

Chili Pepper Fruits: Content and Pattern of Capsaicinoids in Single Fruits of Different Ages

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The content of capsaicinoids differs widely in fruits of an individual plant. This is shown for *Capsicum annuum* var. Cayenne and var. DeArbol and *Capsicum frutescens* var. Hot Siberian, respectively. Three age groups, (i) very young, (ii) medium age, and (iii) older fruits, were studied. A consistent dependence on the node position on the plant for fruit weight and capsaicinoid content of the individual fruits was not observed. These traits do not develop concomitantly and are influenced differently by environmental factors. Therefore, the expression as capsaicinoid content per fruit leads to a different conclusion than a comparison of concentration values (mg/kg). This is exemplified for *C. frutescens* var. Hot Siberian grown in two consecutive years with fruits of lower fruit weight but the same capsaicinoid accumulation in the second year. Higher values for pungency (expressed as mg/kg) would have been the result from the analysis of bulked material. The fatty acid pattern of capsaicinoids is uniform for all fruits from one plant, irrespective of the large variation of total capsaicinoid content.

KEYWORDS: *Capsicum*; individual fruits; fruit weight; capsaicin; dihydrocapsaicin; nordihydrocapsaicin; heterogeneity

INTRODUCTION

The genus *Capsicum* arose in the arid, high elevations of the Andes in South and Central America and was an important part of the diet in the pre-Columbian high cultures (1). After the discovery of the Americas by the Europeans, wild and domesticated *Capsicum* species were spread all over the subtropical world. Their fruits are used as vegetables and as spice and are increasingly popular in ethnic cuisines. Their pungent principle resides in the capsaicinoids, the amides made up of vanillylamine, and characteristic medium chain length fatty acids (Figure 1) (2).

These compounds exhibit interesting pharmacological properties and are discussed as a way to manage obesity (3, 4).

The nonpungent species *Capsicum ciliatum* is basal to all *Capsicum* taxa (5). Polymorphism for pungency is found in the ancestral species *Capsicum chacoense*, *Capsicum baccatum*, and *Capsicum eximium* (6). It is unclear when and how pungency appeared in the genus, providing the plant with a good deterrent against fruit consumption by rodents, but still allowing seed dispersal by birds (7). Nonpungency arose again in the domestication of *Capsicum annuum* at a later time, when a deletion in the *Pun1* locus occurred, giving rise to probably all mild varieties cultivated today (8).

Recent genetic studies elucidated aspects about the regulation of the content of individual capsaicinoids and their sum. From plants of a cross between a mildly pungent *C. annuum* variety and highly pungent *Capsicum chinense* var. Habanero, mature

fruits were harvested from nodes 1–4, bulked per plant, and analyzed for capsaicinoid concentration values (mg/kg) (9). In a cross between a nonpungent and a pungent variety of *C. annuum*, single mature fruits were analyzed for capsaicin and dihydrocapsaicin concentrations (10). The synthesis of the capsaicinoids seems to be controlled by different genes and epistatic effects.

In quantitative trait loci (QTL) analysis of a cross between a pungent *Capsicum frutescens* and a nonpungent *C. annuum* variety (11) and between another pungent *C. frutescens* and a mildly pungent *C. annuum* variety (12), capsaicinoid contents of bulked fruits were compared to polymorphism in DNA from leaf tissue. Five genetic loci on three chromosomes were identified for capsaicin and total capsaicinoid content, four of them also for dihydrocapsaicin content, and an additional one for nordihydrocapsaicin at another location. One of these locations is close to a gene for the branched-chain amino acid transferase (BCAT) implicated in the biosynthesis of the characteristic fatty acids (12).

For mature fruits of *C. annuum* a relationship between pungency and node position on the plant was observed and discussed as resulting from competition for photosynthates (13).

In pungent varieties variable capsaicinoid contents for individual ripe fruits from the same plant have been observed (14). This was investigated further. Removing fruits from the plant may influence the availability of nutrients for the remaining fruits, and using bulked material originating from several fruits will blur information available from individual fruits. We therefore harvested all fruits from the plant on one day, documented their position, and analyzed them individually. Results for three varieties are reported.

