The different interaction of isolate × host entry for leaves and pods is a new finding, but the observed differential reaction of leaves and pods themselves to the pathogen in general confirms previous findings (8, 9, 10, 12). The interaction of isolates × leaves of host entries differs from results at CIAT, possibly due to the different combination of host genotypes and isolates used in their studies (2). These results emphasize the importance of testing leaves and pods of progeny and lines with different isolates in order to develop uniform and wide resistance to the pathogen.

**Literature Cited**


**Studies on the Nitrate Reductase Activities of the Fruit and the Source Leaf in Pepper**

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Additional index words. Capsicum annuum

**Abstract.** Nitrate reductase (NR) activity (NO$_3^-$ produced in the dark and under anaerobic conditions) of 30-day-old fruit of *Capsicum annuum* L. was 2.2% that in tissues of a single leaf adjacent to each fruit (33 vs. 1500 nmoles/hr-g fresh weight). The optimal NR activity in one source leaf could only account for about 17% of the fruit's total nitrogen accumulation, while the fruit's own NR activity was almost negligible. Covered and uncovered fruits did not differ significantly in NR activities.

The developing fruit in maturing pepper plants (56 days after anthesis), account for about 50% of the dry weight increase (4, 10) and serve as the major sink for the accumulation of macroelements (N, P, K, Ca, and Mg) (10). Steer (17) examined in vitro NR activities of expanding leaves of *Capsicum frutescens* L. cv. California Wonder and concluded that most nitrate reduction occurs in leaves. Nitrate reduction in this species was light-dependent and only negligible reduction occurred in the dark (13). We reported on the kinetics of N accumulation in developing pepper fruit and the limited importance of fruit photosynthesis to the growth and N assimilation of developing fruit (1). An average, fully grown fruit (60-days postanthesis) weighed 200 g (fresh weight) and accumulated about 420 mg total N. We are not aware of any studies concerning the relationship between nitrate reduction and total nitrogen accumulation of the fruit. We have examined in vivo NR activities of the fruit (covered or uncovered) and the source leaf to determine the total nitrogen accumulation in 30-day-old fruit. We also studied the influence of glucose, ATP, NADH, succinate, or fumarate on NR activity of leaf and fruit tissue.

Individual 'Keystone Resistant Giant' pepper plants were grown in 15-cm pots containing a 1 soil: 1 sand: 4 vermiculite mix in a temperature-controlled (24 ± 2°C) greenhouse. Each pot received 100 ml of Peter's nutrient solution (8 g P, 33 g NK/liter) once every 15 days and cistern water on the remaining days.

The first flower on the main branch was hand-pollinated, and the remaining flowers and flower buds were removed when they appeared. Fruits on half of the plants were covered with aluminum foil when they were 2 cm in diameter (about 10 days postanthesis). The foil was replaced as necessary to keep the expanding fruits covered. At 30 days following anthesis, fruit and leaf tissue were removed from plants (3 with uncovered and 3 with covered fruit), and NR assays were conducted as described below. Both the uncovered and covered fruit were about the same size and weight at this stage of development, but the covered fruits were etiolated. In subsequent portions of this paper, tissue removed from uncovered fruits will be referred to as green and that from covered fruits as etiolated.

A modified NR assay system was used (6, 7, 16). Preliminary experiments were conducted on fruit and leaf tissue to maximize NR by finding the optimal combination of substrate (KNO$_3$), incubation time, and n-propanol concentration. Once the optimal combination of variables had been determined for leaf and fruit tissue, those conditions were used for the incubation to determine the NR of that organ. The 'source' leaves (leaf closest to the fruit) and fruits were collected 3–4 hr after sunrise and temperature kept constant until assays were conducted. Four disks were removed from each source leaf or fruit using a 6 mm corkborer. The disks then were weighed and NR assays were conducted. The minimal amount of tissue which proved appropriate for the assay was 2 disks. Therefore, assays were run on pairs of disks incubated in 2 ml of medium. The incubation medium (50 mM K-phosphate buffer, 50 mM KNO$_3$, pH 7.5, but no n-propanol) had been added previously to the

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vials and kept in an ice bucket. N-propanol (1%, final concentration) was added after the disks had been placed in the vials. The leaf and fruit disks sank to the bottom of the vial upon vacuum infiltration. The tubes were quickly stoppered and incubated with constant agitation in a water bath maintained at 30°C. This treatment was used to ensure rapid penetration of NOF into the tissue and to produce anaerobic conditions during the subsequent incubation period (7). At the end of the incubation period (1 hr for fruit; 2 hr for leaf tissue), 0.4 ml were withdrawn from each vial for NOF determination.

