Soluble Carbohydrate Levels in Tobacco Systemically Protected Against Blue Mold by Stem Injection with *Peronospora tabacina* or Leaf Inoculation with Tobacco Mosaic Virus

S. Q. Pan, X. S. Ye, and J. Kuc

Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546.
Journal Series Paper 9211222 of the Kentucky Agricultural Experiment Station, Lexington 40546. This work was supported in part by grants from the R. J. Reynolds Tobacco Co. and Cooperative Agreement 43YK-5-0030 of the U.S. Department of Agriculture.
Accepted for publication 25 March 1993.

ABSTRACT

Pan, S. Q., Ye, X. S., and Kuc, J. 1993. Soluble carbohydrate levels in tobacco systemically protected against blue mold by stem injection with *Peronospora tabacina* or leaf inoculation with tobacco mosaic virus. Phytopathology 83:906-909.

Susceptible tobacco plants were systemically protected against blue mold (*Peronospora tabacina*) by stem injection with sporangiospores of *P. tabacina* or inoculation of three to four lower leaves with tobacco mosaic virus (TMV). After stem injection (9–13 days), higher soluble carbohydrate levels were detected in stem tissues at the sites of injection and distant from the sites of injection in *P. tabacina*-injected plants as compared with control plants. Thirteen days after stem injection, higher soluble carbohydrate levels were detected in nonchallenged leaves of *P. tabacina*-injected plants than in those of control plants. The differences in carbohydrate levels between *P. tabacina*-induced and control plants increased as the protection against blue mold increased. After challenge with *P. tabacina*, the soluble carbohydrate levels in challenged leaves of both *P. tabacina*-induced and control plants decreased, but the levels in the induced plants remained higher than those in the control plants during the entire period after challenge. In contrast, there was no difference in soluble carbohydrate levels between TMV-induced and control plants in the inoculated lower leaves or uninoculated upper leaves before or after challenge. This suggests that soluble carbohydrates which accumulated in tobacco plants systemically protected by *P. tabacina* are unlikely to be important in either the induction of resistance or as resistance mechanisms.

There are numerous reports that the development of some plant diseases is restricted by high soluble carbohydrate levels in plants (4–6,11,16,18). Shading or excessive nitrogen can decrease sugar levels and increase disease severity (4,5,16). Increased sugar concentrations, induced by growth in continuous light or application of sugars, have also been reported to reduce disease (4,16,17). It is possible that sugars such as glucose function primarily as a source of energy required for the development of resistance responses rather than as a source of precursors for antifungal compounds such as phytoalexins (17). It has been demonstrated that sugars repress the synthesis of microbial enzymes such as endopolygalacturonase, cellulase, and pectate lyase (2,4), which are involved in pathogenesis. It is possible that the repression of these enzymes by sugars contributes to the reduction of disease symptoms (4).

Susceptible tobacco plants can be systemically protected against blue mold (*Peronospora tabacina*) by stem injection with sporangiospores of *P. tabacina* (1,7,10,14,15,19). Higher soluble carbohydrate levels were observed in those systemically protected plants (9,12,13). Administration of exogenous sugars increased the soluble carbohydrate levels in the plant tissues, but did not elevate the resistance to blue mold (13), suggesting that the higher soluble carbohydrate level was unlikely responsible for the induced resistance in a direct manner. However, it is not clear what causes the systemic increase in soluble carbohydrate levels (13) and whether induction of the resistance is associated with an increase in the soluble carbohydrate level. Recently, we observed that the induced systemic resistance to blue mold could also be achieved by inoculating three to four lower leaves with tobacco mosaic virus (TMV) (8,10,19). This allows us to examine the association of the elevation of soluble carbohydrate level with the induction of resistance, circumventing the possibility that *P. tabacina* secretes β-1,3-glucanase (10), which in turn releases sugars from the plant tissues and contributes to the increase in soluble carbohydrate levels. In the present study, we investigated soluble carbohydrate levels in tobacco plants protected by both *P. tabacina* and TMV before and after challenge with *P. tabacina* in order to determine whether increase of soluble carbohydrate level is associated with induction of systemic resistance.