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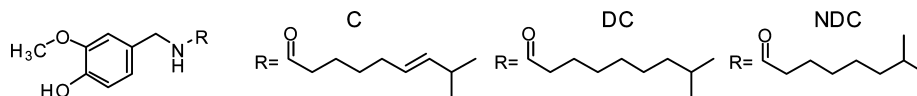


Figure 1. Vanillyllamine and the fatty acid moieties of the three major capsaicinoids, capsaicin (C), dihydrocapsaicin (DC), and nordihydrocapsaicin (NDC).

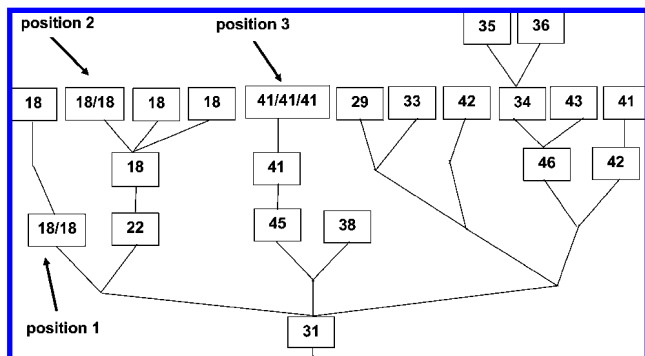


Figure 2. Schematic representation of *Capsicum annuum* var. Cayenne, plant A. Lines represent branches and squares represent fruits, harvested at the indicated age (DAF, days after flowering); positions 1–3 show twins and triplicates.

MATERIALS AND METHODS

Plant Material. Seeds of *C. annuum* var. Cayenne, *C. annuum* var. DeArbol, and *C. frutescens* var. Hot Siberian were obtained from RUEHLEMANNS (Horstedt, Germany). Plants were grown during the summers of 2001 and 2002 in a greenhouse at 28–32 °C day and 22–25 °C night temperatures with supplemental lighting (16 h). They were grown on a mixed soil of Compo SANA (Compo, Muenster, Germany), and Peligran G (Deutsche Perlit, Dortmund, Germany) 80 + 20 (v/v). NPK (Compo) fertilizer was added once a week. Flowers were tagged at blossom opening and self-pollinated within the plant. All fruits present on the plant were harvested on one day, and their exact position, age (days after flowering, DAF), and fruit weight documented (Figure 2). They were immediately frozen in liquid nitrogen and kept at –20 °C.

Sample Preparation and Capsaicinoid Analysis. Single fruits were crushed while frozen. Methanol was added as extraction agent, taking into account the water provided by the fresh fruit. Homogenization was done for 1 min with an Ultra-Turrax (Janke & Kunzel, Staufen, Germany) in a final volume of 30 mL of 75% methanol. The solution was left standing for 30 min. After filtration, HPLC with fluorescence detection and quantification for the three major capsaicinoids, capsaicin (C), dihydrocapsaicin (DC), and nordihydrocapsaicin (NDC), was performed as described previously (15). Single fruits are available only once and cannot be analyzed in duplicate or triplicate. Several times fruits were bulked, minced, and carefully homogenized. This material served for triplicate sampling and analysis to establish the precision of the analytical procedure. The coefficient of variation was always better than 7.5%.

Statistical Analysis. Medians, percentiles, and correlation coefficients for values within groups of fruit were calculated with Microsoft Excel.

RESULTS

Capsaicinoids are synthesized in the placenta of the fruits. The pericarp contributes about 80% of the fruit weight. Placenta and pericarp do not grow at the same rate. If whole fruits are analyzed, the concentration of capsaicinoids (expressed as mg/kg) depends on the weight of all parts of the fruit. It is therefore reasonable to consider capsaicinoids as milligrams per fruit and not as concentration (mg/kg). Thereby, confounding effects due to differences in the thickness of the pericarp and due to water loss in older fruits, partly dried on the plant, are avoided.

