Temperature and CO₂ Level Influence *Potato leafroll virus* Infection in *Solanum tuberosum*

Bong Nam Chung¹*, Sang Wook Koh, Kyung San Choi, Jae Ho Joa, Chun Hwan Kim, and Gopal Selvakumar

National Institute of Horticultural & Herbal Science, Rural Development Administration, Wanju 55365, Korea

(Received on March 13, 2017; Revised on May 22, 2017; Accepted on June 7, 2017)

We determined the effects of atmospheric temperature (10-30 ± 2°C in 5°C increments) and carbon dioxide (CO₂) levels (400 ± 50 ppm, 540 ± 50 ppm, and 940 ± 50 ppm) on the infection of *Solanum tuberosum* cv. Chubaeak by *Potato leafroll virus* (PLRV). Below CO₂ levels of 400 ± 50 ppm, the PLRV infection rate and RNA content in plant tissues increased as the temperature increased to 20 ± 2°C, but declined at higher temperatures. At high CO₂ levels (940 ± 50 ppm), more plants were infected by PLRV at 30 ± 2°C than at 20 or 25 ± 2°C, whereas PLRV RNA content was unchanged in the 20-30 ± 2°C temperature range. The effects of atmospheric CO₂ concentration on the acquisition of PLRV by *Myzus persicae* and accumulation of PLRV RNA in plant tissues were investigated using a growth chamber at 20 ± 2°C. The *M. persicae* PLRV RNA content slightly increased at elevated CO₂ levels (940 ± 50 ppm), but this increase was not statistically significant. Transmission rates of PLRV by *Physalis floridana* increased as CO₂ concentration increased. More PLRV RNA accumulated in potato plants maintained at 540 or 940 ± 50 ppm CO₂, than in plants maintained at 400 ± 50 ppm. This is the first evidence of greater PLRV RNA accumulation and larger numbers of *S. tuberosum* plants infected by PLRV under conditions of combined high CO₂ levels (940 ± 50 ppm) and high temperature (30 ± 2°C).

**Keywords**: carbon dioxide, infection, *potato leafroll virus*, temperature

**Handling Associate Editor**: Ju, Ho-Jong

Climate change models predict progressive increases in the average global temperature of 4.6°C and an average CO₂ concentration to 940 ppm by the year 2100, with regions at higher latitudes warming faster than those at lower latitudes (Intergovernmental Panel on Climate Change, 2014). The dynamics of plant virus epidemics and the losses that they cause are likely to be greatly influenced by the direct consequences of climate change, such as increased temperatures, and indirectly through the increased abundance and activity of vectors (Jones, 2009).

Elevated CO₂ enhances plant photosynthetic rates, resulting in greater production of plant biomass or yield (Ainsworth and Long, 2005; Cure and Acock, 1986). The growth-enhancing effects of elevated CO₂ levels typically increase with rising temperature, and the optimum growth temperatures of several plants have been shown to rise substantially with increasing levels of atmospheric CO₂ (Berry and Bjorkman, 1980; McMurtrie and Wang, 1993; McMurtrie et al., 1992; Stuhlfauth and Fock, 1990). The pool of total soluble sugars and starch in plant leaves also increases at elevated CO₂ levels (Ye et al., 2010). While there is abundant literature describing the effects of temperature on virus accumulation and symptom expression, little is known regarding the responses of crops to virus infection under elevated CO₂ conditions, and few studies have investigated the interactions between plants and viruses under elevated CO₂ conditions.

Potato leaf roll virus (PLRV) is transmitted by *Myzus persicae* in a circulative, nonpropagative manner in which the virus is acquired through gut tissue into the aphid...
Temperature and CO₂ Level Influence PLRV

The PLRV inocula were field isolates of PLRV-infected plants obtained from B. Fenton (James Hutton Institute, Dundee, Scotland), as previously described (Chung and Palukaitis, 2011). Source plants for aphid transmission were PLRV-infected *P. floridiana* plants, which were maintained in insect-proof cages and renewed every month.

