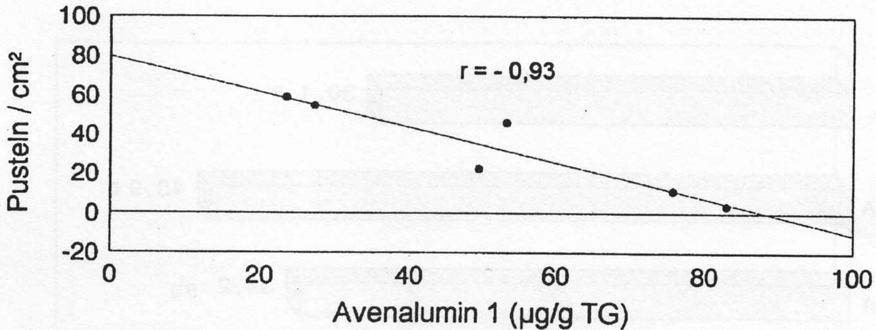

Abb.4. Korrelation zwischen Pusteln und Avenalumingehalt
Vorinokulation mit f. sp. *tritici*, Nachinokulation mit f. sp. *avenae*

Beim einem dritten Versuchsansatz wurde mit beiden Vorinokulationsvarianten eine Befallsverminderung erreicht. Der Unterschied gegenüber der Kontrolle war nur bei ETA signifikant. Vorinokulation mit f. sp. *tritici* ergab einen signifikanten Unterschied zur Kontrolle und eine Befallsverminderung von 40%. Mit f. sp. *hordei* vorinokulierten Varianten wiesen 23% weniger Pusteln auf (Abb. 5).

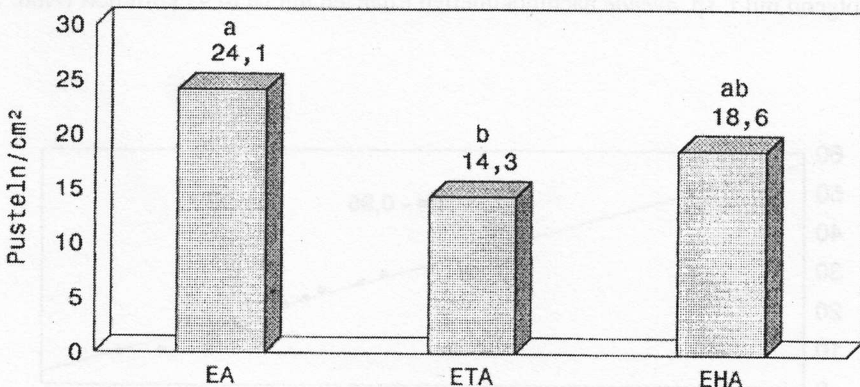

Abb.5. Mehtaubbefall von cv. "Erbgraf" durch *E. graminis* f. sp. *avenae* in Abhängigkeit von einer Vorinokulation mit inkompatiblen ff. sp. von *E. graminis*

EA : Inokulation ohne Vorinokulation mit f. sp. *tritici* und *hordei*
ETA : Inokulation mit f. sp. *avenae* nach Vorinokulation mit f. sp. *tritici*
EHA : Inokulation mit f. sp. *avenae* nach Vorinokulation mit f. sp. *hordei*

Die Avenaluminsynthese war bei mit f. sp. *tritici* vorinokulierten Blättern (ETA) ebenfalls signifikant höher als bei der Kontrollvariante (EA). Nach Vorinokulation mit f. sp. *hordei* (EHA) war kein signifikanter Unterschied zur Kontrolle (EA) feststellbar (Abb. 6).

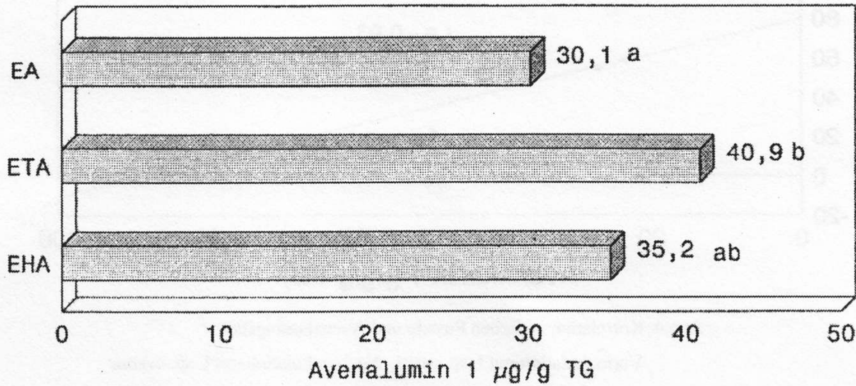

Abb.6. Avenalumingehalt in verschiedenen Varianten nach 8-tägiger Inkubation

EA : Inokulation ohne Vorinokulation mit f. sp. *tritici* und *hordei*

ETA : Inokulation mit f. sp. *avenae* 1 Tag nach der Vorinokulation mit f. sp. *tritici*

