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# Seasonal variation in disease development in tomato under single and mixed infection with *Potato X potexvirus* and *Tobacco mosaic tobamovirus*

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ABSTRACT: Both the initial, and long term symptom responses to single and mixed infections with potato x potexvirus (PVX) and the L (wild strain) and LIIA (attenuated strain) of tobacco mosaic tobamovirus (TMV) were monitored in tomato (*Lycopersicon esculentum* Mill), cultivar GCR 236 (+/+) which were raised under greenhouse conditions at different seasons of the year in Japan. Symptoms expression, both in rapidly as well as in severity varied with the seasons among the treatments. Generally, disease impinged on plant growth and yield at both the cellular and macro levels and resulted in reductions in size of parenchyma and collenchyma cells from the stem, plant height, number of leaves, stem girth and weights of fresh and dry shoot and root as well as fresh fruit. There was also delayed flowering. Both singly and doubly infected plants had relatively better growth and yield during summer season. However, while there were only narrow discrepancies, in disease severity and yield loss, between summer and winter crops singly infected with TMV (L or LIIA), single infection with PVX consistently induced significantly rapid and more severe disease and consequently more growth and yield reductions in winter than in summer crops. For instance, while the percentage loss in fresh fruit weight was ca. 49% and 56% respectively for summer and winter crops under single infection with TMV-L, the loss was 33% and 57% under single infection with PVX, respectively for the two seasons. Nevertheless, simultaneous mixed infection of PVX and the TMV strains induced the most severe symptoms and also led to more growth and yield reductions than single infections with either virus, the season of growth notwithstanding. Compared to the healthy plants, about 90% reduction in total fruit weight per plant was recorded. Furthermore, cross protection was evidenced in plants that were mixed inoculated with the LIIA and the L strain of TMV as they manifested significantly delayed and milder disease compared to plants that were inoculated with the l (i.e. severe strain) alone.

Key words: Virus strains, symptom severity, cross protection, summer and winter crops.

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## Introduction

Tomato, *Lycopersicon esculentum* Mill, an annual crop of the family Solanaceae is an important fruit vegetable cultivated the world over for its edible fruit that is relished, both fresh and in various processed form. Its production, however, is limited by various problems not the least of which are diseases and pests. Among the important disease causing agents are viruses of diverse groups. Simon and Sobulo (1975), identified virus diseases, late blight and *Alternaria* disease as the most common diseases of tomato in West Africa. In Nigeria, the most common and important virus diseases of tomatoes include tomato mosaic virus, tomato bunchy top virus and tomato yellows. Tomato mosaic and tomato yellows virus diseases are the most serious as they can cause as much as 20-90% loss (Lana and Adegbola, 1977).

Generally, viral infections often cause visible symptoms such as various forms of mosaic and distortions with consequent reductions in plant growth and drop yield. Such severe responses have from long being reported for many crops (James, 1974; Hampton, 1975; Whenham et al., 1985). Environmental conditions such as temperature and light variations, season of the year, nutrition and water supply are known to affect the efficiency of infection in some viral-host interaction and may also affect symptom development. The development of red pigment symptom, and time for development in subterranean clover red leaf disease were a function of temperature (Helms et al., 1984). According to Close (1964) PVX replicated optimally in the range between 20°C to 24°C.

More often than not mixed infections by viruses involving even more complex interactions are responsible for severe diseases in plants and more often than not result in serious economic losses to the grower. For instance, tobacco on infection with potato virus X (PVX) and potato virus Y (PVY) normally develop a more severe disease than that induced by the viruses alone (Vance, 1991). Mixed infection of PVX and tobacco mosaic virus (TMV) is also known to induce a more severe disease in TMV-susceptible tomato plants (Balogun, et al., 2002).

Nevertheless, not all mixed infections involving viruses result in more severity of disease. Indeed many mild strains of TMV such as the M11-16 strain (Rast, 1972; Fletcher and Rowe, 1975) have been exploited in control measures of mosaic disease induced by the wild strain of TMV in tomato. Marrou and Migliori (1971) had earlier used cross protection to describe this phenomenon, which however, is usually found only between virus strains that are serologically related and adapted to the same host.

The overall aim of this study was to determine not only the extent of superiority of mixed over single infections, but also evaluate the influence of the seasons of growth, with respect to induction of severe disease symptoms, and the subsequent growth and yield responses in tomato plant.

## Materials and Methods

*Propagation of plants and inoculation with viruses*

Seedlings of cultivar GCR 236 (+/+) (i.e. TMV susceptible) tomato seedlings were raised under greenhouse conditions in all the experiments. Temperature during the summer experimental months was a maximum of 32°C during the day, and 20°C at night. Winter months' low was a minimum of 18°C. natural daylight was the regime for all experiments. Sandy-loam soil or Levington compost, steam-sterilized at 121°C for 30 min and supplemented with N P K fertilizer at the rate of 2:2:1g per liter pot, and some vermiculite at seedling were used in all cases. Plants were watered adequately daily to avoid water stress.