C. annuum Var. **Cayenne.** Two plants were grown in the summer of 2001. All fruits from the plant were harvested on the same day. The distribution on the plant concerning the age

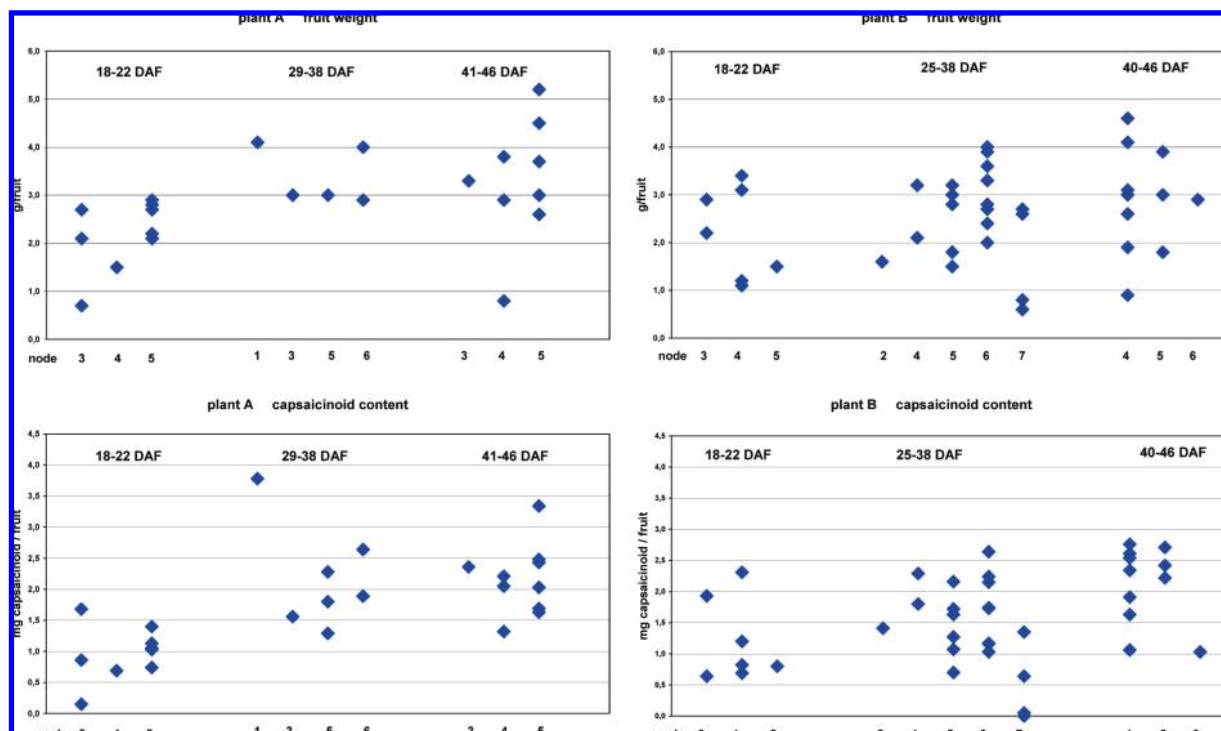


Figure 3. *Capsicum annuum* var. Cayenne: fruit weight and capsaicinoid content for individual fruits. All fruits were harvested from two plants, A and B; three age groups, 18–22, 25–38, and 40–46 DAF; and node positions 1–7.

Table 1. Fruit Weight, Capsaicinoid Content, and Pungency (Capsaicinoid Concentration), According to Node Position^a

age group	18–22 DAF					25–39 DAF					40–46 DAF			
	3	4	5	1	2	3	4	5	6	7	3	4	5	7
node position	3	4	5	1	2	3	4	5	6	7	3	4	5	7
no. of fruits	5	5	6	1	1	1	2	9	11	4	1	11	9	1
total fruit wt (g)	10.6	10.3	14.2	4.1	1.6	3.0	5.3	24.5	35.2	6.7	3.3	30.7	30.3	2.9
total capsaicinoid content (mg)	5.26	5.71	6.15	3.78	1.41	1.56	4.05	13.9	20.1	2.04	2.36	23.0	21.0	1.03
pungency (mg/kg)	500	550	430	920	880	520	770	570	570	300	710	750	690	300

^a Values as sum for all fruits combined from two plants of *Capsicum annuum* var. Cayenne, three age groups as ranges of days after flowering (DAF).