EHA : Inokulation mit f. sp. *avenae* 1 Tag nach der Vorinokulation mit f. sp. *hordei*

Pustelzahl und Avenalumingehalt waren bei allen Vorinokulationsvarianten und nachfolgend mit f. sp. *avenae* nachinokulierten Pflanzen mit r= -0.95 korreliert (Abb. 7).

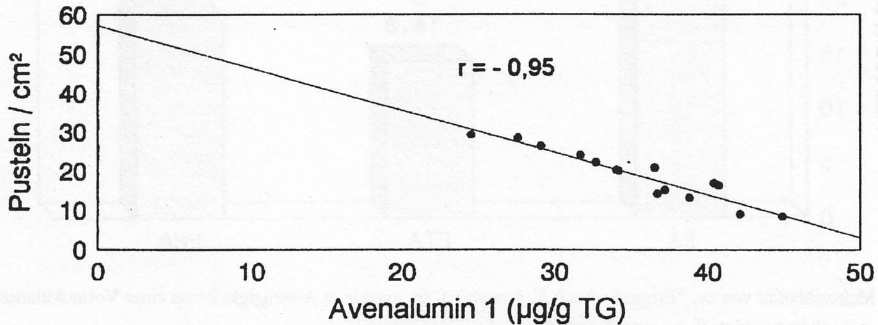

Abb.7. Korrelation zwischen Pusteln und Avenalumingehalt

Vorinokulaiton mit f. sp. *tritici* oder mit f. sp. *hordei*, Nachinokulation mit f. sp. *avenae*

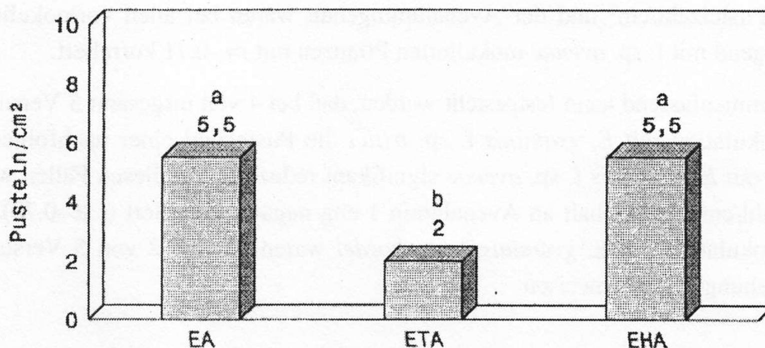

Abb.8. Mehtaubefall an Inokulationsvarianten von cv. "Erbgraf"

EA : Inokulation mit f. sp. *avenae* ohne Vorinokulation
ETA : Inokulation mit f. sp. *avenae* nach Vorinokulation mit f. sp. *tritici*
EHA : Inokulation mit f. sp. *avenae* nach Vorinokulation mit f. sp. *hordei*

Bei einem weiteren Versuchsansatz mit cv. "Erbgraf" zeigte eine Vorinokulation mit f. sp. *tritici* erneut eine signifikant befallsvermindernde Wirkung, die Pustelzahl wurde um 65% reduziert (Abb. 8). Der Avenalumingehalt der mit f. sp. *tritici* vorinokulierten Varianten war im Vergleich zu den Kontrollen signifikant erhöht (Abb. 9). Auch bei diesem Versuch war der Avenalumingehalt nach kombinierter Inokulation (ETA) signifikant höher als bei *E. graminis* f. sp. *avenae* (EA) und f. sp. *tritici* (ET) jeweils allein. Bei den mit f. sp. *hordei* inokulierten Varianten waren die einfache und die mehrfache Inokulation hinsichtlich des Avenalumingehaltes gleich.