The O strain of PVX, and the L and LIIA strain of TMV that were used for the different experiments were multiplied in *Nicotiana tobacum* cv. Xanthi. Purified viruses from the leaves were used to inoculate the primary leaves i..e. the first 2 true leaves from the base of tomato plants at the 5 true-leaf stage by rubbing with a suspension of 200 µg of virus per ml of phosphate buffer, pH 7.0. Control plants were mock inoculated with buffer only. Leaves were dusted lightly with carborundum prior inoculations and washed with running water immediately after inoculations that followed pre-determined treatment designs in which the viruses were inoculated singly and in various combinations. Simultaneous mixed inoculations were carried out by mixing equal volume of inoculum of both viruses and then gently rubbing as usual on

same leaf position as for single inoculations. All plants were kept on platforms in the greenhouse following completely randomized design (CRD) pattern to enable subsequent relevant statistical analysis of variance.

*Disease, plant growth and yield assessment*

Plants were monitored daily to record visible changes such as time of first appearance and type of symptoms and days to flowering among others as previously reported (Balogun et al., 2002). Weekly records of plant height and number of leaves, and stem girth were also taken. Leaf samples for ELISA (to confirm success and level of infection) were collected at various times postinoculation and kept frozen when necessary at –40°C until assayed. At 7 weeks post inoculation, shoots of some plants were cut from the base with a scalpel and weighed while the roots were also removed from the soil, carefully rid of attached soil particles and then weighed. Both shoots and roots were then wrapped individually in papers properly labeled and oven-dried to constant weight at 80°C over 24 h after which the dry weight was taken. Fruits derived from the first round of flowering were harvested and weighed at first sign of ripening from at least four plants in both summer and winter crops. Analysis of variance and multiple comparisons between treatments using Tukey-Kramer HSD test were carried out at $P = 0.05$ level of significance.

*Stem cell length measurement*

The internode between the $8^{th}$ and $9^{th}$ leaves of both healthy and infected plants shoot was excised at harvest after weighing but before oven-drying the shoot in both seasons of growth. They were kept in cellophane bags to prevent early dehydration. At the laboratory, about 1 cm long random segment, of each of the samples was later mounted individually on a DSK micropslicer (Dosaka Emco. Ltd., Japan) and longitudinal thin sections, 10 µm in thickness were cut. Pieces of the sections were temporarily mounted on slides and viewed with a Nikon Phase contrast microscope (Nippon kogaku, Tokyo, japan) equipped with ocular micro calibrator. Lengths of at least 20 of each of parenchyma and collenchyma cells from the cortex, for each sample, were measured and the mean cell length estimated.

*Enzyme Linked Immunosorbent Assay (ELISA) Procedure.*

ELISA for confirmation of infection as well as virus concentration, in both preliminary and substantive experiments of this study was carried out according to the indirect method essentially as described by Koenig (1981). Leaf samples were ground for 1 min using pre-cooled mortar and pestle in freshly prepared 0.02 M sodium carbonate ($Na_2CO_3$) in the ratio of 1g of tissue; 10ml buffer. The homogenate was centrifuged for 10 min at 10,000 rpm and the supernatant was removed carefully and was then diluted in two-fold steps in the homogenizing buffer from 1:100 to 1:12,800 for all treatment samples.

Sample preparations, as described above, were coated directly unto Corning microtiter plates and incubated at room temperature for 1h. Washing and blocking with Tris-buffered Saline (TBS-T) (50mM Tris-HCl, pH 7.6; 0.15M NaCl; 0.05% $NaN_3$; 0.05% Tween 20) was done 4 times at three min interval and antibody against relevant viruses were added accordingly at 5 µg/ml final concentration in TBS-T. Goat anti rabbit 1gG- alkaline phosphatase conjugate (Biosource International, Camarillo Ca. USA) was used as the second antibody at 1:2,000 dilution. Colour was developed with p- nitro phenyl phosphate at 1mg/ml of 10% diethanolamine, pH 9.8. Absorbance was measured using a 405 nm filter of microplate photometer (Corona Electric, Tokyo, japan). The concentration of each virus in the samples was estimated from a standard curve established using purified virus preparations that had been passed through sucrose gradients and concentration measured spectrophotmetrically (Hitachi U-1100 spectrophotometer).

## Results

*Effects of treatments on symptom expression*

All virus inoculated plants manifested systemic symptoms. However, there were differences in the symptoms manifested and the time of their appearance after inoculation (Table 1). Plants inoculated with PVX alone expressed same chlorotic mottling in addition to becoming subsequently stunted compared to

the healthy control regardless of the season of growth. However, whereas first appearance of symptoms, as yellowish spots, was as early as 5 days post inoculation (dpi) in plants inoculated in late autumn and winter, it was as late as 15 dpi in plants inoculated during hotter periods of late spring and summer. Symptoms persisted in subsequent new leaves.