MATERIALS AND METHODS

Plants and pathogens. Tobacco (*Nicotiana tabacum* L. 'Ky 14') plants were grown in a greenhouse with a photoperiod of 14 h with daylight supplemented with sodium light at a temperature of 25–33 C. Seeds were planted in Pro-Mix BX (Premier Peat Corp. Marketing, New York, NY) in small trays, and seedlings were watered with a 0.005% 15-16-17 (N-P-K) fertilizer solution (Peters Fertilizer, W.R. Grace and Co., Fogelsville, PA). After 4 wk, seedlings were transplanted to pots (6.5-inch pots for small plants and 10-inch pots for plants taller than 80 cm) containing Pro-gro 300 (Pro-gro Product Inc., Elizabeth City, NC) and fertilized three times a week with 0.15% 15-16-17 (N-P-K) Peters fertilizer. *Peronospora tabacina* D. B. Adam isolated in Kentucky in 1979 and 1982 (14,15), designated as isolates 79 and 82, respectively, was maintained by weekly transfers of sporangiospores on young Ky 14 tobacco plants in a growth room with a 16-h photoperiod under fluorescent and incandescent light at 20–25 C. Purified TMV in water was kindly provided by J. Shaw, Department of Plant Pathology, University of Kentucky.

Induction and challenge. The plants were induced by stem injection of 5×10^5 sporangiospores per milliliter of *P. tabacina* isolate 82 in the greenhouse (14) or by inoculation with 25 μg per milliliter of TMV on three to four lower leaves of plants at the 9- to 10-leaf stage in a growth room with a 14-h photoperiod under white fluorescent and incandescent light at 23 C (19). Control plants were stem-injected with water or mock-inoculated. Unless indicated otherwise, leaves above the sites of injection with *P. tabacina* or inoculation with TMV were challenged with 5×10^4 spores per milliliter of *P. tabacina* isolate 79 as described in previous reports (14,19).

Tissue sampling and analysis for soluble carbohydrates. Leaf tissues were sampled by periodically excising small areas of the lamina of young but mature leaves (leaf position number 3–5 from the apex) with scissors, avoiding large veins. Stems were

© 1993 The American Phytopathological Society

sampled by excising with a razor blade patches of tissue external to the woody xylem including the epidermis, cortex, outer phloem, and cambium. Samples were immediately frozen at −80 C. Frozen tissues were weighed rapidly and then immersed in boiling 80%, v/v, aqueous ethanol for 10 min. After cooling, the volume was adjusted for evaporation losses, and the tissue-ethanol slurry was homogenized with sand using a mortar and pestle. The homogenate was filtered through Whatman GF/A glass fiber filters (Whatman Ltd., Maidstone, England), and total soluble carbohydrate was determined spectrophotometrically by the phenol-sulfuric acid method (3). Glucose in 80%, v/v, aqueous ethanol was used as a standard, and results are expressed as glucose equivalents.

RESULTS AND DISCUSSION

Protection against blue mold. In order to examine the association of the increase in soluble carbohydrate level with the induction of resistance to blue mold, susceptible tobacco plants were induced by stem injection with sporangiospores of *P. tabacina* or leaf inoculation with TMV. At various times after induction, the plants were assayed for the soluble carbohydrate levels and concurrently challenged with *P. tabacina*. The induced systemic resistance was observed just as documented previously (10,15,19). After induction with TMV and *P. tabacina* (12 and 21 days, respectively), the induced resistance reached a maximum and the plants were protected 90% on the basis of the diameter of lesions and area of leaves covered with lesions as compared with controls.