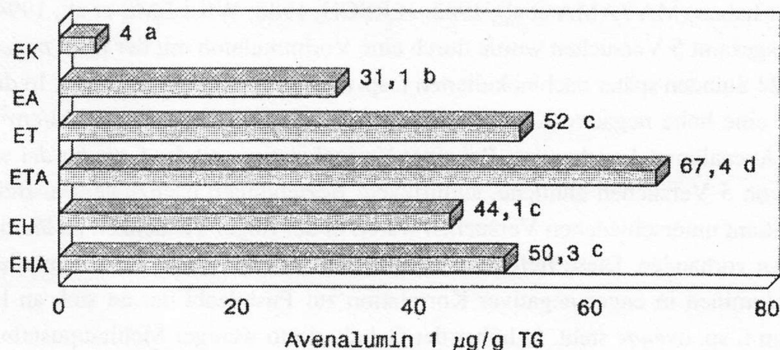

Abb. 9. Avenalumingehalt verschieder behandelten und mit *E. graminis* f. sp. *avenae* inokulierter Pflanzen von cv. "Erbgraf" nach 8-tägiger Inkubation

EK : Unbehandelte Kontrolle
EA : Inokulation f. sp. *avenae* ohne Vorinokulation
ET : Vorinokulation mit f. sp. *tritici*
ETA : Inokulation mit f. sp. *avenae* nach Vorinokulation mit f. sp. *tritici*
EH : Vorinokulation mit f. sp. *hordei*
EHA : Inokulation mit f. sp. *avenae* nach Vorinokulation mit f. sp. *hordei*

Die Pustelzahl/cm^2 und der Avenalumingehalt waren bei allen vorinokulierten und nachfolgend mit f. sp. *avenae* inokulierten Pflanzen mit r= -0.71 korreliert.

Zusammenfassend kann festgestellt werden, daß bei 4 von insgesamt 5 Versuchen eine Vorinokulation mit *E. graminis* f. sp. *tritici* die Pustelzahl einer nachfolgenden Inokulation mit *E. graminis* f. sp. *avenae* signifikant reduzierte. In diesen Fällen waren die Pustelzahl/cm^2 und Gehalt an Avenalumin 1 eng negativ korreliert ($r \geq -0.70$). Bei einer Vorinokulation mit *E. graminis* f. sp. *hordei* waren nur bei 2 von 5 Versuchen solche Beziehungen nachzuweisen.

DISKUSSION

Es wurde versucht, mit den an Hafer inkompatiblen ff. sp. *tritici* und *hordei* durch Vorinokulation eine Resistenz gegen die 24 Stunden später nachinokulierte kompatible f. sp. *avenae* auszulösen. Das dies an Getreide mit den jeweils inkompatiblen ff. sp. von *E. graminis* bzw. avirulenten Stämmen eines an sich kompatiblen Erregers grundsätzlich möglich ist wurde schon berichtet (CHAUDHARY et al., 1983; THORDAL-CHRISTENSEN & SMEDEGARD-PETERSEN, 1988a, b; SMEDEGARD-PETERSEN et al., 1992; VILICH-MEYER & WELTZIEN, 1989, 1990). Diese Veröffentlichungen beschreiben allerdings nur das Phänomen der induzierten Resistenz, ohne Kausalanalyse der stofflichen bzw. morphologischen Ursachen. Im vorliegenden Fall ist dies anders, denn die induzierte Resistenz kann zu den Avenaluminen in Beziehung gesetzt werden, die offensichtlich eine Abwehrfunktion gegen obligat biotrophe pilzliche Krankheitserreger haben (MAYAMA et al., 1982; JERSCH, 1986; WILLEMS et al., 1994). In vier von insgesamt 5 Versuchen wurde durch eine Vorinokulaton mit der f. sp. *tritici* den Befall der 24 Stunden später nachinokulierten f. sp. *avenae* signifikant reduziert. In diesen Fällen war eine hohe negative Korrelation von $r \geq -0.90$ zwischen Pustelzahl/cm^2 und Gehald an Avenalumin 1 vorhanden. Bei einer Vorinokulation mit der f. sp. *hordei* waren nur bei 2 von 5 Versuchen ähnliche, signifikante Beziehungen nachzuweisen. Bei den nich signifikant unterschiedenen Versuchen waren in der Regel Tendenzen zu ähnlichen Beziehungen vorhanden. Diese Befunden belegen klar, daß die Höhe der Akkumulierung von Avenaluminen in enger negativer Korrelation zur Pustelzahl der an sich an Hafer kompatiblen f. sp. *avenae* steht. Je höher der Gehalt, desto weniger Mehltaupusteln sind vorhanden. Wenn schon die kompatible f. sp. in solchem Maße durch Avenalumine gehemmt wird, dann darf angenommen werden, daß die Abwehr der inkompatiblen ff. sp. *tritici* und *hordei* an Haferblättern entscheidend durch die Höhe der von ihnen selbst induzierten Avenalumingehalten bestimmt wird. Die in der vorliegenden dargestellten Werte belegen eindrucksvoll diese Schlußfolgerung.