Table 1: Symptoms development, during summer and winter periods, in cv GCR 236 tomato plants under single or mixed infection with PVX and TMV.

| Treatment combinations | No. of days to appearance of symptom in summer experiments (dpi) | No. of days to appearance of symptom in winter experiments (dpi) | Leaf position with the first symptoms | Symptoms description |
|---|---|---|---|---|
| PVX alone | 8 (15*) | 5 (9) | L4/5 | Yellowing, rugose |
| TMV-L alone | 5 (7) | 6 (9) | L5/6 | Severe mosaic |
| TMV-LIIA alone | 21 (x) | 21 (x) | L7/8 | Very mild mosaic |
| TMV-L plus L | 18 (28) | 21 (28) | L9/10 | Mild to moderate mosaic |
| PVX plus TMV | 5 (7) | 4 (7) | L4/5 | Mosaic, rugose, necrotic lesions |
| PVX plus TMV | 5 (8) | 5 (7) | L4/5 | Mosaic, rugose, necrotic lesions |
| Healthy control | n.s | n.s | n.s | No symptoms |

*Numbers in parentheses indicate time of appearance of severe symptom
n.s. = No symptoms
n.r. = Not recorded
x = No severe symptom
dpi = day postinoculation

Plants infected with TMV-L alone manifested severe mosaic, which appeared as early as 5 dpi regardless of the season, in the youngest leaves. It became more prominent at 9 dpi and thereafter on subsequent new leaves. The lower, fully expanded leaves at inoculation, including the inoculated ones, remained symptomless. Plants inoculated with the LIIA strain remained symptomless for at least 3 weeks post inoculation and then a mild mosaic appeared on the uppermost leaf. The subsequent leaves manifested it.

Plants simultaneously inoculated with PVX and the TMV-L or TMV-LIIA strains manifested characteristic distortions and necrosis on the uppermost leaf as well as on the stem area close to the distorted leaves. The first indication was also as early as 5 dpi in autumn/winter experiments. The symptoms became fully apparent at 7 dpi and by the end of two weeks led to death in weaker plants and those inoculated at a very early stage of growth, during both seasons of growth. Plants that survived were seriously stunted. The mock-inoculated plants, which served as control, as expected, remained healthy.

*Effects on plant growth*

In both summer and winter crops, the final height of plants, under all treatments i.e. whether infected singly or doubly, was significantly lower than for the healthy control plants. The infected plants as well as the control, however, appeared to have fared better during the summer season than during the winter period (Tables 2, 3 and Fig. 1). Among singly infected plants, those with TMV-LIIA alone were significantly taller. Although mixed infected plants mostly did not differ significantly from one another, simulatenous mixed inoculation with PVX and TMV-L apparently elicited relatively more damaging response than

mixed infection with TMV-LIIA and PVX. In both seasons, plants co-infected with L plus LIIA strains were not different from those with LIIA alone. In addition, the plants were significantly taller than those infected with the L strain alone (Tables 2 and 3).

Table 2: Some growth parameters in healthy and diseased tomato plants under single and mixed infection with TMV and PVX during summer.

| Treatment combination | Height at harvest (cm) | Number of leaves at harvest | Final stem diameter (mm) | Stem cell Length (µm) Parenchyma | Collenchyma | Parenchyma/ collenchyma ratio |
|---|---|---|---|---|---|---|
| PVX alone | 60.8d | 19.5bc | 9.1b | 122.5c | 170.8d | 0.72a |
| TMV-L alone | 67.8c | 18.3c | 8.2b | 126.0b | 182.5c | 0.69ab |
| TMV-LIIA alone | 93.2b | 21.0b | 9.6b | 129.5b | 194.0b | 0.67b |
| TMV-L plus TM | 87.8b | 20.3bc | 9.3b | 126.3b | 191.3bc | 0.66b |
| PVX plus TMV-I | 48.3e | 12.0d | 6.3c | 109.5d | 151.5e | 0.72a |
| PVX plus TMV-I | 51.3e | 12.3d | 6.7c | 110.5d | 156.5e | 0.71a |
| Healthy control | 101.4a | 23.8a | 13.0a | 134.6a | 219.0a | 0.61c |

Figures followed by the same letter in a column are not significantly different at P = 0.05, Tukey-Kramer HSD Test.

Each value is a mean of 4 plants at 49 days postinoculation.