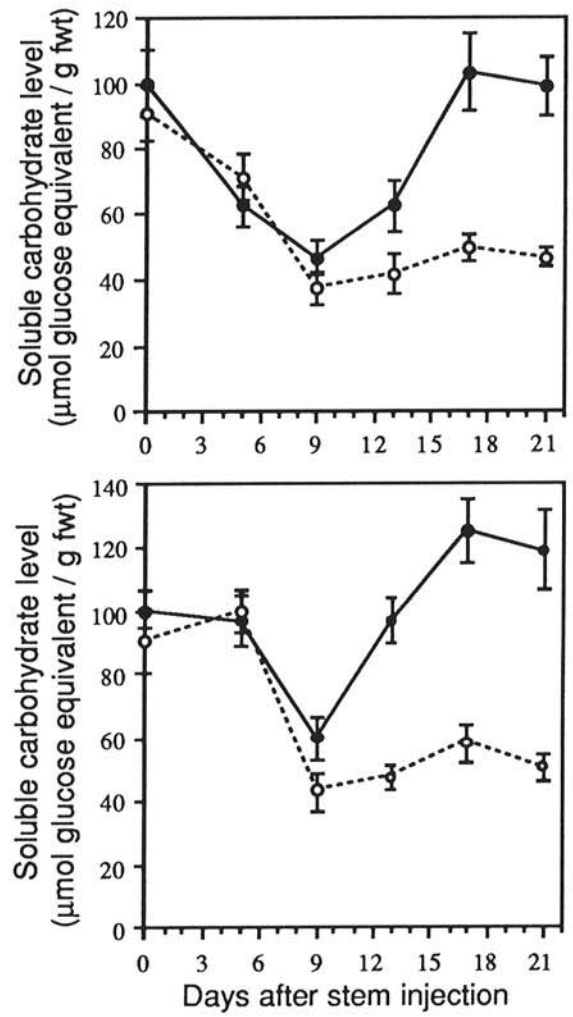

Fig. 1. Soluble carbohydrate levels in stem tissues of nonchallenged tobacco plants stem-injected with *Peronospora tabacina* (●) or water (○). The soluble carbohydrate levels were detected in the stem tissues near injection sites (upper) and in the stem tissues remote from injection sites (lower). Data in the figure represent means of triplicate determinations with three plants per treatment. The experiment was repeated twice, and data from a representative experiment are presented. The bars indicate the standard errors.

Soluble carbohydrate level in plants induced by *P. tabacina*. Stem injection with *P. tabacina* resulted in slowly expanding necrosis in the stem near the site of injection. The necrosis became visible 4 days after injection. After injection (9–13 days), soluble carbohydrate levels were higher in the necrotic stem tissues of *P. tabacina*-injected plants as compared with the green stem tissues at the same position in the controls (Fig. 1, upper panel). The differences in soluble carbohydrate levels at the sites of injection between the induced and control plants increased with the time (Fig. 1, upper panel).

Similarly, 9–13 days after injection, soluble carbohydrate levels were higher in the green stem tissues (remote from sites of injection) of the induced plants relative to the controls, and the differences in soluble carbohydrate levels between the induced and control plants also increased with time (Fig. 1, lower panel).

Thirteen days after injection, higher soluble carbohydrate levels were detected in the leaves of induced plants than in those of the controls (Fig. 2). This was also the time that the induced resistance in tobacco was first detected (10,15). The differences in the carbohydrate levels between the induced and control plants increased with time (Fig. 2); accordingly, the protection also increased with time (10,15). It appeared that the onset and the level of induced systemic resistance were correlated with the elevation of soluble carbohydrate levels in the systemically protected leaves.

In order to examine the response of induced plants to challenge, the soluble carbohydrate levels were detected in the plants challenged with *P. tabacina* 21 days after injection. The soluble carbohydrate levels in challenged leaves of both induced and control plants decreased after challenge, but the levels in the induced plants remained higher than those in the controls during the entire period after challenge (Fig. 2).