ÖZET

YULAFTA KÜLLEME ETMENİ *Erysiphe graminis*'e KARŞI INKOMPATIBLE FORM SPECIES ÖNİNOKULASYONU YOLUYLA DAYANIKLILIĞIN UYARILMASI ÜZERİNDE ARAŞTIRMALAR

Bu çalışmada yulaf bitkilerinin bu bitkide patojen olmayan inkompatible *E. graminis* f. sp. *tiritici* ve *E. graminis* f. sp. *hordei* ile önceden inokule edilmeleri halinde kompatible etmen *E. graminis* f. sp. *avenae*'ye karşı dayanıklılık responsları araştırılmıştır.

Araştırmada dayanıklılığın uyarımı yulafta varlığı bilinen fitoaleksinlerden Avenaluminlerin niceliklerinin değişimi ve püstül sayısındaki azalmalar yoluyla değerlendirilmiştir.

Sonuçta;

- İnokompatibl (uyumsuz) *E. graminis* f.sp. *tritici* ve *E. graminis* f.sp. *hordei* konidileri ile yulaf yapraklarının öninokulasyonu genellikle 24 saat sonra kompatibl (uyumlu) f. sp. *avenae* tarafından oluşturulan hastalığın şiddetini ve önemli derecede etkilemiştir.

- Hastalık şiddetinin durumunda yaprakta püstül sayısı ile Avenalumin içeriği arasında negatif bir korelasyon var olmuştur ($r = \geq - 0.70$).

- Bu sonuçlar sayesinde yulaftaki uyarılmış dayanıklılığın biyokimyasal karakterinin Avenalumin oluşumuna bağlı olduğu ortaya konmuştur.

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Determination of PVX and PVS Symptoms on Some Test Plants and Identification of These Viruses Using dsRNA Analysis

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ABSTRACT

This study was conducted to determine the symptoms of potato X (PVX) and potato S (PVS) viruses on some test plants, and to identify these viruses on the basis dsRNA analyses from infected plant leaves. In this study *Datura stramonium* and *Chenopodium quinoa* were used for isolation, *Nicotiana glutinosa* and *N. clevelandii* used for multiplication of PVX and PVS, respectively. Mechanical inoculation results showed that PVX caused local lesion on *Chenopodium album, Chenopodium amaranticolor* and *C. quinoa;* necrotic local lesions on *Gomphrena globosa;* systemic symptoms on *D. stramonium, Lycopersicon esculentum, Nicotiana benthemiana, N. debneyii, N. glutinosa, N. rustica, N. sylvestris, N. tabacum* Samsun NN, *N. tabacum* Samsun Typ, *N. tabacum* White Burley, *N. tabacum* Xanthii and *Physalis floridana.* However, PVX did not exhibit any visual symptoms on *N. clevelandii.* PVS was able to show local lesions on *C. album, C. amaranticolor* and *C. quinoa;* systemic symptoms on *N. benthemiana, N. clevelandii, N. debneyii, N. glutinosa, N. sylvestris, N. tabacum* Samsun Typ, *N. tabacum* White Burley and *N. tabacum* Xanthii, and no symptom on *D. stramonium, G. globosa, L. esculentum, N. rustica, N. tabacum* Samsun NN and *P. floridana* plants. dsRNA of PVX were isolated from both *N. glutinosa* and *N. tabacum* Xanthii plants, and dsRNA of PVS were obtained from *C. quinoa* plants. Our results demonstrated that only single band was detected from PVX infected plant leaves, but dsRNA profile was not involved in PVS.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most important vegetable crops of cultivation and industry to feed human population in the world as a basic food (Esendal, 1990). Therefore it has the forth plant among the other crop plants for the worlwide production (Anonymous, 1992). One of the important factors which limits the productivity of potato in Turkey is diseases. Agrios (1997), stated that diseases cause annualy 21.8% yield loses from potato production. Shepard and Claflin (1975), pointed out that propagation of potato by tuber make it more sensitive to plant pathogens. Whereas it was reported that nearly 50 viruses and viroids besides the other pathogens cause diseases on potatoes (Peter et al., 1981; Hooker, 1982).

To increase or at least to maintain the same level in potato production, it is necessary that potato seed must be changed in every three years and virus-free seeds should be used for planting (Courtbaoui, 1984). The previous studies demonstrated that chemical applications for management of diseases causes virus was not effective (Jayasinghe, 1988).

The objectives of this study, to determine the symptoms of PVX and PVS on test plants, and to identify the causal agents by isolation and analysis of the replicative form (dsRNA) of subgenomic RNAse derived from the genomic RNA.