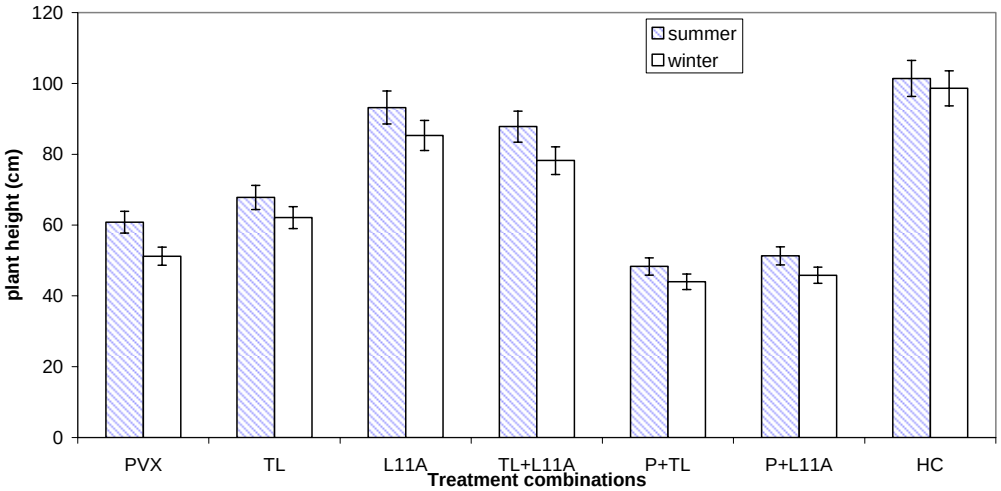


**Fig 1: Comparative height of tomato plants singly or doubly infected with PVX and TMV during summer and winter periods.**

TL and L11A = TMV alone; TL+ L11A = double inoculation of the two TMV strains P+L11A and P+TL = Simultaneous mixed inoculation; HC= Healthy control. Error bars represent 5% of the individual data value.

Table 3: Some growth parameters in healthy and diseased tomato plants under single or mixed infection with PVX and TMV during winter.

| Treatment combinations | Height at harvest (cm) | Number of leaves at harvest | Final stem diameter (mm) | Stem cell Length µm) Parenchyma | Collenchyma | Parenchyma/ collenchyma ratio |
|---|---|---|---|---|---|---|
| PVX alone | 51.2d | 18.8c | 7.2cd | 103.8d | 153.8d | 0.68b |
| TMV-L alone | 62.1c | 15.8d | 7.5c | 118.3c | 176.3c | 0.67b |
| TMV-LIIA alone | 85.3b | 23b | 9.6b | 125.8b | 194.0b | 0.65b |
| TMV-L plus TMV-LIIA | 78.2b | 23b | 9.3b | 123.3bc | 191.3b | 0.64b |
| PVX plus TMV-L | 44.0d | 10.5e | 5.8d | 99.0d | 149.5d | 0.66b |
| PVX plus TMV-LIIA | 45.8d | 11.0e | 5.9d | 101.5d | 154.5d | 0.66b |
| Healthy control | 98.6a | 26.5a | 12.5a | 132.8a | 216.0a | 0.61a |

Figures followed by the same letter(s) in a column are not significantly different at P = 0.05, using Tukey-Kramer HSD Test. Each value is a mean of 4 plants at 49 days postinoculation.

Considering stem cell length, healthy plants had significantly longer stem parenchyma and collenchyma cells than the infected ones in both seasons. The control was an average of 134.8 µm (parenchyma) and 219 µm (collenchyma) for plants grown in summer (Table 2), and 132.8 µm and 216 µm respectively for plants grown during winter (Table 3). The shortest cells, during summer, were an average of 109.5 µm (parenchyma) and 151.5 µm (collenchyma) recorded in plants doubly infected with PVX and TMV-L. The trend was as for plant height with singly infected plants having longer cells than mixed infected ones at the region of the stem examined.

Considering the lengths of the parenchyma cells in relation to those of the collenchyma, it is shown that healthy plants have significantly lower parenchyma : collenchyma length ratio compared to the infected ones, which did not differ significantly among one another (Tables 2 and 3). Higher ratios indicate that the viral infections generally suppressed the extension of the stem cortical collecnchyma cells more than it did that of parenchyma cells in the infected plants in both seasons of growth.

A general increase with time in the number of leaves per plant was recorded, in all treatments, up to 6 weeks postinoculation regardless of the season of growth. As shown in Tables 2 and 3, comparison of the number of living leaves among treatments showed the control plants, followed by those with LIIA alone, with the highest number of leaves which averages 24 and 21 respectively during summer. The lowest number (12) was recorded on doubly infected plants. During winter experiments, the range was 11 (in PVX + TMV-L and PVX + LIIA treatments) and 27 in healthy plants (Table 3 and Fig. 2). Plants singly infected with PVX or TMV-L, however, did not differ significantly from each other.

The effect on stem girth followed the same trend as the number of leaves. Healthy control plants had significantly thicker stems that infected plants at harvest (tables 2 and 3). Plants singly infected with LIIA had medium sized stems that were significantly thicker than those of plants with PVX or TMV-L alone but not of those plants doubly infected with the two strains of TMV (i.e. LIIA + TMV-L). Stem diameter ranged between 6.3mm and 13.0 mm in summer experiments, and 5.8 mm and 12.5 mm in winter

experiments. In both cases, mixed infected plants had the thinnest stems while the control had the thickest ones.