Nitrite concentration was determined by adding 0.3 ml of 1% sulfanilamide reagent and 0.3 ml of 0.02% N-naphthylethylene diamine HCl (3n) solution. The absorbance was read at 540 nm after 20 min. NR activity is expressed as the rate of NOF produced per hr per gram fresh weight. Various metabolites (glucose, NADH, ATP, succinate, or fumarate at 2, 4, 6, 8, and 10 mM final concentration) were added to the incubation medium and vacuum-infiltrated to check for possible stimulation of NOF reduction by leaf and fruit tissue.

It is widely accepted that photosynthetic cells that are able to assimilate nitrate in the dark can use carbohydrates or other reduced compounds for the reduction of nitrate and nitrite (9). In order to maximize in vivo NR activities of the leaf and fruit tissue, various metabolites were added separately to the incubation medium and the results are presented in Table 1. Both the leaf and fruit’s NOF production was inhibited by NADH. NOF produced following the addition of 6 mM NADH was 22% of the control in the case of the leaf. The comparable value for fruit was 73%, and there was no apparent difference between green and etiolated fruit in this respect. This inhibition is consistent with earlier reports of in vitro inactivation by incubation with NADH (and reversal of the inhibition by ferricyanide) in tobacco cells (19), spinach (12), and in rice plants (8). Two and 10 mM ATP, the most effective concentrations, increased NOF production by 22% and 35% from leaf and fruit disks, respectively.

Sawhney et al. (15) found that infiltration with either ATP or fructose-1,6 diphosphate resulted in a significant accumulation of NOF in the absence of air and proposed that photosynthesis may regulate NOF reduction by supplying ATP.

Provision of glucose to the leaf or fruit (green) increased NOF production but had no effect on etiolated fruit. Two and 10 mM glucose, the most effective concentrations, increased NOF production by about 71% and 73% from leaf and fruit tissues, respectively. The stimulation of NR activity by glucose could result either from the production of NADH by glycolysis and/or citric acid cycle dehydrogenases (14) or through the influence on the amount of NOF entering the metabolic NOF2 pool from the external medium (2).

Mulder et al. (11), Klepper et al. (7), and Sawhney et al. (14) reported significant increases in NOF production following the addition of Kreb’s cycle intermediates. For example, an increase of 35% and 48% following the addition of succinate and fumarate, respectively, was reported for wheat seedlings (14). In the present experiments, succinate (4-10 mM) increased NOF production from the fruit disks by about 30%. Succinate up to 6 mM had no marked effect, but 10 mM decreased NOF production by 50% from the leaf disks. Fumarate had little or no effect on either fruit or leaf tissue (data not shown). Because NR is substrate-inducible, it usually is believed that changes in activity can be attributed to changes in the level of enzyme protein (3, 18). It is important to note that the results presented above concerning the changes in NR activities (increase or decrease) induced by various intermediary metabolites are based on the catalytic production of NOF from NO3 and not on enzyme protein. It is probable that all of the changes could be mediated by a modulation of enzyme activity rather than by alteration of enzyme protein levels.

Although ATP, glucose, and succinate enhanced in vivo NR activities of fruit tissue, the increase was insufficient to account for the total reduced nitrogen deposited in the fruit. This observation suggests that the major site for NOF assimilation is the leaf rather than the fruit. The in vivo nitrate reductase assay showed that pepper leaf disks readily reduced NOF to NO3 in the dark and under anaerobic conditions. This is consistent with the earlier report by Steer (17) who investigated in vitro NR activities of expanding leaves of C. frutescens and concluded that most NOF reduction occurs in leaves.

As mentioned in the introduction, an average, fully grown pepper plant (60 days postanthesis) weighs 200 g (fresh weight) and accumulated about 420 mg (30 mmoles) total N (1). Although biomass and total N accumulated up to day 55, about 62% of the total biomass and N accumulation was achieved by day 30; a midpoint between anthesis and the red-ripe stage. The maximum rate of nitrogen deposition was 25 mg (1.79 mmolles)/day and occurred between days 20 and 25, from then on the rate of increase slowed down. Time-course experiments conducted during the growth of the fruit revealed that the in vivo NR activity of the source leaf reached a maximum of 1.5 umolmes/h/g fresh weight when the fruit was 30-days-old and then leveled off (Fig. 1). We did not observe any decline in the NR activity of the source leaf during the next 20 days. These findings are consistent with the observations that fruit development retards senescence and protein degradation in aging pepper leaves (5).