MATERIALS and METHODS

Plant materials infected by PVX exhibiting symptoms of mosaic, swelling between veins and rolling leaves (Bercks, 1970; Şahtiyancı, 1972; Kurçman, 1979; McDonald, 1984; Wright et al., 1994), and infected plants by PVS showing symptoms deepening of veins, curving leaves, and mottling, bronzing or rugosity of the foliage were collected from potato fields (Wetter, 1971; Şahtiyancı, 1972; Wright et al., 1994), in Aşkale, Horasan, İspir, Narman, Oltu, Pasinler and Tortum districts of Erzurum region.

The collected plants samples were kept at -27 ^{o}C for further tests. *D. stramonium* which is the systemic host of PVX, and *N. glutinosa,* were used for isolation and multiplication of PVX, respectively (Bercks, 1970; Allison and Shalla, 1973; Krachanova et al., 1978; Kurçman, 1979; Talens, 1979; Grama et al., 1981; Çıtır, 1982). On the other hand, *C. quinoa* and *N. clevelandii* plants were used for isolation and multiplication of PVS, respectively (Wetter, 1971; Goth and Weeb, 1974; Hiruki, 1975; Slack, 1983; Dolby and Jones, 1987). The identifying of PVX and PVS were determined according to their symptoms developments on the test plants listed in Table 1.

The test plants were inoculated as using mechanical inoculation method in which 1 ml of phospate buffer (0.01 M pH: 7.2) 1% of 2-mercaptoethanol were mixed with 1 gr of infected plant leaves. The prepared inoculums were applied on the three plants with 3-4 leaves of each test plants species powdered with 500 mesh-carborandum. The inoculated plants were incubated about 4 weeks under greenhouse condition and observed for symptoms development.

The isolation of dsRNA of PVX were performed from 7 gr leaf tissues of *N. glutinosa* and *N. tabacum* Xanthii plants, and dsRNA of PVS were isolated from *C. quinoa* plants (Morris and Dodds 1979). The plants used in this experiment were inoculated with the test plants with viruses 10 days ago isolations.

For this study, the leaf samples were homogenised in the mixture of 1xSTE buffer solution (0.1 M NaCl, 0.05 M Tris, 0.001 EDTA, 6.8 pH) including 1 % bentonit, 1 % SDS (Sodium Dodecyl Sulphate), phenol and chloroform - pentanol (25:1 v/v).

The mixture was centrifuged (10000 rpm) for 20 min. The supernatant was washed through CF-11 cellulose column with 60 ml of STE buffer solution containing 16 % ethanol, and dsRNA were separated by washing the column with STE buffer without ethanol. The ds RNAs of the viruses were electrophoresed in 1.2 % of agarose

gel for 3 hour at 100 V in 1xTBE buffer (0.1 M Tris, 0.089 M Boric acid, 0.002 M EDTA; pH: 8). The λ DNA Hind III were used as size of the marker in kb and then the gel stained with Ethidium Bromide and dsRNA bands were visualised under UV light.

RESULTS and DISCUSSION

The symptoms PVX on the plants of *C. amaranticolor, C. quinoa, D. stramonium, G. globosa, L. esculentum, N. glutinosa, N. rustica, N. tabacum* Samsun NN, *N. tabacum* Samsun Typ, *N. tabacum* White Burley and *N. tabacum* Xanthii, were shown in Table 1. These symptoms observed on these plants conformed by the findings by Bercks (1970), Allison and Shalla (1973), Kurçman (1979), Talens (1979), Grama et al. (1981), Çıtır (1982). It has been found that PVX caused local lesion on *C. album* and systemic mosaic on *P. floridana*. However, Çıtır (1982) recorded that PVX did not cause symptoms on these test plants. The difference between the results of these two test plants can be thought from the age of test plants, at the time of inoculation performed and the differences in the greenhouse condition after inoculation as well as from the differences in the race of viruses used in this experiment. The symptoms of PVX (Table 1) were observed on the plants of *N. benthemiana, N. clevelandi, N. debneyii* and *N. sylvestris*, However according to our knowledge, there is no regard in the literature about occurrence of disease caused by PVX on these plants.

Table 1. Time table of the symptoms caused by PVX and PVS viruses on some test plant.