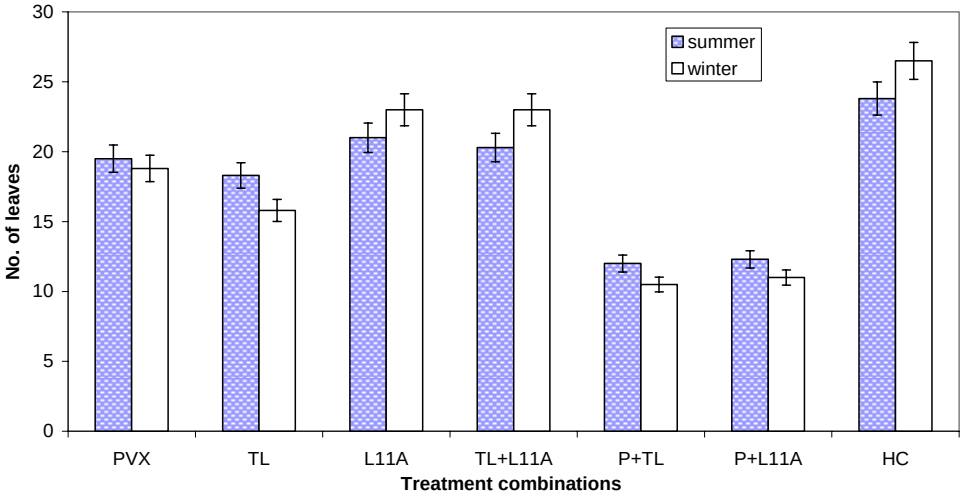


**Fig 2: Comparative mean number of leaves in tomato plants singly or doubly infected with PVX and TMV during winter and summer periods.**

TL and L11A = TMV alone; TL+ L11A = double inoculation of the two TMV strains P+L11A and P+TL = Simultaneous mixed inoculation; HC= Healthy control. Error bars reprrent 5% of the value.

*Effects on yield components*

Samples for measurement of fresh weight of tops were taken for all treatments at 49 days postinoculation. As with most growth parameters, the healthy plants had significantly higher fresh and dry matter weight than infected plants (Tables 4 and 5). Plants with TMV-LIIA alone and those with combined TMV-LIIA plus TMV-L infection, significantly out-weighed other plants with severe infections.

The percent dry matter composition of the shoots as well as the percent loss in weight, based on the control values for both categories of experiments, are shown in Tables 4 and 5. These values followed the trend of the absolute values of fresh and dry weight. As high as 72% loss of dry matter was recorded in winter experiments while the highest in summer experiments was ca. 43% indicating the influence of the season of growth on disease severity. Fig. 3 illustrates the discrepancies in fresh shoot weight between summer and winter crops.

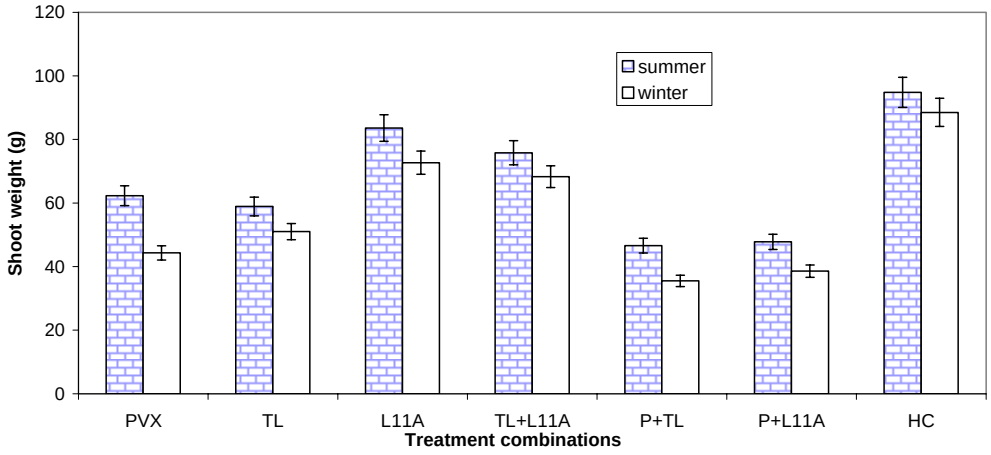


**Fig 3: Comparative fresh shoot weight of tomato plants singly and doubly infected with PVX and TMV during summer and winter periods.**

TL and L11A = TMV alone; TL+ L11A = double inoculation of the two TMV strains P+L11A and P+TL = Simultaneous mixed inoculation; HC= Healthy control. Error bars reprrent 5% of the value.