Table 2. Fruit Weight, Capsaicinoid Content, and Pungency (Capsaicinoid Concentration) for Single Fruits Grown at the Same Position (Twins, Triplets) on *Capsicum annuum* Var. Cayenne, Plant A (Figure 2)

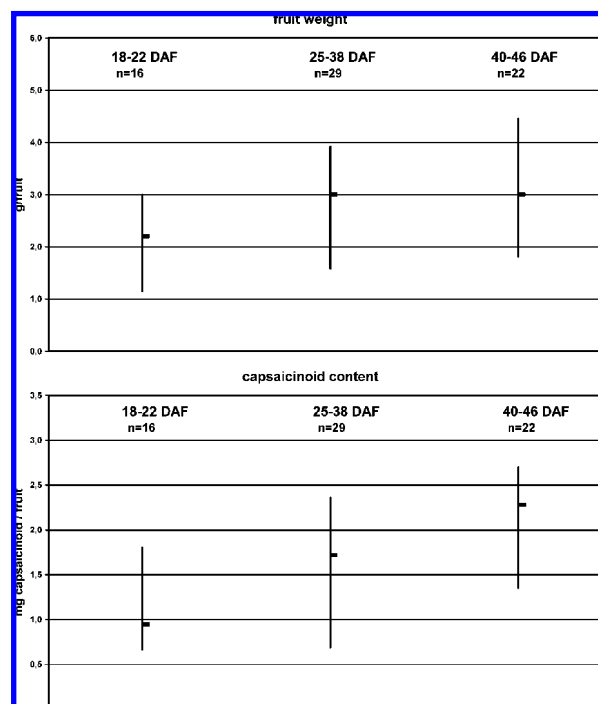
position	age (DAF)	node	fruit wt (g)	capsaicinoid content (mg)	pungency (mg/kg)
1	18	3	0.7	0.15	210
			2.1	0.86	410
2	18	5	2.1	0.74	350
			2.9	1.05	360
3	41	5	2.6	2.03	780
			3.7	1.63	440
			4.5	2.48	550

of the fruit was variable (Figure 2). For a defined day after flowering up to 12 fruits, only 1, or no fruit were obtained; a representative sampling per day was not possible. They were therefore grouped as very young fruits (18–22 DAF), slightly older fruits (≥ 29 DAF from plant A and ≥ 25 DAF from plant B), and fruits considered to be ripe (≥ 40 DAF). We compared these three groups for both plants separately with respect to the node position. For each plant they are of the same genetic origin and experienced the same environmental influences, for example, light and nutrition. Neither fruit weight nor capsaicinoid accumulation showed a consistent dependence on node position (Figure 3).

In plant A both traits showed an increase from the very young to the older fruits. In plant B the large variation in the values for the individual fruits obscured this trend. The range of values was the same for both plants. The extremes were then excluded for the comparison. Normal distribution of values was not assured; therefore, values for the median and percentiles instead of mean and standard error were calculated for the fruits from both plants. The bars in the figures depict the values for 80% of the fruits, ranging from the 10th to the 90th percentile (Figure 4).

In young fruits (18–22 DAF) the pericarp, which contributes mainly to the fruit weight, and the placenta containing the capsaicinoids are not yet fully developed. The median is low for both traits, the value for the 90th percentile reaching to just the median of the next age group. In the two other age groups, the median for fruit weight remains at 3.0 g, with a skewed range for both groups. Capsaicinoid contents differ between the two age groups. Only a few old ripe fruits exhibit a low capsaicinoid content; this results in a higher median for the group >40 DAF and also affects the value for the 10th percentile, but much less the 90th percentile.

Fruit weight and capsaicinoid content show a correlation ($r = 0.81$) in the young fruits only; both develop at about the same rate. This is not observed in the other age groups. In ripe fruits only 26% of the variance ($r = 0.51$) in capsaicinoid content is explained by fruit weight. The expression for pungency as concentration value (mg/kg) is confounded by the inconsistent contribution of the pericarp.

**Figure 4.** Fruit weight and capsaicinoid content, median and range from the 10th to the 90th percentile. Individual fruits were from two plants, *Capsicum annuum* var. Cayenne.

The dependence on node position had been observed by Zewdie and Bosland (13) for the capsaicinoid concentration (mg/kg) obtained for bulked fruits. To allow a comparison of our data with their results, we included all fruits from both plants. For a specified node and age group, fruit weights (g) and capsaicinoid contents (mg) for all harvested fruits were summed, and then the value was calculated for the concentration (mg/kg) (Table 1). This approach mimics the analysis of bulked fruit material.