Host plants	PVX		PVS	
	Symptoms	Day	Symptoms	Day
Chenopodium album L.	1	12-14	1	14-17
Chenopodium amaranticolor Coste	1	8-9	1	14-17
Chenopodium quinoa Wild	1	9-10	1-2	14-17
Datura stramonium L.	3	13-15	0	-
Gomphrena globosa L	4	5-7	0	-
Lycopersicon esculentum Mill	3	8-9	0	-
Nicotiana benthemiana L	5	8-9	6	16-18
Nicotiana clevelandii Gray	0	12-14	6	14-16
Nicotiana debneyii Domin	7	10-12	6	10-14
Nicotiana glutinosa L	8-9	7-9	10-11	12-15
Nicotiana rustica L	12	11-12	0	-
N. sylvestris Spegaz et Comes Grav	12	-	13.2	13-15
Nicotiana tabacum L. Samsun NN	12	12-13	0	-
N.t. L. Samsun Typ	9	10-11	2-11	14-16
N.t. L. White Burley	9	9-10	2-11	13-15
N.t. L. Xanthii	9	8-9	2-11	11-13
Physalis floridana Rvbd	8	9-10	0	-

0: No symptoms
1: Local lesions
2: Yellowing
3: Mosaic
4: Necrotic local lesions
5: Ring spot
6: Color brightness
7: Distortion and stunting
8: Severe Mosaic
9: Vein clearing
10: Dwarfing
11: Distortion of younger leaves
12: Mild mosaic
13: Leaf deformation

The symptoms of PVS (Table 1) were observed on the test plants of *C. album, C. amaranticolor, C. quinoa, D. stramonium, G. globosa, L. esculentum, N. clevelandi, N. debneyii, N. rustica, N. tabacum* Xanthii and *P. floridana* were shown in (Table 1). These smyptoms observed on these plants conformed by the foundlings by Wetter, 1971; Goth and Webb, 1974; Hiruki, 1975; Çıtır, 1982; Slack, 1983; Dolby and Jones, 1987. On the other hand, PVS symptoms on *N. glutinosa, N. tabacum* Samsun NN, *N. tabacum* Samsun Type and *N. tabacum* White Burley (Table 1) were not observed by Çıtır (1982). The differences between our study and Çıtır (1982), may probably be due to the same factors mentioned above for PVX. Furthermore, in our data, the results demonstrated that PVS is able to cause systemic mosaic symptoms on *N. benthemiana* and no symptoms on *N. sylvestris*. As far as we know these plants were not tested in previous studies.

In our study, a dsRNA profile of PVX belonging to potex virus group was obtained from both, *N. glutinosa* and *N. tabacum* Xanthii plants, and no bands obtained from as used healthy control plant (Fig 1). The profiles obtained from diseased leaves always presented a single intense band corresponding to a molecule of estimated size about 6.6 kbp. These finding also supported by the previous reports (Valverde et al., 1986).

On the other hand, single band of PVS belonging to carlavirus group was isolated from both infected and healthy control plants of *C. quinoa* (Fig 2). This result indicated that the band is found in our study did not belong to PVS. Previous report showed that molecular weight of dsRNA of PVS supposed to be $4.8x10^6$ (Valverde et al., 1986). The date suggest that the band may be host plant originated DNA.

Brunt (1988), demonstrated that the concentration of various virus groups in plant tissues were different such as the concentration of PVX in belonging to potexvirus group was determined as 250-3000 mg/kg. PVS in carlavirus group 30-1000 mg/kg and PVY in potyviruses group 5-200 mg/kg. In our study, we were able to detect PVS using the standard ds RNA isolation method. It is possible that concentration of PVS virus in infected plant samples used in our study was quite low to detect. Thus, it is necessary to try different dsRNA isolation methods or different detection and visualisation method or gel.

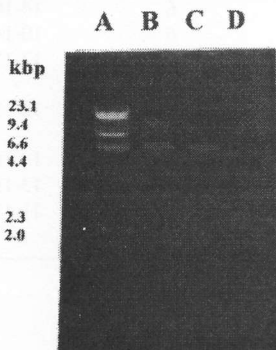

Figure 1.A: λ DNA Hint III, B: *N. glutinosa* infected with PVX, C: *N. tabacum* xanthii infected with PVX, D: Healthy *N. glutinosa*

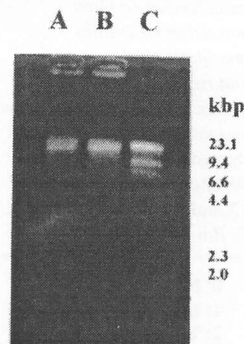

Figure 2.A: Healthy *C.quinoa*, B: *C. quinoa* infected with PVS, C: λ DNA Hint II

ÖZET

PATATES X ve S VİRÜSLERİNİN BAZI TEST BİTKİLERİNDE NEDEN OLDUĞU SİMPTOMLARIN BELİRLENMESİ ve BU VİRÜSLERİN dsRNA ANALİZİ ile TANIMLANMASI