Virus-free *M. persicae* cultures were maintained on *Nicotiana tabacum* cv. Samsun plants in insect cages in a 20°C growth chamber. To transmit the virus, single *M. persicae* of the second or third instar were used for each plant. No pre-acquisition starvation period was given for PLRV, and the virus acquisition period was 3 days. The duration of feeding on test plants was 3 days. Then the aphids were killed by spraying pesticide.

The primer sets used for RT-PCR and qPCR have previously been described (Chung et al., 2016). RT-PCR was used to determine virus infection in plants after inoculation. Total RNA used for RT-PCR or qPCR was prepared with an RNeasy Mini Kit. The RT-PCR reaction was performed as previously described (Chung and Palukaitis, 2011). To quantify the absolute copy numbers of PLRV, we constructed a standard curve employing known concentrations of *in vitro* transcripts. Synthesis of RNA transcripts was performed using mMESSAGE mMACHINE T7 (Ambion, Austin, TX, USA) according to the manufacturer’s instructions. Clones of PLRV 627/MACHINE T7 (Ambion, Austin, TX, USA) according to the manufacturer’s instructions. Clones of PLRV 627/pGEM-T plasmid DNA were linearized with the *SalI* restriction enzyme and treated with *T7* RNA polymerase.

Absolutes levels of viral RNA are expressed as the number of viral copies per nanogram of total RNA. For qPCR, 40 ng DNase-treated total RNA (Ambion) was reverse-transcribed (Promega ImProm-II RT, Madison, WI, USA) using a gene-specific primer. We added 8 μL cDNA mix prior to subsequent qPCR. All of the reactions proceeded in a C1000 Touch Thermal cycler (Bio-Rad, Hercules, CA, USA) using the SYBR-Green method (Universal SYBR-Green Supermix, Bio-Rad) according to the following protocol: 1 cycle at 95°C for 30 s; and 39 cycles at 95°C for 10 s, 60°C for 30 s, and 65-95°C in increments of 0.5°C and at intervals of 5 s. We used the qPCR analysis program CFX Manager 3.1 (Bio-Rad).

Table 1. Effects of atmospheric temperature on *Potato leafroll virus* (PLRV) infection during establishment of infection under ambient CO₂ concentration in *Solanum tuberosum* cv. Chubaek

| No. of plants inoculated per temperature treatment | % of plants infected with PLRV |
|-----------------------------------------------|-------------------------------|
|                                               | 10 ± 2°C | 15 ± 2°C | 20 ± 2°C | 25 ± 2°C | 30 ± 2°C |
| 59                                            | 49 ± 12.9 b* | 42 ± 8.9 b | 58 ± 9.9 a | 31 ± 4.6 c | 27 ± 3.3 c |

*Means with same letters are not significantly different (Duncan’s multiple range test, *P* < 0.05).*
The SAS 4.2 statistical package (SAS Inc., Cary, NC, USA) was used for data analysis.

We determined the effects of atmospheric temperature (10-30 ± 2°C in 5°C increments) and CO₂ concentration (400 ± 50 ppm, 540 ± 50 ppm, and 940 ± 50 ppm) on PLRV infection in a growth chamber. The results showed that PLRV infection was influenced by both temperature and CO₂ concentration. Below CO₂ levels of 400 ± 50 ppm, both the PLRV infection rate and PLRV RNA content increased as the temperature increased to 20 ± 2°C; however, both of these factors declined at temperatures higher than 20°C (Table 1, Fig. 1). When CO₂ concentra-

tion was increased to 940 ± 50 ppm, comparatively more plants were infected by PLRV at 30 ± 2°C than at 20 or 25 ± 2°C. PLRV RNA content was unchanged over a temperature range of 20-30 ± 2°C (Fig. 2).

