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Temperature and CO_2 Level Influence *Potato leafroll virus* Infection in *Solanum tuberosum*

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We determined the effects of atmospheric temperature (10-30 ± 2°C in 5°C increments) and carbon dioxide (CO_2) levels (400 ± 50 ppm, 540 ± 50 ppm, and 940 ± 50 ppm) on the infection of *Solanum tuberosum* cv. Chubaek by *Potato leafroll virus* (PLRV). Below CO_2 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 CO_2 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 CO_2 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 CO_2 levels (940 ± 50 ppm), but this increase was not statistically significant. Transmission rates of PLRV by *Physalis floridana* increased as CO_2 concentration increased. More PLRV RNA accumulated in potato plants maintained at 540 or 940 ± 50 ppm CO_2, 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 CO_2 levels (940 ± 50 ppm) and high temperature (30 ± 2°C).

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Climate change models predict progressive increases in the average global temperature of 4.6°C and an average CO_2 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 CO_2 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 CO_2 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 CO_2 (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 CO_2 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 CO_2 conditions, and few studies have investigated the interactions between plants and viruses under elevated CO_2 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

hemocoel and then exits through salivary tissues (Gray and Banerjee, 1999). In this study, the effects of increased temperature and CO_2 levels on PLRV infection and PLRV RNA content in both the aphid vector and plant tissues were examined using *Solanum tuberosum* as a plant host.

To examine the effects of atmospheric temperature on PLRV infection at CO_2 levels of 400 ± 50 ppm and 940 ± 50 ppm, 30-59 *S. tuberosum* cv. 'Chubaek' plants (7-8 cm in height) were inoculated with PLRV using *M. persicae*, and maintained in a 20 ± 2°C growth chamber. After 3 days, aphids were killed by chemical spray and the plants were moved to growth chambers where the temperature was maintained at 10-30 ± 2°C (in 5°C increments). PLRV RNA was quantified using real-time quantitative reverse transcription polymerase chain reaction (qPCR).

To examine the effects of atmospheric CO_2 concentration on PLRV infection, 30-48 plants (7-8 cm in height) were inoculated with PLRV using *M. persicae*, and grown in a growth chamber at 20 ± 2°C. After 3 days, aphids were killed by chemical spray and plants were moved to 20 ± 2°C growth chambers where CO_2 levels were adjusted to 400, 540, and 940 ppm (± 50 ppm). PLRV RNA was quantified using qPCR. We also examined the effects of atmospheric CO_2 on PLRV acquisition by the natural vector *M. persicae*. We allowed aphids to acquire the virus by feeding on PLRV-infected source plants (*Physalis floridiana*) at different CO_2 concentrations within the range 400-940 ppm (± 50 ppm) in a growth chamber, and then transferred single virus-loaded aphids onto individual test plants (*P. floridiana*). The plants were maintained at 20 ± 2°C until virus infection was determined 10 days later. Approximately 46-56 plants were used to test the transmission ability of the aphids. The *M. persicae* PLRV RNA content was determined using qPCR with 30 aphids, and total RNA extracted from individual aphids was determined using an RNeasy Plant Mini Kit (Qiagen, Hilden, Germany).

Stem cuttings approximately 7-8 cm in length were rooted in soil and used for PLRV infection. *P. floridiana* seedlings were used to test the transmission efficiency of PLRV by *M. persicae*. The plants were grown in 'Barok' commercial soil in an insect-proof glasshouse.

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. floridana* 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/pGEM-T plasmid DNA were linearized with the *Sal*I restriction enzyme and treated with T7 RNA polymerase. Absolute 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 CO_2 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 CO_2 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 CO_2 concentration. Below CO_2 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 CO_2 concentration 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).

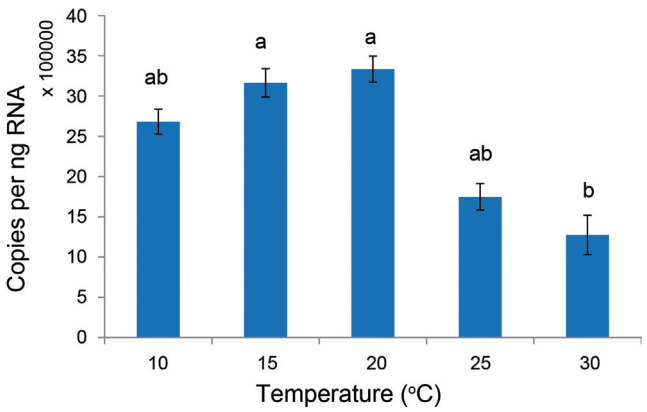

Fig. 1. Real-time quantitative PCR analysis of *Potato leafroll virus* (PLRV) RNA content affected by atmospheric temperature during establishment of virus infection in *Solanum tuberosum* cv. 'Chubaek' in a growth chamber (400 ± 50 ppm CO_2). Standard error bars are shown in charts. Different letters in charts are significantly different at a 5% level using Duncan's multiple range test.