As with shoot weight, on absolute value and percentage basis, the control had significantly higher fresh and dry root weight than the other treatments at harvest. As usual, plants with PVX and the TMV strains combined had the smallest weights followed by the singly infected ones. Among these, plants with TMV-LIIA alone had the highest fresh, dry and percent dry weight (Tables 4 and 5). Dry matter percentage ranged between 11.4 and 20.4 during summer (table 4) and 11.9 and 19.1 during winter (Table 5).

The record of the average number of days postinoculation to flower appearance showed that healthy plants did not bloom significantly earlier than singly infected plants and those with TMV-L + TMV-LIIA in both seasons. However, plants with mixed infection of PVX and any of the TMV strains had significant delay of several days (Tables 4 and 5). The response in plants mixed infected during winter was more drastic, with a delay of more than 10 days after those with TMV-L only that flowered at an average of 36 dpi. Apart from this very long delay, rate of flower abortion was also high and indeed, many of the doubly infected plants did not flower at all before the termination of the experiments.

The average number of edible fruits as at first harvest was 3 in both healthy plants and those infected with LIIA only during winter. This was not significantly different from those of singly infected plants, which had an average of 2 each. This group, however, significantly differed from plants doubly infected with PVX and TMV, which had a mean value of 0.5 fruits per plant (Table 5). In summer crops as well, the trend was the same. Healthy plants had an average of 4 fruits, which did not differ significantly from those of singly infected plants. Mixed infected plants also had significantly fewer edible fruits than those of other treatments (Table 4).

In summer experiments, as shown on Table 4, the mean total fruit weight per plant as well as the average weight of a fruit were significantly higher in the control than in other groups. Mixed infected plants had the lowest weights. The highest total weight per plant was 653g while the lowest was ca. 20g. In winter experiments, the control plants as well as those inoculated with the mild strains of TMV i.e. TMV-LIIA, had significantly the same total fruit weight per plant, and average weight of fruit. The mixed infected plants had the lowest weights and highest losses based on both total fruit weight per plant and average weight per fruit (Table 5). Unlike in the summer period, of the singly infected plants those with PVX had the lowest total fruit weight and average weight of fruit (ca. 265g and 115g respectively). Generally, apparently heavier fruit were produced in both healthy and infected plants during summer than in winter period (Fig. 4).

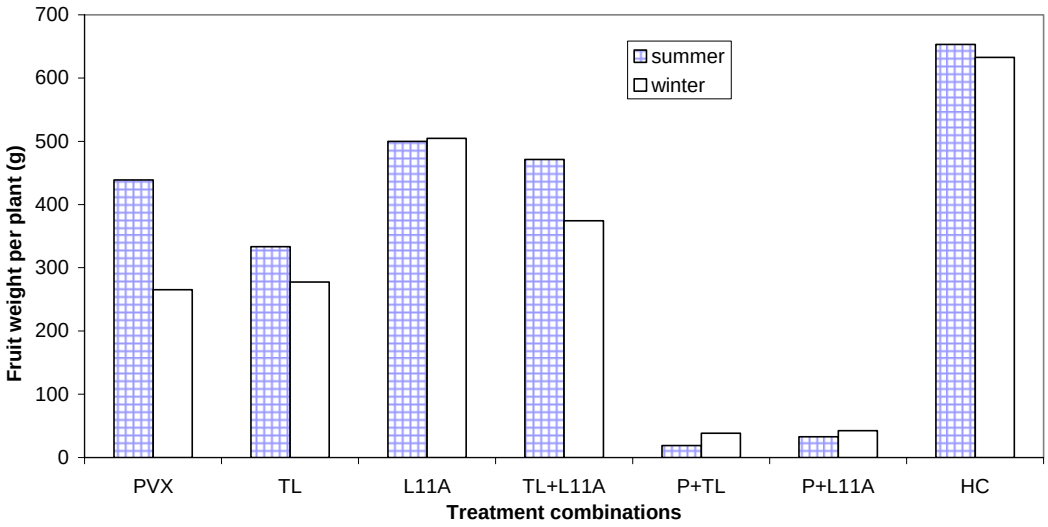


**Fig 4: Comparative total fresh weight of edible fruits in tomato plants singly or doubly infected with PVX and TMV during summer and winter periods**

TL and L11A = TMV alone; TL+ L11A = double inoculation of the two TMV strains P+L11A and P+TL = Simultaneous mixed inoculation; HC= Healthy control. Error bars reprrent 5% of the value.

## DISCUSSION

The results of the various experiments have shown that seasons of growth have substantial influence on disease development and the growth and yield responses of plants to infection by the different strains of virus used in this study. Symptoms produced by the same viruses in infected plants varied dramatically in days to initial appearance and severity depending on the growth season. Although all experiments were carried out under normal greenhouse conditions, the effect of the seasonal changes was apparent. For instance, whereas the appearance of PVX symptom was delayed substantially during summer, compared to the winter period, that of TMV manifested at about the same time post inoculation in both seasons. The cumulative effects apparently contributed, especially, to PVX infected plants doing relatively better, growth- and yield-wise, during summer than during winter. As noted by Matthews (1991), the complex factors including day length, light intensity, air and soil temperatures, and water supply among others that change during seasonal cycle, will affect plant growth and thus the disease produced by a given virus, and the extent to which it replicates.

