Effects of Onion Yellow Dwarf and Leek Yellow Stripe Viruses on Symptomatology and Yield Loss of Three French Garlic Cultivars

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ABSTRACT

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This study was conducted to determine the effect of two potyviruses, onion yellow dwarf virus (OYDV) and leek yellow stripe virus (LYSV), on the symptoms, growth, and potential yield loss of garlic (*Allium sativum*). For 2 consecutive years, the impact on leaf length, pseudostem diameter, and bulb weight was evaluated after mechanical inoculation of cultivars Messidrome, Germidour, and Printanor, the three main garlic cultivars grown in France. The reduction in bulb weight due to OYDV ranged from 39% for Germidour to about 60% for the two other cultivars. For LYSV, the reduction in bulb weight was less on Messidrome (17%) and Germidour (26%) than on Printanor (54%). Coinfection with both viruses further reduced growth and bulb weight. When cloves originating from bulbs infected by each virus alone or a mixture of both viruses were planted, results indicated that such chronic infection induced further yield reduction. An assay designed to evaluate the role of LYSV inoculation date on yield revealed that yield losses were the lowest for late-season infections. However, yield loss was greater than 30% when the inoculation was performed at the end of April, the time when natural contamination generally occurs in southern France. A comparison of the impact of mixed infections of OYDV and LYSV from different origins suggested that the isolates did not differ significantly in their effects on yield loss.

Due to exclusive vegetative propagation, all commercial garlic cultivars tend to accumulate complexes of viruses (3,6,19). The disease known as "garlic mosaic" results in leaf striping, growth reduction, and yield loss (20). For a long time, the literature has been confusing because several potyviruses, carlaviruses, or other filamentous viruses were incompletely described, and their respective roles in the disease syndrome were fragmentary or unreliable. This was partially caused by difficulties in isolation of specific entities and a lack of appropriate tools, such as specific antiserum, for their identification.

Onion yellow dwarf potyvirus (OYDV) has been clearly identified as a pathogenic component of the garlic mosaic virus complex (6). Specific garlic strains of OYDV (OYDV-G) have been detected and characterized in cultivars originating from most garlic-producing countries (15). OYDV is now recognized worldwide as a major element of the virus disease complex. The first report of leek yellow stripe virus

(LYSV) in commercial varieties of garlic was in 1987 (21), but it is now known that this potyvirus was present in New Zealand as early as 1981 as a component of the garlic yellow streak complex (15). Specific strains of LYSV (LYSV-G) have now been identified in most temperate and tropical countries (1,5,15,22). Several commercial varieties have been shown to be coinfecting with both viruses (17). Two carlaviruses, garlic common latent virus (GCLV) and shallot latent virus (SLV), have been characterized on the basis of physical and serological properties (1,16) and have also been identified as elements of the garlic virus complex. GCLV has been found mainly in cultivars from Europe and South America, whereas SLV is mainly in cultivars from Asia and the ex-USSR (2). No particular symptom or effect has been associated with infection by either of these two viruses. A new type of flexuous virus has been identified in garlic plants in the past 6 years. Several viruses seemed to coinfect most garlic clones, which made a specific identification difficult. Nevertheless, their characterization is in progress (1,7,14), and several strains appear to have a close serological relationship with the two garlic mite-borne viruses described by Van Dijk (18). These viruses, presently grouped as Gar V type viruses (12), are not reported to be associated with any particular symptom.