In the 16 very young fruits, having experienced competition for nutrients by fruits already present on the plant, nodes 3 and 4 bore comparable fruit weight and capsaicinoid amount, resulting in pungencies of 500 and 550 mg/kg. At node 5 the higher total fruit weight resulted in a slightly lower pungency, 430 mg/kg. For the 29 fruits in a later developmental stage (25–38 DAF), node 6 bore more fruits with more weight and capsaicinoids compared to node 5, but with the same pungency, 570 mg/kg. The 22 oldest fruits, in the beginning without much competition for nutrients, exhibited comparable weight, capsaicinoids, and pungency at node positions 4 and 5. Individual very pungent fruits are found at node 1 (31 DAF) and node 2 (25 DAF), but also at node 4 (38, 40, and 42 DAF). A low pungency level, assumed as being due to a deficiency in photosynthates or nutrients, is seen only for the fruits at node 7.

The differences in pungency cannot be explained consistently, and the supply of nutrients at different node positions did not determine the capsaicinoid content. This is even more evident in the comparison of “twins” and “triplets”, fruits of the same

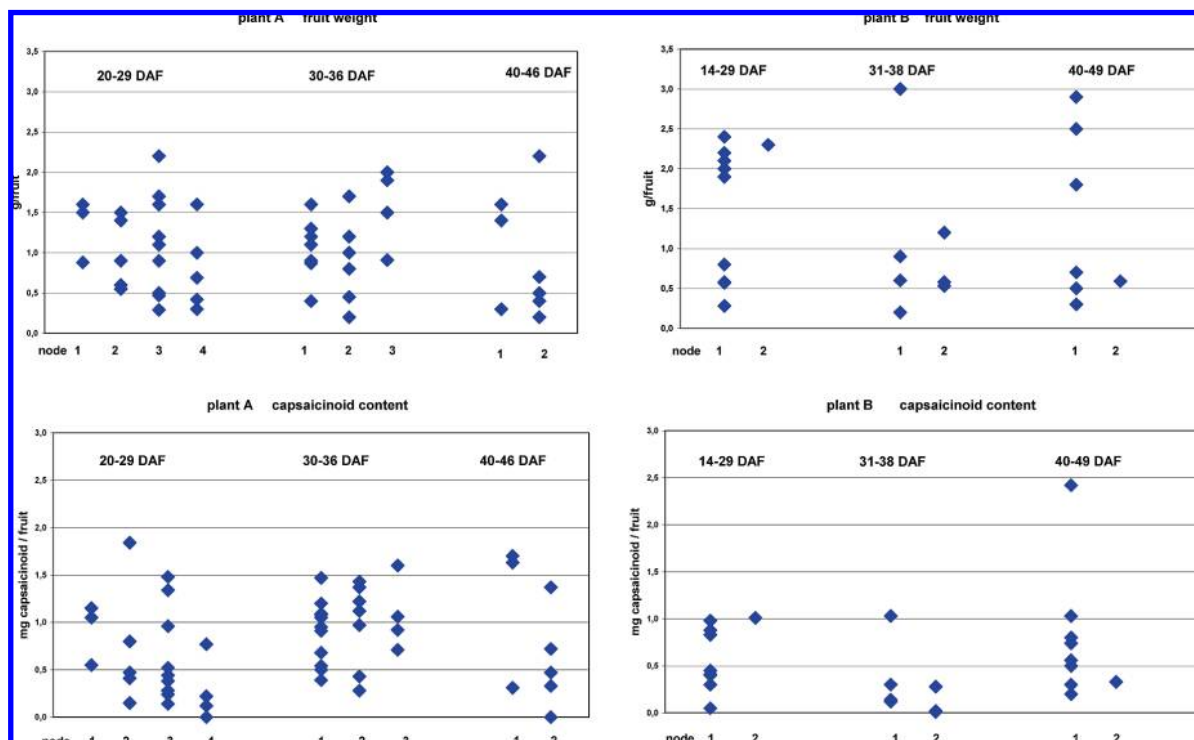


Figure 5. *Capsicum annuum* var. DeArbol: fruit weight and capsaicinoid content from individual fruits. All fruits were harvested from two plants, A and B; three age groups, 14–29, 30–38, and 40–49 DAF; and node positions 1–4.