The effects of atmospheric CO₂ concentration on PLRV acquisition by M. persicae and PLRV RNA content in plant tissues was investigated in a growth chamber at 20 ± 2°C. M. persicae PLRV RNA content increased slightly at elevated CO₂ levels (940 ± 50 ppm); however, this increase was not statistically significant (Fig. 3). The transmission rates of PLRV in P. floridana increased as CO₂ concentration increased (Table 2). More PLRV RNA accumulated in tissues of plants maintained at 540 or 940 ± 50 ppm than in those maintained at 400 ± 50 ppm (Fig. 3).

PLRV infection in S. tuberosum plants was influenced by both temperature and CO₂ concentration. The PLRV infection rate and PLRV RNA content were highest in plants maintained at 15-20 ± 2°C after inoculation in a growth chamber at 400 ± 50 ppm CO₂. The highest virus titer was observed at 18-20°C, which is the optimum temperature for S. tuberosum plant growth (Haverkort, 1990). In another study using P. floridana, the PLRV infection rate was highest at 25 ± 2°C, and greater PLRV levels were evident in plants maintained at 20-25°C (Chung et al., 2016).

Previous studies have shown that virus infection is relatively good when the host plants are maintained at their optimum growth temperature. Singh et al. (1988) observed that high relative humidity and high temperature (25-30°C) increased virus transmission by 30-35% while the PLRV infection rate increased with higher (30°C) post-inoculation temperatures in P. floridana. Swenson (1968) similarly observed that more pea plants were
Temperature and CO\textsubscript{2} Level Influence PLRV

Infected with bean yellow mosaic virus when grown at 30°C than when grown at 24°C or 15°C after inoculation. The optimal temperature for viral RNA replication in cells infected by soil-borne wheat mosaic virus is 17°C (Ohsato et al., 2003). Mangrauthia et al. (2009) determined that the optimum temperature for symptom development in PRSV-infected papaya is 26-31°C, supporting their results by showing that purified PRSV-HC-Pro recombinant protein bound 21 nt ds miRNA duplexes more efficiently at ambient temperature (25°C) than at high (35-45°C) or low (15°C) temperatures.

Relatively low temperatures (15-20°C) tended to delay symptoms in *Potato virus Y-O* (PVY-O)- or *Potato virus A* (PVA)-infected *N. benthamiana* plants (Chung et al., 2016), and in PRSV-infected papaya (Mangrauthia et al., 2009). Relatively high temperatures (40-45°C) also delayed symptom development in PRSV-infected papaya plants (Mangrauthia et al., 2009). Similar results have been reported in other host–virus systems. Melon necrotic spot virus symptoms were observed to diminish as temperature increased from 20 to 25°C (Kido et al., 2008), and less severe banana streak virus symptoms and lower virus titers were detected in plants grown at 28-35°C than in those grown at 22°C (Dahal et al., 1998). PVY-O and PVA symptoms in *N. benthamiana* plants grown at 30°C were attenuated compared to those of plants grown at temperatures below 25°C (Chung et al., 2016).

In this study, we found that an increase in atmospheric CO\textsubscript{2} concentration (940 ± 50 ppm) resulted in the infection of more potato plants by PLRV at 30 ± 2°C than at 20 or 25 ± 2°C; PLRV RNA content was unchanged over a temperature range of 20-30 ± 2°C. *M. persicae* PLRV RNA content increased, but not significantly, as atmospheric CO\textsubscript{2} concentration increased during PLRV acquisition from source plants. Consequently, PLRV transmission rates in *P. floridana* plants increased. More PLRV RNA accumulated in potato plants maintained at 540 or 940 ± 50 ppm CO\textsubscript{2} than in those maintained at 400 ± 50 ppm CO\textsubscript{2} at 20 ± 2°C.