Studies on the effect of garlic viruses on symptomatology or yield loss have been

hampered by their uncertain identification and by the difficulties in isolating them to obtain single virus strains. Data on the influence of these viruses on yield were obtained by comparison of so-called virus-infected and virus-free garlic, the latter originating from meristem-tip culture of the original cultivar (20), but the identity of the viruses in the infected stocks was unknown. Agronomic evaluation of garlic clones following mechanical inoculation was done by Messiaen et al. (11), but the virus populations of the sources of inoculum were not identified. All authors reported yield losses ranging from 25 to 50%. Previous trials to separate OYDV-G and/or LYSV-G from GCLV or SLV by inoculation from single local lesions or aphid transmission from coinfecting garlic plants have failed (H. Lot, *unpublished results*). Moreover, garlic strains of both potyviruses were shown to have a high level of host specificity and cannot infect onion (OYDV-G) or leek (LYSV-G). Hence, infection of other hosts as a means to free these viruses of contaminants has not been successful. Furthermore, OYDV from onion and LYSV from leek cannot infect or very occasionally infect garlic plants (15; H. Lot, *unpublished results*).

The objective of this work was to evaluate the effects of well-defined OYDV-G, LYSV-G, and mixtures of these viruses on symptoms, growth, and yield of the most important garlic cultivars grown in France.

MATERIALS AND METHODS

Plant material. Messidrome and Germidour, two autumn cultivars, and Printanor, a spring cultivar with high dormancy, are nonbolting cultivars (8). The cloves used in our experiments were from F2 plants multiplied under conditions required by the technical rules of the French certification scheme (9). This generation of plants was maintained in insect-proof screenhouses where numerous observations and random checks for virus infection by enzyme-linked immunosorbent assay (ELISA) were performed. Neither OYDV nor LYSV was detected in any plants.

Virus isolates. OYDV-VC was originally isolated from a Violet de Cadours plant showing severe yellow striping. This plant was tested by ELISA and immuno electron microscopy (IEM) and found to be OYDV infected and LYSV free, but it was also infected by GCLV. LYSV-58 was obtained from a Messidrome plant with a

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mild green mosaic collected in a field of certified seed-bulbs. The plant was positive for LYSV and negative for OYDV by ELISA and immunosorbent electronmicroscopy (ISEM). It was also infected with GCLV. Both isolates were mechanically inoculated on Messidrome F2 plants and maintained by vegetative propagation under insect-proof conditions. The isolates referred to as FR82 and FR93 were mixtures of OYDV and LYSV that differed serologically on the basis of decoration tests in IEM (*data not shown*). They originated from separate plants of cv. Fructidor collected in commercial production fields in 1982 and 1993, and maintained through vegetative multiplication.

Virus transmission. Young leaves of chronically infected garlic plants at the four- to five-leaf stage were triturated in 0.03 M Na₂HPO₄ containing 0.2% Na-diethylthio-carbamate (DIECA) (1:4, wt/vol). Extracted sap was mixed with 400-mesh Carborundum (75 mg/ml) and activated charcoal (75 mg/ml) before being rub-inoculated on two basal leaves of plants at the six- to seven-leaf stage (autumn cultivars) or the five-leaf stage (spring cultivar). In most cases, a second inoculation was done about 2 weeks later on the two leaves just above those inoculated previously. In the case of inoculation with two isolates (double inoculation), sap extracts of both source plants were mixed to obtain the same concentration of each individual virus as in extracts containing a single virus.

Serological assays. Antiserum against OYDV was prepared in our laboratory after purification of an onion OYDV isolate. Antisera against LYSV (As 1083) prepared from a leek isolate (510), and against GCLV (As 292), also prepared from a leek isolate, were kindly provided by H. J. Vetten and D.-E. Lesemann, BBA, Braunschweig, Germany. Serological detection was achieved by the direct double-antibody sandwich (DAS) ELISA (4). Three different leaves were sampled from individual plants. Plant extracts were prepared with a roll press. IEM techniques (decoration and ISEM) were as described by Delecalle and Lot (6).