Bu çalışma, patates X virüsü (PVX) ve patates S virüsü (PVS)'nün bazı test bitkilerinde oluşturduğu simptomların belirlenmesi ve bu virusların dsRNA analizi ile tanılanması amacıyla yapılmıştır. PVX'in izolasyonunda *Datura stramonium,* çoğaltımında *Nicotiana glutinosa* bitkisi kullanılmıştır. Yapılan mekaniksel inokulasyonlar sonucunda PVX'in *Chenopodium album, C. amaranticolor* ve *C. quinoa* bitkilerinde lokal lezyon; *Gomphrena globosa*'da nektorik lokal lezyon; *D. stramonium, Lycopersicon esculentum, Nicotiana benthemiana, N. debneyii, N. glutinosa, N. sylvestris, N. tabacum* Samsun NN, *N. tabacum* Samsun Typ, *N. tabacum* White Burley, *N. tabacum* Xanthii ile *Physalis floridana* bitkilerinde ise sistemik simptomlara neden olduğu, buna karşı *N. clevelandii* ve *N. rustica* bitkilerinde ise herhangi bir simptoma neden olmadığı belirlenmiştir. PVS virüsünün izolasyonunda *C. quinoa,* çoğaltımında ise *N. clevelandii* bitkisi kullanılmıştır. Yapılan mekaniksel inokulasyonlarda, PVS'nin *C. album, C. amaranticolor* ve *C. quinoa* bitkilerinde lokal lezyon; *N. benthemiana, N. clevelandii, N. debneyii, N. glutinosa, N. sylvestris, N. tabacum* Samsun Typ, *N. tabacum* White Burley ile *N. tabacum* Xanthii bitkilerinde sistemik simptomlara neden olduğu, buna karşı *N. rustica, G. globosa, D. stramonium, L. esculentum, N. tabacum* Samsun NN ve *P. floridana* bitkilerinde ise herhangi bir simptom oluşturmadığı belirlenmiştir. PVX'e ait dsRNA'lar, *N. glutinosa* ve *N. tabacum* Xanthii, bitkilerinden izole edilmiştir. PVS'ye ait dsRNA'lar ise *C. quinoa* bitkisinden izole edilmiştir. Sonuçda, PVX'in tek banda sahip dsRNA profili elde edilirken, PVS virüsüne ait dsRNA profili elde edileme-miştir.

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Occureance of Hop Stunt Viroid (HSVd) on Pomegranate *(Punica granatum)* Trees in Turkey

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ABSTRACT

Hop student viroid (HSVd) has been found a number of herbaceous and woody hosts, such as grapevine, *Citrus* and *Prunus* plants. 10 pomegranate (*Punica granatum*) trees were checked about viroid containment. Using sPAGE and RT-PCR methods HSVd was obtained from 2 pomagranate cultivars (Çekirdeksiz, Beynarı). This is a first report that HSVd on pomegranate trees in Turkey.

INTRODUCTION

Pomegranate *(Punica granatum)* was produced worldwide in many commercial plantings. In Turkey there were 2.350.000 pomegranate trees planted and pomegranate production was 56.000 ton in 1996 (Anonymous, 1996).

Viroids are the smallest known pathogens of plants. They are single-stranted, circular, highly structured, rod like RNAs without any protein capsid (Diener 1987, Semancik 1987). The known viroid species were suggested to belong into two families; Avsunviroidae and Pospiviroidae. Hop stunt viroid (HSVd) were included into the Pospiviroidae family and Hop stunt viroid genus (Flores et al., 1998) HSVd has been found in a wide range of hosts including hop, cucumber, grape, citrus, plum, peach (Shikata, 1990) and recently apricot, almond and pomegranate (Astruc et al., 196). This paper is a first record presents the occureance of HSVd in pomegranate trees in Turkey.

MATERIALS and METHODS

Plant Material

Ten Pomagranate trees were selected from the collection parsel, Alata Horticultural Research Institute, for checking viroid profile. Young leaf samples from pomegranate trees were collected and used for viroid detection.

Nucleic acid extraction and purification

Viroid RNAs were extracted according to Semancik et al., (1998) using young leaves. Preparation of 2 M LiCl soluble nucleic acid were applied to CF-II cellulose col-

umns. Viroid RNAs were detected and isolated by sPAGE (Semancik and Harper, 1984; Rivera Bustamente et al., 1986) followed by staining with silver for greater sensitivity in viroid detection (Igloi, 1983).

cDNA Synthesis

Viroid RNAs were amplified by RT-PCR (Sambrook, et al., 1989; Yang et al., 1992) using one pair HVSd-spesific synthetic oligonucleotide identical to homologeus residues 271-288 (5'-CTGGATCCGCGGCAGAGGCT-3') and coplementary 283-302 (5'-CCGGATCCTCTCTTGAGCCCT-3'). First strand cDNA was synthesized from RNA extracts by reverse transcriptase (Visvader and Symons, 1985). The dsRNA was amplified by PCR using the method previously described by Semancik et al. (1983). Aliquots of the amplified DNA products were analysed in %2 agarose gels.