Previous experiments indicated that glucose and fructose accounted for most of the elevated soluble carbohydrate level (13). Neither glucose nor fructose inhibited germination of *P. tabacina* sporangiospores at concentrations found in tobacco; fructose inhibited spore germination at levels approximately six-

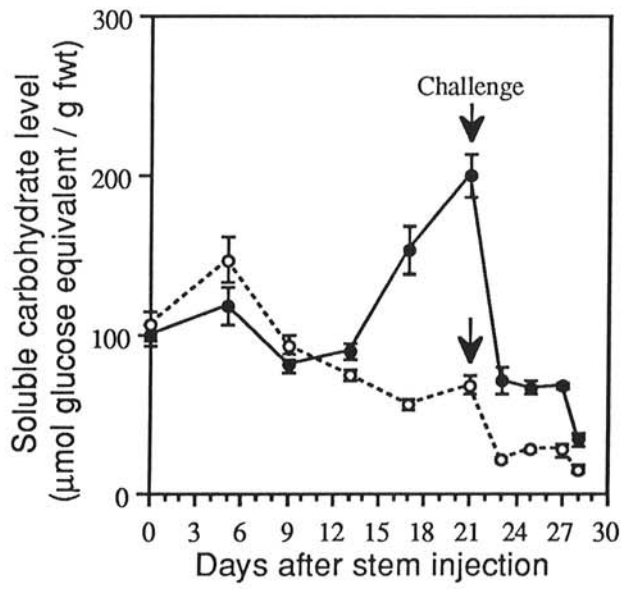

Fig. 2. Soluble carbohydrate levels in systemically protected leaves at various time intervals after stem injection with sporangiospores of *Peronospora tabacina* and challenge with *P. tabacina* (challenged 21 days after induction). Tobacco plants were stem-injected with *P. tabacina* (●) or water (○). Data in the figure represent means of triplicate determinations with three plants per treatment. The experiment was repeated twice, and data from a representative experiment are presented. The bars indicate the standard errors.

fold greater than the highest levels detected in the tissues (13). Administration of glucose, fructose, sucrose, or mixtures of the sugars to the tobacco plants through the petiole increased the soluble carbohydrate levels but did not reduce disease symptoms (13). Direct application of the sugars on the tobacco leaves also did not reduce their susceptibility (13). This suggests that an elevated soluble carbohydrate level is unlikely to be directly responsible for the induced systemic resistance.

Soluble carbohydrate level in plants induced with TMV. Three to four lower leaves of tobacco plants were inoculated with TMV or mock-inoculated. Localized necrotic lesions became visible 2 days after inoculation and continued to enlarge for several days. The soluble carbohydrate levels in the inoculated lower and uninoculated upper leaves were determined at various times after treatment. There was no difference in soluble carbohydrate level in the lower (inoculated) leaves between the induced and control plants during the entire period of induction (Fig. 3). No difference in soluble carbohydrate level was also detected between upper leaves of induced and control plants before or after challenge with *P. tabacina* (Fig. 4). However, the resistance induced by TMV was as good as the resistance induced by *P. tabacina*. This further confirmed that higher soluble carbohydrate levels are not directly responsible for induced systemic resistance. Furthermore, while the soluble carbohydrate levels in the necrotic stem tissues (the sites of induction) of *P. tabacina*-induced plants were elevated, the levels in the TMV-inoculated leaves (the sites of induction) were similar to those in the leaves of control plants, suggesting that the elevated soluble carbohydrate levels were also not associated with the induction of resistance.