Our results are consistent with those of a recent study, in which barley yellow dwarf virus titer increased by 36.8% in the leaves of infected plants grown under higher CO\textsubscript{2} concentrations than ambient levels (Trezbiicki et al., 2015). Del Toro et al. (2015) reported that at elevated CO\textsubscript{2} concentrations, viral titers for cytomegalovirus markedly increased as the proportion of total plant protein content increased in leaf disks, albeit less so for PVY and three *Potato virus X* (PVX) constructs. Similarly, elevated CO\textsubscript{2} concentrations alleviated damage to *N. tabacum* plants due to PVY infection, or delayed viral spread to some extent (Ye et al., 2010). Elevated CO\textsubscript{2} concentration also decreased the incidence of tomato yellow leaf curl virus (TYLCV) (by 14.6% in 2009 and 11.8% in 2010), disease severity (by 20.0% in 2009 and 10.4% in 2010), and lev-

**Fig. 3.** Real-time quantitative PCR analysis of *Potato leafroll virus* content of *Myzus persicae* (left) and *Solanum tuberosum* cv. Chubak (right) as influenced by atmospheric CO\textsubscript{2} concentration during establishment of virus infection in a growth chamber (20 ± 2°C). Standard error bars are shown in charts. Different letters in charts are significantly different at a 5% level using Duncan’s multiple range test.

**Table 2.** Effects of atmospheric CO\textsubscript{2} concentration during acquisition of *Potato leafroll virus* (PLRV) by *Myzus persicae* on virus transmission efficiency of PLRV in *Physalis floridana* in a growth chamber (20 ± 2°C).

| No. of plants inoculated per CO\textsubscript{2} treatment | % of plants infected with PLRV |
|-----------------------------------------------------------|-------------------------------|
| 400 ± 50 ppm                                              | 52.6 ± 1.84 c                 |
| 540 ± 50 ppm                                              | 64.4 ± 0.78 b                 |
| 940 ± 50 ppm                                              | 86.6 ± 0.04 a                 |

*Means with different letters are significantly different (Duncan’s multiple range test, *P* < 0.05).
levels of TYLCV coat protein in tomato leaves (Huang et al., 2012), reportedly due to an increase in salicylic acid and jasmonic acid at high CO$_2$ concentration.

Few studies have considered how insect fitness is altered by changes in host plant physiology induced by elevated CO$_2$ concentration. According to a recent report (Sun et al., 2015), plant stomatal closure improves aphid feeding at elevated CO$_2$ levels. Elevated CO$_2$ concentrations were also shown to upregulate an abscisic-acid-independent enzyme, carbonic anhydrase, which led to a further decrease in stomatal aperture in aphid-infested plants. These effects enhance phloem-feeding time. Based on the above report, we suggest that the accumulation of high amounts of PLRV RNA in *M. persicae* at elevated CO$_2$ concentrations (940 ± 50 ppm) observed in this study may have been due to increased feeding time. Further studies are needed to determine whether the increase in the number of PLRV-infected plants and in PLRV RNA content in plant tissue due to high temperature and CO$_2$ concentration significantly increases damage to potato crops.

Acknowledgments

This work was performed with the support of the Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01024602), Rural Development Administration, Republic of Korea.

References

Ainsworth, E. A. and Long, S. P. 2005. What have we learned from 15 years of free-air CO$_2$ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO$_2$. *New Phytol.* 165:351-371.

Berry, J. and Bjorkman, O. 1980. Photosynthetic response and adaptation to temperature in higher plants. *Annu. Rev. Plant Physiol.* 31:491-543.

Chung, B. N. and Palukaitis, P. 2011. Resistance to multiple viruses in transgenic tobacco expressing fused, tandem repeat, virus-derived double-stranded RNAs. *Virus Genes* 43:454-464.

Chung, B. N., Canto, T., Tenllado, F., Choi, K. S., Joa, J. H., Ahn, J. J., Kim, C. H. and Do, K. S. 2016. The effects of high temperature on infection by *potato virus Y*, *potato virus A*, and *potato leafroll virus*. *Plant Pathol.* J. 32:321-328.

Cure, J. D. and Acoc, B. 1986. Crop responses to carbon dioxide doubling: a literature survey. *Agr. Forest Meteorol.* 38:127-145.

Dahal, G., Hughes, J. d’A. and Thottappilly, G. 1998. Effect of temperature on symptom expression and reliability of banana streak badnavirus detection in naturally infected plantain and banana (*Musa* spp.). *Plant Dis.* 82:16-21.