RESULTS and DISCUSSION

Using sPAGE analysis one band was detected from 2 local pomegranate cultivars (Çekirdeksiz, Beynarı) which had the same electrophoretic mobility with hop stunt viroid (HSVd). No viroid band was found other 8 pomegranate trees. Fig 1 shows the size determination of RT-PCR products of pomegranate trees. The viroid band had 300 nucleotide in size which is spesific for HSVd.

Hop stunt viroid was first described as the causal agent of the stunt disease of hops in Japon. Than it has been found in several plant species like citrus, pear, peach and plum. Some of these plants showed spesific disorders such as hop stunt (Shikata, 1990), dapple fruit of plum and peach (Sano et al., 1989) and citrus cachexia (Semancik et al., 1988). However some hosts like grapevine and apricot the infectious appeared to be latent (Kofalvi et al., 1997). HSVd on pomegranate was first reported by Austruc et al., (1996) but there was no data about the effect of this viroid. Cvd-IIa and citrus cachexia viroid (CCavd) which were the members of HSVd family was found Satsuma and Clementine mandarins (*Citrus reticulata* Blanco) in Çukurova Region of Turkey (Önelge et a., 1994). But there wasn't any other study about the other plant groups in Turkey. In this study the pomegranate trees which contain the HSVd did not show any disorder except dwarfing. Fruits of these trees were examined but there wasn't any disorder identified.

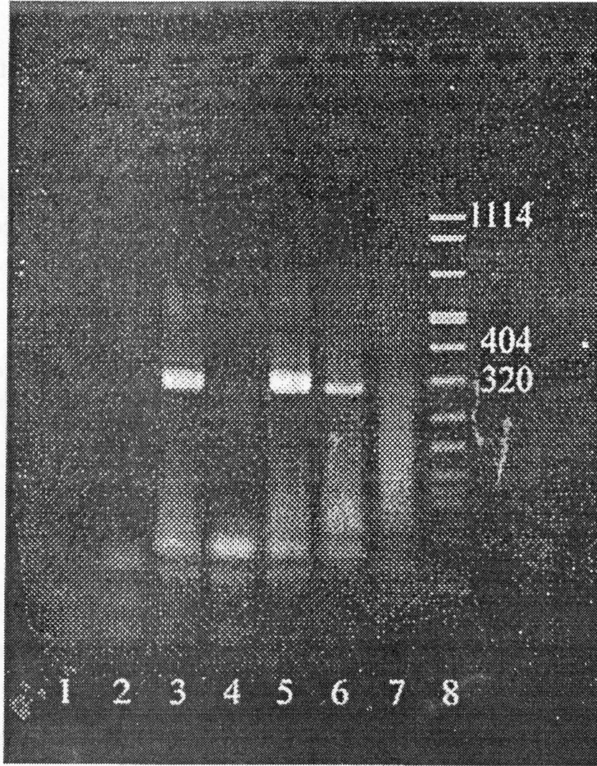

Figure 1. RT-PCR products, in a 2% agarose gel stained with ethidium bromide synthesized from extracts of pomegranate. Lane 1, 2, 4 healty pomegranate samples. Lane 3, 5 pomegranate samples infected with HSVd. Lane 6 HSVd as a positive control and lane 7 negative control. Lane 8 DNA molecular size markers are Hpa II and Dra I plus Hind III restriction fragments of plasmid pUCBM21.

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ÖZET

TÜRKİYE'DE NAR AĞAÇLARINDA HOP STUNT VİROİDİNİN VARLIĞI

Hop stunt viroidi (HSVd) otsu ve odunsu, bağlarla, *Citrus* ve *Prunus* bitkileri, olmak üzere çok sayıda konukçuda bulunmaktadır. 10 Nar *(Punica granatum)* ağacı viroid içeriği bakımından testlenmiştir. sPAGE ve RT-PCR yöntemleri kullanılarak HSVd'nin varlığı iki nar kültuvarında (Çekirdeksiz, Beynarı) belirlenmiştir. Bu çalışma Türkiye'de narlarda HSVd'nin narlarda HSVd'nin varlığı bildiren ilk rapordur.

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