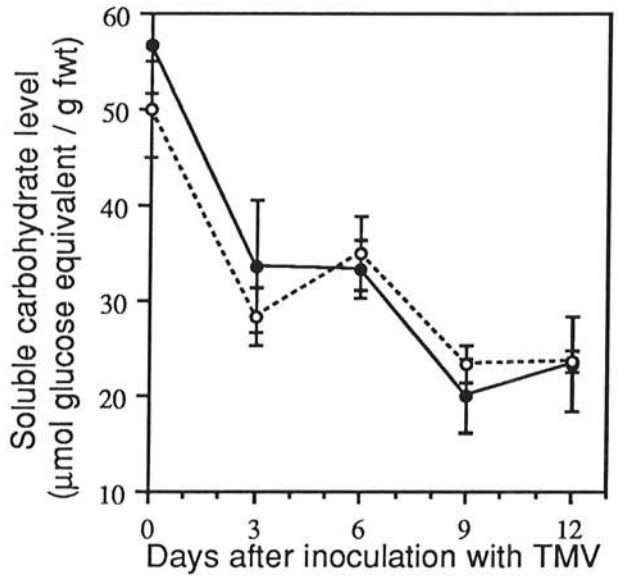

Fig. 3. Soluble carbohydrate levels in TMV-inoculated (•) or mock-inoculated (○) leaves of nonchallenged tobacco plants at various time intervals after inoculation. Data in the figure represent means of triplicate determinations with three plants per treatment. The experiment was repeated twice, and data from a representative experiment are presented. The bars indicate the standard errors.

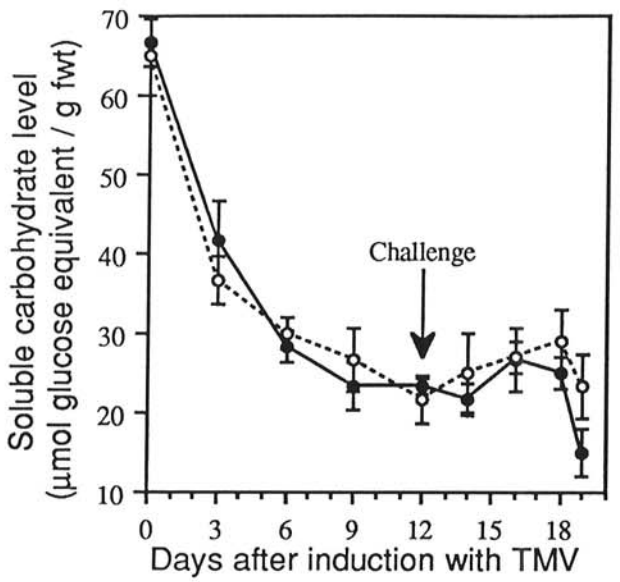

Fig. 4. Soluble carbohydrate levels in upper leaves at various time intervals after inoculating three to four lower leaves with TMV (induction) (•) or mock-inoculation (○), followed by challenge with *Peronospora tabacina* on the upper leaves uninfected with TMV. Data in the figure represent means of triplicate determinations with three plants per treatment. The experiment was repeated twice, and data from a representative experiment are presented. The bars indicate the standard errors.

The soluble carbohydrate levels decreased in the leaves of both *P. tabacina*-injected and control plants upon challenge with *P. tabacina* (Fig. 2). This is likely due to the moving of plants from the greenhouse to the growth room where the light intensity was much lower. The TMV-induced plants were induced and challenged in the growth room, and the soluble carbohydrate levels remained virtually constant even upon challenge with *P. tabacina* (Fig. 4). Soluble carbohydrate levels in both TMV-inoculated and control plants decreased when the plants were moved from the greenhouse to the growth room for induction (Figs. 3 and 4). This suggests that the challenge did not reduce the soluble carbohydrate levels in the *P. tabacina*-induced plants, but the reduced photosynthesis due to lower light intensity in the growth room decreased the soluble carbohydrate levels in the *P. tabacina*-injected and control plants when they were challenged in the growth room.