Field experiments. Two experiments were performed in 1994 and 1995 to evaluate the effects of OYDV, LYSV, and a mixture of these viruses on the three cultivars. Because of the direct relationship between the size of the planted cloves and bulb size at harvest, the cloves were graded according to weight. In both assays, the mean clove weight was about 8 g for Messidrome and Germidour, and about 5 g for Printanor. The field plot each year consisted of two randomized blocks, each block containing four main plots for the four inoculation treatments (noninoculated, OYDV-VC, LYSV-58, and OYDV-VC + LYSV-58). Each main plot contained three subplots, one for each garlic cultivar. Sub-

plots were separated by 70 cm on all sides. Each subplot consisted of two rows, with 10 cloves in each row. Cloves were planted 10 cm apart within rows, with 40 cm between rows.

In the 1994 experiment, cloves of Messidrome and Germidour were planted on 16 November, and cloves of Printanor were planted on 15 December. The 1995 experiment planting dates were 2 December and 12 December, respectively. These field experiments were conducted in an insect-proofed greenhouse. Mechanical inoculations were performed on 3 and 16 March 1994 and on 6 and 20 March 1995, respectively. Symptoms were recorded weekly, starting in April. Growth was evaluated at the beginning of June by recording the length of the youngest mature leaf (from the soil level to the tip) and the diameter of the pseudostem at 4 cm from the soil level. The bulbs were harvested when plants were considered mature, i.e., when two-thirds of the leaves were dried up (9). Bulbs were stored at ambient temperature and weighed about 1 month after harvest (dry weight). Treatment effects were evaluated with 1 factor ANOVA where treatments had significant effects ($P \leq 0.05$). Treatment means were compared according to Tukey's honest significant difference test using the Statistica software (StatSoft Inc., Tulsa, OK).

To evaluate the effect on bulb weight of virus infection when cloves were infected (chronic infection), an experiment was done in 1995 in an open field using cloves harvested from plants infected in the 1994 trial. Due to the important effect of the viruses on bulb size, the clove grade could not be the same in all virus-cultivar combinations. The weights ranged from 3 to 5 g when singly infected and 2 to 4 g when doubly infected. Four main plots of 150 plants were planted on 15 November 1995 and covered with insect-proof gauze. Each plot contained plants originating from cloves harvested from one of four treatments (i.e., noninoculated, OYDV-VC, LYSV-58, and OYDV-VC + LYSV-58). Each main plot contained three subplots, one for each garlic cultivar. Each subplot contained five rows of 10 plants, with the same spacing conditions as described above. The number of germinated cloves was recorded on 20 April 1996.

In 1995, we conducted two experiments to evaluate the role of time of infection with LYSV and of the nature of the isolate on symptomatology, rate of infection, and bulb weight. Both experiments were conducted in an open field with Printanor, a cultivar harvested in mid-July. The first experiment consisted of two randomized blocks planted on 10 January. Each block contained four plots for the three inoculation dates (22 March, 21 April, 25 May) and the noninoculated, with the same spacing conditions as described above. The plants were inoculated with LYSV-58 on

the two basal green leaves. The second experiment consisted of two randomized blocks planted on 10 January; each contained six plots for the six treatments (inoculated with a mixture of OYDV and LYSV [OYDV-VC + LYSV-58, FR82, or FR93], OYDV-VC, LYSV-58, and non-inoculated). In both trials, plots were covered with gauze after planting.

RESULTS

Rate of infection. In all experiments, F2 plants to be inoculated were individually tested by ELISA 2 days before inoculation to ensure the absence of OYDV and LYSV. All plants appeared free of these viruses.

In the 1994 and 1995 assays to evaluate the effects of virus infections on the three garlic cultivars, two ELISAs were performed about 40 and 75 days after the second inoculation to record the rate of infection and to reveal any contamination. In both assays, most of the plants inoculated with a single virus were positive after the first serological test (87 to 100%), regardless of the virus or the cultivar. In the doubly inoculated plants, most of the plants appeared infected with OYDV (95%), but LYSV was detected in only 60% of the plants (25% for Messidrome). After the second ELISA, 100% of singly inoculated plants of the three cultivars and 100% of the doubly inoculated plants for cvs. Germidour and Printanor were positive for OYDV and LYSV. However, LYSV could not be detected in 8% of the Messidrome plants inoculated with both viruses.