Del Toro, F. J., Aguilar, E., Hernández-Walías, F. J., Tenllado, F., Chung, B. N. and Canto, T. 2015. High temperature, high ambient CO$_2$ affect the interactions between three positive-sense RNA viruses and a compatible host differentially, but not their silencing suppression efficiencies. *PLoS One* 10:e0136062.

Gray, S. M. and Banerjee, N. 1999. Mechanisms of arthropod transmission of plant and animal viruses. *Microbiol. Mol. Biol. Rev.* 63:128-148.

Haverkort, A. J. 1990. Ecology of potato cropping systems in relation to latitude and altitude. *Agric. Syst.* 32:251-272.

Huang, L., Ren, Q., Sun, Y., Ye, L., Cao, H. and Ge, F. 2012. Lower incidence and severity of tomato virus in elevated CO$_2$ is accompanied by modulated plant induced defence in tomato. *Plant Biol.* 14:905-913.

Intergovernmental Panel on Climate Change. 2014. Climate change 2014: the physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, UK. 996 pp.

Jones, R. A. 2009. Plant virus emergence and evolution: origins, new encounter scenarios, factors driving emergence, effects of changing world conditions, and prospects for control. *Virus Res.* 141:113-130.

Kido, K., Tanaka, C., Mochizuki, T., Kubota, K., Ohki, T., Ohnishi, J., Knight, L. M. and Tsuda, S. 2008. High temperatures activate local viral multiplication and cell-to-cell movement of melon necrotic spot virus but restrict expression of systemic symptoms. *Phytopathology* 98:181-186.

Mangrauthia, S. K., Singh Shakyia, V. P., Jain, R. K. and Praveen, S. 2009. Ambient temperature perception in papaya for papaya ringspot virus interaction. *Virus Genes* 38:429-434.

McMurtrie, R. E. and Wang, Y. P. 1993. Mathematical models of the photosynthetic response of tree stands to rising CO$_2$ concentrations and temperatures. *Plant Cell Environ.* 16:1-13.

McMurtrie, R. E., Comins, H. N., Kirschbaum, M. U. F. and Wang, Y. P. 1992. Modifying existing forest growth models to take account of effects of elevated CO$_2$. *Aust. J. Bot.* 40:657-677.

Ohhsato, S., Miyanishi, M. and Shirako, Y. 2003. The optimal temperature for RNA replication in cells infected by soil-borne wheat mosaic virus is 17°C. *J. Gen. Virol.* 84:995-1000.

Singh, M. N., Paul Khurana, S. M., Nagaich, B. B. and Agrawal, H. O. 1988. Environmental factors influencing aphid transmission of *potato virus Y* and *potato leafroll virus*. *Potato Res.* 31:501-509.

Stuifhaut, T. and Fock, H. P. 1990. Effect of whole season CO$_2$ enrichment on the cultivation of a medicinal plant, *Digitalis lanata*. *J. Agron. Crop. Sci.* 164:168-173.

Sun, Y., Guo, H., Yuan, L., Wei, J., Zhang, H. and Ge, F. 2015. Plant stomatal closure improves aphid feeding under elevated CO$_2$. *Glob. Chang. Biol.* 21:2739-2748.

Swenson, K. G. 1968. Relation of environment and nutrition to plant susceptibility to bean yellow mosaic virus by aphid transmission. Vol. 106. Agricultural Experiment Station, Or-
Trezbicki, P., Nancarrow, N., Cole, E., Bosque-Pérez, N. A., Constable, F. E., Freeman, A. J., Rodoni, B., Yen, A. L., Luck, J. E. and Fitzgerald, G. J. 2015. Virus disease in wheat predicted to increase with a changing climate. *Glob. Chang. Biol.* 21:3511-3519.

Ye, L., Fu, X. and Ge, F. 2010. Elevated CO$_2$ alleviates damage from *potato virus Y* infection in tobacco plants. *Plant Sci.* 179:219-224.