By multiplying the in vivo NR activity of the leaf with its fresh weight, which averaged 3 g, and by multiplying the resulting value for 30 days (here it is assumed that NR activity is maintained at maximum levels throughout a 24 hr day), the highest possible amount of NOF produced over the 30-day period is 3.24 mmoles. The amount of nitrogen accumulated by the fruit over the same period is 18.57 mmoles. In reality, 3.24 mmoles is an over estimation because nitrate reduction is light-dependent and only negligible reduction occurs in C. frutescens in the dark (13). Furthermore, the increase in leaf NR activity during the fruit development phase was steady till day 30 (postanthesis) and then leveled off (see Fig. 1). Even if the maximum NR activity of the source leaf observed on day 30 (or 40 or 50) were to operate throughout the growth of the fruit it could account for only about 17% of the fruit’s total N accumulation observed on day 30. If there were a tight source-sink relationship in the present experiments, one would expect most of the fruit’s 18.57 mmoles N to be imported from this source leaf. The question thus arises as to the source of the remaining nitrogen. Because the fruit’s own NR activity was almost negligible, the remaining nitrogen deposited in the fruit could be attributed to the

![Fig. 1. Changes in the in vivo leaf nitrate reductase activity during the fruit development phase of pepper plant (days postanthesis). Vertical bars represent SE.](image)

Table 1. The influence of NADH, ATP, glucose, or succinate on in vivo NR activity of leaf and fruit tissue.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conc (mM)</th>
<th>Leaf</th>
<th>Fruit</th>
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<tbody>
<tr>
<td>NADH</td>
<td>0</td>
<td>36</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1050</td>
<td>25</td>
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</tr>
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<td>Glucose</td>
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</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>10</td>
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<tr>
<td></td>
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<td>33</td>
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</table>

*Each value is the average of 3 replicates, and comparable results were obtained in 2 separate experiments.*
involvement of additional leaves. The flower-pruned plants possessed about 12 leaves per fruit on the main stem in our experiments. Assuming that each leaf possessed comparable NR activity to that of the measured leaf, it follows that there was more than adequate amounts of NR in the shoot to account for the reduced nitrogen accumulated in mature fruits. This favorable relationship between estimated leaf NR activity and nitrogen accumulation by the fruit negates the necessity for NO₃ reduction in pepper roots where no NR activity was observed (13).

Literature Cited


Inheritance of Bacterial Spot Resistance in Pepper

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Additional index words. Xanthomonas campestris pv. vesicatoria, Capsicum annuum

Abstract. All possible crosses, excluding reciprocals, among 3 bacterial-spot-resistant plant introductions of pepper and the susceptible 'Yolo Wonder' were evaluated for resistance in the F₁ along with the backcross (BC) F₁s derived from backcrosses to the susceptible cultivar. PI 322719 carries a single dominant gene for resistance that is independent of the one carried by PI 163192. The resistance of PI 163192 is more complex; it is independent of the resistance of PI 322719 but may be associated with that of PI 163192. Nonsegregating, resistant families were recovered in the F₁s of all crosses and backcrosses.

Bacterial spot, caused by Xanthomonas campestris pv. vesicatoria (Dodge) Dye, is a serious problem of commercial cultivars of pepper. Sowell (2) reported resistance in a number of plant introductions from India, including PI 163184, 163189, 163192, 183922, 244670, and 246331. Cook and Stall (1) confirmed the resistance of these PI s and reported that the resistance of PI 163192 was due to a single, dominant gene. The other resistant introduction, when crossed with susceptible lines, produced F₁s having intermediate reactions to the pathogen and probably had multigenic resistance. Sowell and Dempsey (3) reported resistance in PI 322719, another introduction from India that has larger fruit than the other resistant introductions. No cultivar carrying resistance from any of these sources has been released. The resistance reaction does not in any case amount to total immunity.

It would be desirable to combine different genes that act in a complementary fashion to achieve a higher level of resistance. If a practical level of immunity could be achieved, then the population of the pathogen would be reduced and thus less able to produce resistance-breaking variants. The first step, and purpose of this work, must be to find sources of resistance that have different genetic bases.

Constraints of time and space limit the number of populations that can be evaluated simultaneously. We chose 3 PIs thought most likely to have different genes for resistance: 1) PI 163192 because it had been studied previously; 2) PI 322719 because of its overall phenotypic difference and large fruit size; and 3) PI 163189 as a representative of those introductions thought to have multigenic resistance. 'Yolo Wonder' (YW) was included as a susceptible parent carrying the desirable attributes of cultivated pepper. All possible crosses, excluding reciprocals, were made among the 4 parents. The F₁ hybrids of resistant x susceptible pairings were backcrossed to the susceptible parent and all F₁s were selfed to produce an F₂ generation.

The method to be used in the genetic study was a major concern. Since resistance is not absolute and the level of infection in a test is difficult to control precisely, we doubted that resistant and susceptible plants could be identified with sufficient precision for a genetic study of backcross (BC) and F₂ generations. Individual plant ratings would be critical in these. Two preliminary tests were run. The growing medium (pH 5.5–5.6) in these and subsequent tests was a composted mixture of peatmoss, soil, vermiculite, and...