Symptomatology and growth reduction due to different virus infections. Symptoms related to infection by OYDV + LYSV and OYDV alone appeared first as a clear yellow striping, by mid-April, on Messidrome and Germidour, and 1 week later on Printanor. Symptoms on LYSV-infected plants were distinct at that time and became more conspicuous by the end of April. By 15 May (40 days after the first inoculation), the type and severity of symptoms according to the different cultivar-virus combinations could be summarized as follows: for Messidrome, plants infected with OYDV exhibited severe yellow and light green striping on most leaves, with premature drying of the leaf tip. On Germidour, faint chlorotic to yellow streaking was noticed. On Printanor, striping was clear on older leaves but less distinct on young leaves. Plants infected with LYSV showed light yellow stripes on the distal part of older and intermediate leaves of the three cultivars, but on the young leaves, a faint green mosaic was hardly discernible. On doubly inoculated plants, symptoms were similar or more pronounced than on plants infected with OYDV alone, particularly for Messidrome.

Symptoms became more severe with increasing incubation. Two weeks before maturity, distinction between LYSV- and

OYDV-infected plants was nearly impossible on the basis of leaf symptoms.

In the 1994 and 1995 experiments, the different viruses produced similar effects on the three cultivars. Results of the 1995 assay are presented in Table 1. The double infection severely reduced growth and pseudostem diameter in proportions comparable to that of the OYDV infection, except for Germidour, for which the double infection was more deleterious than the single infection with OYDV. Physiological maturity (two-thirds of leaves dried up) was directly correlated to severity of symptoms. Harvest date was a further reflection of the influence of viral infection. For instance, in the 1995 trial, harvest dates for the autumn cultivars were 17 June for the doubly inoculated plants and 5, 7, and 12 days later for the OYDV-infected, LYSV-infected, and noninfected plants, respectively. The spring cultivar was harvested on 22 June for the doubly inoculated plants and 10, 12, and 15 days later for the OYDV-infected, LYSV-infected, and noninfected plants, respectively.

Yield loss. The mean bulb weights of the noninoculated plants in the 1994 trial were those expected for each cultivar, and in 1995 were slightly lower (Table 2). The influence of the different infections on the cultivars appeared very similar in the two experiments. Losses due to OYDV on Messidrome and Printanor were considerable (about 55 to 65%) and were less on Germidour (30 to 48%). The result of LYSV infection was less severe on the autumn cultivars Messidrome and Germidour (about 17 to 28%) but was considerable on the spring cultivar Printanor (50 to 60%). When doubly inoculated, all cultivars were severely affected, with losses of 56%, and up to 84% in the case of Printanor.

An analysis of the weight of individual cloves from infected bulbs revealed that the effect was different depending on virus and cultivar. For the 1994 trial, on plants inoculated with OYDV, LYSV, OYDV + LYSV, or noninoculated, the respective mean weights of cloves were 3.8, 4.4, 3.9, and 5.8 g for Messidrome, 5.8, 4.9, 4.0, and 6.8 g for Germidour, and 2.3, 2.2, 1.3, and 4.0 g for Printanor. In 1995, we used these cloves to evaluate the influence of the infection when infected cloves were planted, trying to select cloves of similar size to minimize the effect of clove weight on bulb weight. The rate of germination was 94 to 100% for the three cultivars when infected by OYDV or LYSV, but was 70% for Messidrome and 60% for Germidour and Printanor when doubly inoculated. Planting infected cloves resulted in a major effect on the mean bulb weights. The yield losses due to that chronic infection ranged from 42 to 71% for OYDV, 39 to 62% for LYSV, and 80 to 91% for OYDV + LYSV (Table 3).