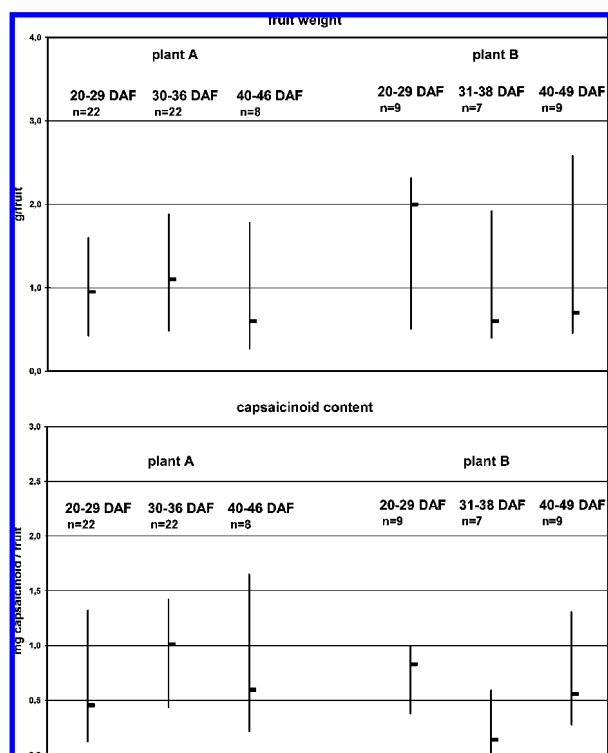


Figure 6. Fruit weight and capsaicinoid content, median and range from the 10th to the 90th percentile. Individual fruits were from two plants, *Capsicum annuum* var. DeArbol.

age grown together at the same position of the plant (Table 2). They show a great variability concerning fruit weight, capsaicinoid content, and pungency (capsaicinoid concentration).

Capsicum annuum Var. DeArbol. Two plants were grown in the summer of 2001. Plant A produced 52 fruits between 20 and 46 days old, located at nodes 1–4. Plant B developed only two nodes and 25 fruits (20–49 DAF) and 1 much younger

Table 3. Fruit Weight, Capsaicinoid Content, and Pungency (Capsaicinoid Concentration) for Single Fruits Grown at the Same Position (Twins, Triplets) on *Capsicum annuum* Var. DeArbol

position	age DAF	node	fruit wt (g)	capsaicinoid content (mg)	pungency (mg/kg)
1/plant A	22	2	0.9	0.47	520
			1.4	0.80	570
			1.5	1.84	1230
2/plant A	35	1	0.4	0.54	1350
			0.9	0.39	430
			1.1	0.51	460
3/plant B	35	2	0.5	0.28	560
			0.6	0.02	30
4/plant B	40	2	0.4	≤0.01	≤30
			0.5	0.72	1440
			2.2	1.37	620
5/plant B	41	3	1.8	0.80	440
			2.5	1.03	410
			2.9	0.74	250

fruit (14 DAF). The age of the fruits at the day of harvest was different from that for *C. annuum* var. Cayenne. Therefore, the fruits were assigned differently to three age groups of comparable numbers: young fruits, ≤29 DAF; medium age fruits, ≥30 DAF; and old fruits, ≥40 DAF.

Again, fruit weight and capsaicinoid content varied greatly and did not depend on node position. Fruits from plant B contained less capsaicinoid, with the exception of one older, small, and rather pungent fruit (45 DAF, 0.7 g, 2.42 mg of capsaicinoid) (Figure 5).

The median and the range from the 10th to the 90th percentile for both traits are shown for both plants separately (Figure 6). The single very young fruit from plant B (14 DAF) was omitted from the young fruit group. When the range for fruit weight in