Evidence of cross protection was equally manifest in this study as co-inoculation of the attenuated strain (LIIA) and the wild one (L) of TMV not only led to delay in symptom appearance by more than four weeks but even on manifesting it was considerably milder than that recorded in plants infected with TMV-L alone. Cassels and Herrick (1977) also reported the same pattern of symptom development in tomato doubly inoculated with the M11-6 (mild) and the O strains of TMV. Indeed, TMV-LIIA had been used in Japan to protect greenhouse crops against the wild strain of TMV (Oshima et al., 1965). However, for LIIA strain to be useful for cross protection purposes, timing of inoculation is crucial.

Reduction in plant size is said to be the most general symptom induced by virus infection. According to Matthews (1991) there is probably some slight general stunting of growth even with masked or latent infections where the systemically infected plant show no obvious sign of disease. This observation was also true in this study. For instance, although plants infected with TMV-LIIA alone showed only very mild mosaic symptoms, yet about 12% and 13% reductions, compared to the control, in both plant height and leaf number per plant was recorded during summer and winter respectively. Mixed infection with PVX and the TMV strains, however, caused more reduction in height and number of leaves than single infections.

Table 4: Root, Shoot and Fruit yield in healthy and diseased tomato plants under single and mixed infection with PVX and TMV during summer.

| Treatment combinations | Shoot Weight (g) | | Root Weight (g) | | % Dry Matter* in flowering | | | Edible Fruits per Plant at 1st harvest | | | % loss of fruit |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fresh | Dry | Fresh | Dry | Shoot | Root | (dpi) | Mean No. | Total wt. (g) | Ave wt. (g) | Yield per plant** |
| PVX alone | 62.3c | 7.1d | 7.8c | 1.0c | 11.5c | 13.4cd | 31.3b | 3.0a | 438.8b | 145.7bc | 32.8 |
| TMV-L alone | 58.9c | 6.5d | 10.1b | 1.5b | 10.9c | 15.3bc | 32.8b | 2.5a | 333.5b | 132.9c | 49 |
| TMV-LIIA alone | 83.6b | 13.4b | 10.4b | 1.6b | 16.1ab | 15.8b | 31.3b | 3.3a | 500.0ab | 153.9b | 23.5 |
| TMV-L plus TMV-LIIA | 75.8b | 11.8c | 10.5b | 1.7b | 15.6b | 15.9b | 31.5b | 3.3a | 471.0b | 145.3bc | 27.9 |
| PVX plus TMV-L | 46.6d | 4.8e | 7.3c | 0.8c | 10.3c | 10.8e | 43.5b | 0.5b | 19.0c | 19.0d | 97.1 |
| PVX plus TMV-LIIA | 47.8d | 5.0e | 7.0c | 0.8c | 10.5c | 11.7de | 42.5b | 0.8b | 33.0c | 33.0d | 94.9 |
| Healthy control | 94.8a | 17.3a | 13.1a | 2.5a | 18.2a | 18.7a | 32.0b | 3.8a | 653.3a | 175.1a | 0 |

Figures followed by the same letter in a column are not significantly different at P = 0.05, Tukey-Kramer HSD test. Each value is a mean of plant.
*Comparison among treatments was based on log-transformed data.
**Absolute difference between the values of the control and the respective infection treatment expressed as a % of the control based on the total fruit weight.

Table 5: Root, Shoot and fruit yield in healthy and diseased tomato plants under single or mixed infection with PVX and TMV during winter.

| | Shoot weight | | Root Weight (g) | | % Dry Matter in Flowering | | | Edible fruits Per plant at 1st harvest | | | % loss of fruit |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment combinations** | **Fresh** | **Dry** | **Fresh** | **Dry** | **Shoot** | **Root** | **(dpi)** | **Mean No.** | **Total wt. (g)** | **Ave wt. (g)** | **Yield per plant**** |
| PVX alone | 44.3cd | 5.2e | 7.8c | 1.1c | 11.3c | 13.7c | 29.3d | 2.3a | 265.2bc | 115.3c | 57.3 |
| TMV-L alone | 51.0c | 6.8d | 10.1b | 1.6b | 13.3b | 15.8b | 36.0b | 2.1a | 277.5c | 132.1bc | 56.1 |
| TMV-LIIA alone | 72.7b | 11.9b | 10.4b | 1.7b | 16.4a | 16.0b | 32.3c | 2.8a | 504.7a | 180.3a | 20.2 |
| TMV-L plus TMV-LIIA | 68.3b | 9.6c | 10.5b | 1.7b | 14.0b | 16.4b | 30.8cd | 2.2a | 374.2b | 170.1ab | 40.8 |
| PVX plus TMV-L | 35.5e | 4.4e | 7.1c | 0.8d | 12.2bc | 11.9d | 48.3a | 0.5b | 38.3d | 38.3d | 93.9 |
| PVX plus TMV-LIIA | 38.6de | 4.5e | 6.8c | 0.9cd | 11.6c | 12.4cd | 47.0a | 0.5b | 42.5d | 42.5d | 93.3 |
| Healthy control | 88.5a | 16.0a | 13.1a | 2.5a | 18.1a | 19.1a | 33.0c | 3.4a | 632.5a | 186a | 0 |