Influence of the time of infection by LYSV. In the previous experiments, infec-

tions were ensured by two inoculations, in early March and again 2 weeks later. Because natural contaminations by aphids generally occur from mid-April in southern France, we compared the effect of the date of infection. Symptoms appeared at the end of May and the end of June in the first two inoculation trials (22 March and 21 April), but no symptoms were discernible at the time of harvest on the plants inoculated at the end of May. ELISAs indicated that all plants were infected 2 months after inoculation for plants inoculated on 22 March and 21 April. None of the plants inoculated on 23 May were infected according to ELISAs done on 27 June and 10 July. The effect on the bulb weight was highly de-

pendent on infection time (Table 4). When inoculated in March, the reduction (44.7%) was comparable to reductions observed in previous assays. The inoculation in April resulted in more than 30% yield reduction. No significant effect was observed in the late-season inoculations when compared with the noninoculated plants.

Comparison of the effect of different isolates. Because we could not obtain another isolate of OYDV free of LYSV, we compared the effect of mixtures of the two viruses. We used FR82, FR93, and an artificial mixture of OYDV-VC and LYSV-58, as well as OYDV-VC and LYSV-58 alone. Inoculations conducted on 22 March resulted in 100% infected plants with all

Table 1. Comparison of leaf length and pseudostem diameter of three garlic cultivars infected by onion yellow dwarf virus (OYDV), leek yellow stripe virus (LYSV), and a mixture of these viruses

Treatment	Messidrome		Germidour		Printanor	
	Leaf length (cm) ^y	Pseudostem diam. (mm) ^y	Leaf length (cm)	Pseudostem diam. (mm)	Leaf length (cm)	Pseudostem diam. (mm)
OYDV	76.6 b ^z	14.7 b	78.8 c	15.5 c	83.1 c	14.8 c
LYSV	93.7 a	19.7 a	90.4 b	18.2 b	86.6 b	16.6 b
OYDV + LYSV	72.1 b	14.1 b	75.8 d	13.7 d	82.3 c	14.6 c
Noninoculated	95.4 a	20.7 a	95.3 a	20.3 a	95.7 a	18.4 a

^y Mean value obtained for 40 plants per treatment.

^z Values in the same column followed by the same letter are not significantly different ($P \leq 0.05$) based on Tukey's honest significant difference test.

Table 2. Comparison of bulb weight of three garlic cultivars infected by onion yellow dwarf virus (OYDV), leek yellow stripe virus (LYSV), and a mixture of these viruses

Treatment	Messidrome		Germidour		Printanor	
	Mean bulb weight (g) ^x	Loss (%) ^y	Mean bulb weight (g)	Loss (%)	Mean bulb weight (g)	Loss (%)
Trial 1994						
OYDV	38.8 c ^z	57.5	68.8 c	31.6	38.8 b	55.3
LYSV	75.1 b	17.7	72.1 b	28.2	35.4 b	59.3
OYDV + LYSV	34.0 c	62.8	44.5 d	55.7	13.4 c	84.5
Noninoculated	91.3 a	...	100.5 a	...	86.8 a	...
Trial 1995						
OYDV	29.0 c	65.1	45.2 c	47.7	32.2 c	58.8
LYSV	69.2 b	16.6	65.0 b	24.8	40.1 b	48.6
OYDV + LYSV	27.0 c	66.8	30.5 d	64.7	22.6 d	71.0
Noninoculated	83.0 a	...	86.3 a	...	78.1 a	...

^x Mean values obtained for 40 plants per treatment. Bulbs were weighed about 1 month after harvest.

^y Percent loss when compared with the noninoculated control.

^z Values in the same column followed by the same letter are not significantly different ($P \leq 0.05$) based on Tukey's honest significant difference test.