LITERATURE CITED

1. Cruickshank, I. A. M., and Mandryk, M. 1960. The effect of stem infestation of tobacco with *Peronospora tabacina* Adam on foliage reaction to blue mould. J. Aust. Inst. Agric. Sci. 26:369-372.
2. Dean, R. A., and Timberlake, W. E. 1989. Production of cell wall-degrading enzymes by *Aspergillus nidulans*: A model system for fungal pathogenesis of plants. Plant Cell 1:265-273.
3. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.
4. Horton, J. C., and Keen, N. T. 1966. Sugar repression of endopolygalacturonase and cellulase synthesis during pathogenesis by *Pyrenochaeta terrestris* as a resistance mechanism in onion pink root. Phytopathology 56:908-916.
5. Lukens, R. J. 1970. Melting-out of Kentucky Bluegrass, a low sugar disease. Phytopathology 60:1276-1278.
6. Lyles, W. E., Futrell, M. C., and Atkins, I. M. 1959. Relationship between reaction to race 15B of stem rust and reducing sugars and sucrose in wheat. Phytopathology 49:254-256.
7. Mandryk, M. 1963. Acquired systemic resistance to tobacco mosaic virus in *Nicotiana tabacum* evoked by stem injection with *Peronospora tabacina* Adam. Aust. J. Agric. Res. 14:315-318.
8. McIntyre, J. L., Dodds, A., and Hare, J. D. 1981. Effects of localized infection of *Nicotiana tabacum* by tobacco mosaic virus on systemic resistance against diverse pathogens and an insect. Phytopathology 71:297-301.
9. Pan, S. Q., Ye, X. S., and Kuc, J. 1989. Relationship of β-1,3-glucanase and total soluble carbohydrate to the immunization of tobacco against blue mould caused by *Peronospora tabacina*. (Abstr.) Phytopathology 79:1150.
10. Pan, S. Q., Ye, X. S., and Kuc, J. 1991. Association of β-1,3-glucanase activity and isoform pattern with systemic resistance to blue mold in tobacco induced by stem injection with *Peronospora tabacina* or leaf inoculation with tobacco mosaic virus. Physiol. Mol. Plant Pathol. 39:25-39.
11. Patil, S. H., Hedge, R. K., and Anahosur, K. H. 1985. Role of sugars and phenols in charcoal rot resistance of Sorghum. Phytopathol. Z. 113:30-35.
12. Pe'er, S., and Cohen, Y. 1987. Sugar accumulation in tobacco plants systemically protected against blue mold (*Peronospora tabacina*). Phytoparasitica 15:307-315.
13. Salt, S. D., Pan, S. Q., and Kuc, J. 1988. Carbohydrate changes

in tobacco systemically protected against blue mold by stem infection with *Peronospora tabacina*. Phytopathology 78:733-738.
14. Tuzun, S., and Kuc, J. 1985. A modified technique for inducing systemic resistance to blue mold and increasing growth in tobacco. Phytopathology 75:1127-1129.
15. Tuzun, S., and Kuc, J. 1985. Movement of a factor in tobacco infected with *Peronospora tabacina* Adam which systemically protects against blue mold. Physiol. Plant Pathol. 26:321-330.
16. Vidhyasekaran, P. 1974. Possible role of sugars in restriction of lesion development in finger millet leaves infected with *Helminthosporium tetramera*. Physiol. Plant Pathol. 4:457-467.
17. Ward, E. W. B. 1989. Susceptibility of immature soybean leaves to *Phytophthora* species. Physiol. Mol. Plant Pathol. 34:393-402.
18. Wysong, D. S., and Hooker, A. L. 1966. Relation of soluble solids content and pith condition to *Diplodia* stalk rot in corn hybrids. Phytopathology 56:26-35.
19. Ye, X. S., Pan, S. Q., and Kuc, J. 1989. Pathogenesis-related proteins and systemic resistance to blue mould and tobacco mosaic virus induced by tobacco mosaic virus, *Peronospora tabacina* and aspirin. Physiol. Mol. Plant Pathol. 35:161-175.