Figures followed by the same letter in a column are not significantly different at P = 0.05, Tukey-Kramer HSD Test. Each value is a mean of 4 plants.
*Comparison among treatments was based on log-transformed data.
**Absolute difference between the values of the control and the respective infection treatment expressed as a % of the control based on the total fruit weight.

Other results in this study have also further confirmed that seasonal variations had considerable influence on disease development and the consequent yield response especially in single infections with otherwise relatively mild virus like PVX in tomato. For instance, plants singly infected with PVX during winter had a yield loss of about 57% and 38% based on total fruit weight and average weight of a fruit respectively. These, however, contrasted significantly with about 33% and 17%, which were recorded during the summer period. The relative apparent higher loss in the winter compared to the summer period is illustrated in Figure 5. As shown by ELISA, the prevailing condition (cooler temperatures etc.) during winter enhanced PVX replication and hence also the faster manifestation of more severe symptom, relatively earlier, in winter than in summer (data not shown). That apparently culminated in more growth and yield loss compared to the other season.

Season of growth (winter and summer), however, appeared to have less significant influence on the severity of disease induced by single TMV infection and its mixed infection with PVX, as attested to by the extent of growth and yield loss in the different seasons. Based on the total fruit weight per plants, mixed infection, for instance, led to between 935% and 97% loss regardless of the season of growth. Balogun (2002) had shown that PVX concentration is enhanced considerably in a mixed infection with TMV in cv Fukuju no. 2 tomato, a common Japanese cultivar.

It is true that results in this study were obtained under conditions that were controlled to some extents. It is possible, therefore, that results might vary in the field. The fact, however, that factors that favour the establishment of a mixed infection in tomato can be met easily even in field environment of the tropics, and considerations of the potential economic loss to the grower has made this situation worth studying.

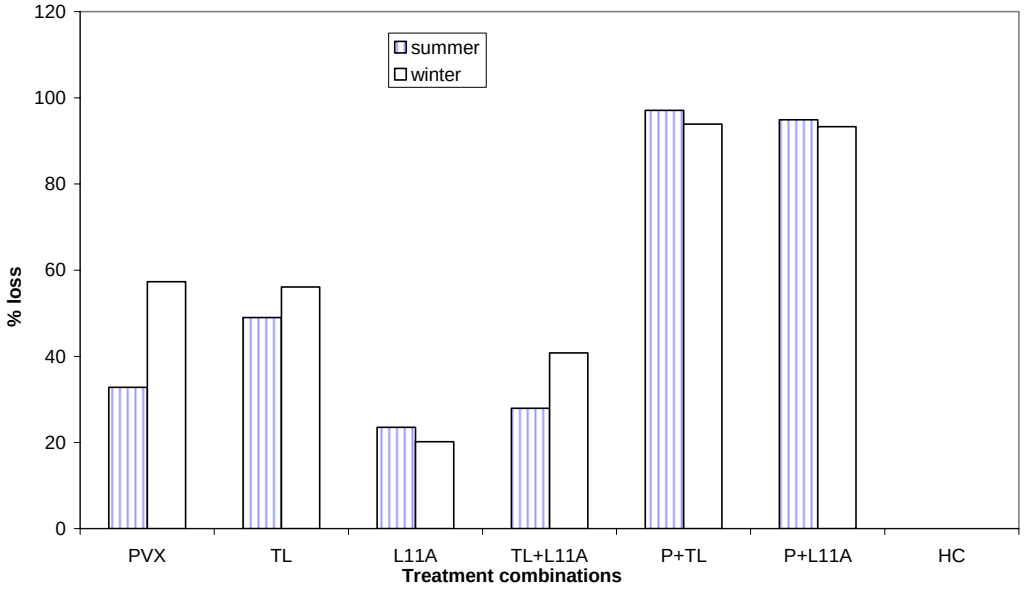


**Fig 5: Comparative % loss of fresh edible fruit weight in tomato singly or doubly infected with PVX and TMV during summer and winter periods**

TL and L11A = TMV alone; TL+ L11A = double inoculation of the two TMV strains P+L11A and P+TL = Simultaneous mixed inoculation; HC= Healthy control. Error bars reprrent 5% of the value.

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