The effects of atmospheric CO_2 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 CO_2 levels (940 ± 50 ppm); however, this increase was not statistically significant (Fig. 3). The transmission rates of PLRV in *P. floridana* increased as CO_2 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 CO_2 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 CO_2. 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

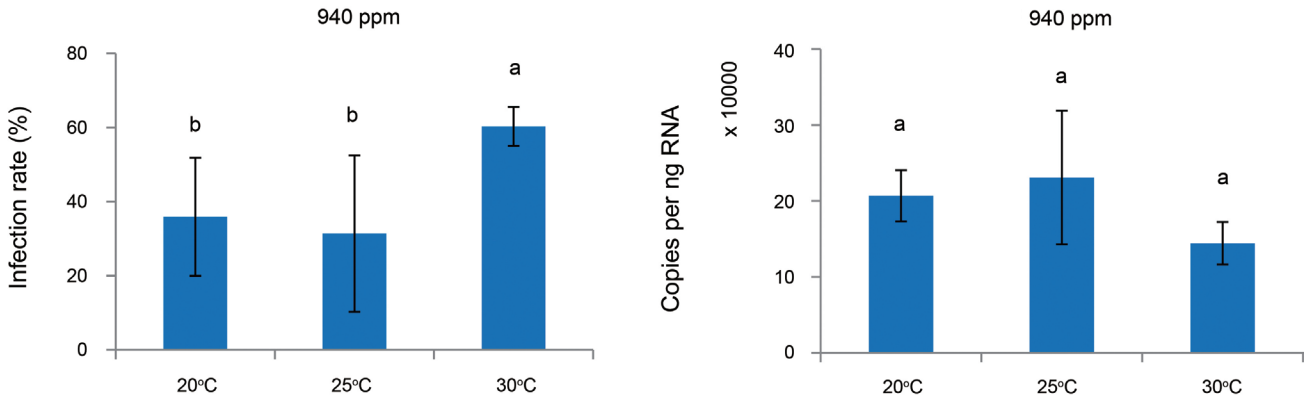

Fig. 2. Effects of atmospheric temperature (20°C, 25°C vs. 30°C ± 2°C) on infection rate and *Potato leafroll virus* RNA content of *Solanum tuberosum* cv. Chubaek in a growth chamber (940 ppm ± 50 ppm CO_2). Standard error bars are shown in charts. Different letters in charts are significantly different at a 5% level using Duncan's multiple range test.

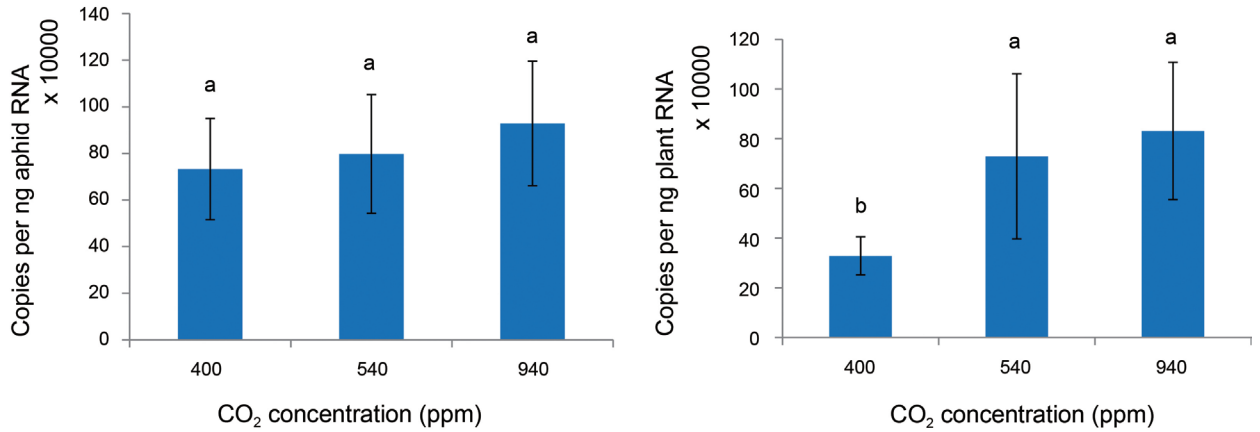

Fig. 3. Real-time quantitative PCR analysis of *Potato leafroll virus* content of *Myzus persicae* (left) and *Solanum tuberosum* cv. Chubaek (right) as influenced by atmospheric CO_2 concentration during establishment of virus infection in a growth chamber (20 ± 2°C). Standard error bars are shown in charts. Different letters in charts are significantly different at a 5% level using Duncan's multiple range test.

Table 2. Effects of atmospheric CO_2 concentration during acquisition of *Potato leafroll virus* (PLRV) by *Myzus persicae* on virus transmission efficiency of PLRV in *Physalis floridiana* in a growth chamber (20 ± 2°C)

No. of plants inoculated per CO_2 treatment	% of plants infected with PLRV		
	400 ± 50 ppm	540 ± 50 ppm	940 ± 50 ppm
46-56	52.6 ± 1.84 c*	64.4 ± 0.78 b	86.6 ± 0.04 a

*Means with different letters are significantly different (Duncan's multiple range test, $P < 0.05$).

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_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_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_2 than in those maintained at 400 ± 50 ppm CO_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_2 concentrations than ambient levels (Trezbicki et al., 2015). Del Toro et al. (2015) reported that at elevated CO_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_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_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-

els 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.

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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 Acock, 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-Walias, 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 Shakya, 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.

Ohsato, 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.

Stuhlfauth, 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-

egon State University, USA. 23 pp.
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.