Table 3. Comparison of bulb weight of three garlic cultivars chronically infected by onion yellow dwarf virus (OYDV), leek yellow stripe virus (LYSV), and a mixture of these viruses^w

Treatment	Messidrome		Germidour		Printanor	
	Mean bulb weight (g) ^x	Loss (%) ^y	Mean bulb weight (g)	Loss (%)	Mean bulb weight (g)	Loss (%)
OYDV	23.44 c ^z	70.6	49.7 b	42.6	36.9 b	56.2
LYSV	48.61 b	39.0	38.2 c	55.9	32.2 b	61.8
OYDV + LYSV	14.9 d	81.3	18.2 d	78.9	7.7 c	90.8
Noninoculated	79.7 a	...	86.5 a	...	84.32 a	...

^w Infected cloves were harvested from the 1994 trial.

^x Mean values obtained for about 50 plants per treatment except for doubly inoculated Germidour and Printanor (30 to 35 plants).

^y Percent loss when compared with the noninoculated control.

^z Values in the same column followed by the same letter are not significantly different ($P \leq 0.05$) based on Tukey's honest significant difference test.

inocula. The reductions in bulb weight due to OYDV-VC, LYSV-58, and to the artificial mixture were in the same range as that of other trials. FR 82 and FR 93 both caused yield losses of about 55%. However, these losses were less than those caused by the artificial mixture of OYDV-VC and LYSV-58 (Table 5).

DISCUSSION

This work was the first attempt to compare the effect of virus infection on garlic with clearly identified inocula. We have shown that there is an important influence of two potyviruses on symptoms, plant vigor, and yield potential (bulb and clove sizes) for three cultivars. OYDV, the first potyvirus of garlic identified with a worldwide distribution, caused severe symptoms and a great reduction of bulb weight. Germidour, which showed some delay in symptom expression and an average yield loss of 39%, has a lower susceptibility to OYDV than did Messidrome and Printanor (about 60% yield loss). Our data for Germidour and Messidrome (25 and 56% yield loss) are similar to those of Messiaen et al. (11) for Germidour and Thermidrome when inoculated with extracts of "infected" garlic that they called garlic mosaic virus. It is likely that their inoculum contained only OYDV.

Until now, the information available on the effects of LYSV infection has only concerned symptoms (15). This lack of data could be due to the difficulty in iso-

lating LYSV free of OYDV from garlic and also in achieving mechanical infection of garlic plants with the virus (S. Green, *personal communication*). Its effect could have been overlooked because symptoms elicited even 4 to 5 weeks after inoculation (i.e., probably after natural contamination) are milder than those caused by OYDV. In fact, our results shows that, after a long incubation and shortly before maturity, plants exhibit yellow stripes comparable to those due to OYDV. Nevertheless, the effect on growth is less important. The resulting yield losses, 17.1 and 26.5% on average for Messidrome and Germidour, respectively, were important but far less than those due to OYDV for the same cultivars (61.3 and 39.7%). Printanor was extremely susceptible to LYSV, with yield losses of about 54%.

Symptoms related to double infection were similar to those caused by OYDV, but they were more intense, especially with increasing incubation time. An aggravated effect on yield was obvious, as bulbs from doubly infected plants weighed about half of those from LYSV-infected plants. Coinfection induced 10% decrease of bulb weight when compared with OYDV infection for Messidrome, but the reduction was considerably enhanced in Germidour and Printanor.

The most severe effect of infections, whether with a single virus or the mixture of both, could be attributed to early infection, as the severity of a virus disease is

often correlated with the time of infection. This assumption was corroborated by the results of our assay to determine the effect of the time of inoculation with LYSV for the spring cultivar Printanor. When inoculation occurred on 21 April, the loss was high but about 30% less than in plants inoculated a month earlier. The latest artificial infection did not affect the plants. Plants inoculated on 23 May did not reveal virus infection based on ELISAs performed 35 and 45 days after inoculation. This was probably due to a low virus content and/or irregular virus distribution reflecting some resistance to infection of the mature plants. However, a few of these plants were infected, because some cloves from the harvested bulbs appeared to be infected (*data not shown*).

LYSV-G strains differing in severity on garlic were evaluated by Van Dijk (15), who assumed that diversity of symptoms on plants infected with OYDV-G could be explained by differences in aggressivity. We could not study the reactions of our cultivars to various isolates of OYDV or LYSV because additional isolates were not available. Therefore, we only compared the effect of two natural mixed inocula of both potyviruses with an artificial mixture of OYDV + LYSV. Printanor was used because it was obtained after selection of mericlones from Fructidor, a cultivar regarded as tolerant to virus infection because it manifests mild symptoms and good yield when chronically infected (6,10). The infection by the two mixed inocula, FR82 and FR 93, from Fructidor resulted in a 55% decrease of the bulb weight compared with the 69% reduction caused by the artificial mixture. These data indicate that Printanor is not tolerant to the virus complex and that the virulence of the complex from the chronically infected Fructidor was not really attenuated.

Our results show that plants originating from cloves of infected plants suffer greater yield losses than plants originating from healthy cloves that are mechanically inoculated. The effect is particularly obvious on Messidrome and Germidour originating from LYSV-infected cloves. However, these yield losses are overestimated because the size of the cloves planted could not be identical for all treatments.

The present study demonstrates the impact of two viruses that affect garlic worldwide. We have shown the deleterious effect of LYSV and confirmed the effect of OYDV. However, one of the shortcomings of our work is that the OYDV-G and LYSV-G isolates were contaminated by GCLV. On the other hand, the symptom-free clones were also contaminated by this latent virus. Although this carlavirus does not induce noticeable symptoms (16), one can not exclude the possibility that it interacts with one or both potyviruses. Symptom aggravation of LYSV-infected leek due to SLV has been reported (15). Further

Table 4. Effect of time of infection by leek yellow stripe virus (LYSV) on bulb weight of garlic cultivar Printanor

Virus	Inoculation date	Infected plants (%) ^w	Mean bulb weight (g) ^x	Loss (%) ^y
LYSV	22 March	100	49.4 c ^z	44.7
LYSV	21 April	100	61.0 b	31.7
LYSV	23 May	0	91.2 a	0
Noninoculated	...	0	84.4 a	...

^w Enzyme-linked immunosorbent assays (ELISA) were performed on 22 May and 27 June for plants inoculated on the first and second dates, on 27 June and 10 July for the latest inoculation. Non inoculated plants were checked in all tests.

^x Mean values obtained from 40 plants per treatment. Bulbs were weighed about 1 month after harvest.

^y Percent loss when compared with the uninfected control.

^z Values in the same column followed by the same letter are not significantly different ($P \leq 0.05$) based on Tukey's honest significant difference test.

Table 5. Effect of various isolates on bulb weight of garlic cultivar Printanor

Treatment	Infected plants (%) ^w	Mean bulb weight (g) ^x	Loss (%) ^y
OYDV-VC + LYSV-58	100	27.9 c ^z	68.8
FR 82	100	40.5 b	54.7
FR 93	100	40.4 b	54.8
OYDV-VC	100	42.2 b	52.8
LYSV-58	100	49.4 b	44.7
Noninoculated	0	89.4 a	...

^w Inoculations were performed on 22 March. Enzyme-linked immunosorbent assays (ELISA) were performed on 22 May.

^x Mean values obtained from 40 plants per treatment. Bulbs were weighed about 1 month after harvest.

^y Percent loss when compared with the uninfected control.

^z Values in the same column followed by the same letter are not significantly different ($P \leq 0.05$) based on Tukey's honest significant difference test.

efforts are required to obtain pure strains of the numerous viruses infecting garlic, and studies are needed to examine their individual effects. Improvements will come from the increasing number of well-defined specific antibodies and from a reliable virus indexing of mericlones. Different regeneration techniques may lead to the production of plants infected with a single virus strain that can be used for direct comparative studies or virus strain isolation.

The demonstration of the significant impact of these two potyviruses and their rapid spread by aphids (13) emphasize the need for the growers to use certified seed-bulbs produced under an appropriate certification scheme.

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