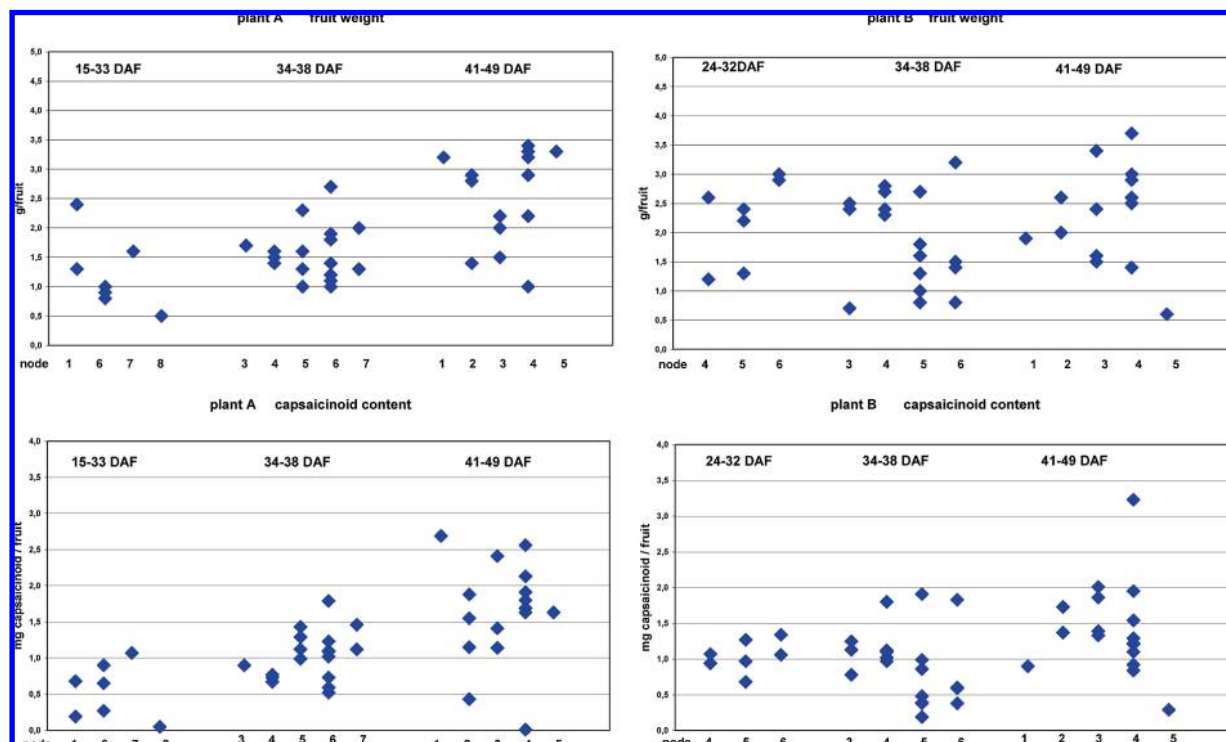


Figure 7. *Capsicum frutescens* var. Hot Siberian: fruit weight and capsaicinoid content from individual fruits. All fruits were from two plants, A and B; three age groups, 15–33, 34–38, and 41–49 DAF; and node positions 1–8.

plant A is considered, the three age groups do not differ, although the median for the fruits ≥ 40 DAF is lower than for those ≤ 36 DAF. For plant B the range for fruit weight was larger if compared to plant A; several heavy young fruits elevate the median for the young fruits to a very high value, 2 g/fruit. Capsaicinoid contents in fruits from plant A showed about the same large but skewed range for the three age groups, with a high median for the fruits 30–36 DAF. In contrast, capsaicinoid contents of fruits from plant B lay in a smaller range, with the lowest median for the fruits 30–36 DAF. Because of these differences, the values for fruits from both plants are not combined as was done for the Cayenne variety.

Fruits were obtained as twins and triplets from both plants. They displayed again a large variation in fruit weight, total capsaicinoid content, and pungency (capsaicinoid concentration) (Table 3).

***Capsicum frutescens* Var. Hot Siberian.** Two plants were grown in the summer of 2001. Fruits were grouped according to young, medium, and old age. Boundaries were chosen to obtain comparable numbers per group. Many fell in a narrow age group, 34–38 DAF; only a very few were younger than 33 DAF. The third group was composed of ripe fruits, 41 DAF and older. The distribution on the plant again was variable. For both plants the range for fruit weight and capsaicinoid content was large and did not depend on the node position (Figure 7).

Both traits, fruit weight and capsaicinoid content, were higher in the oldest age group (Figure 8).

There were too few young fruits (< 24 DAF, $n = 4$) for a reasonable comparison with the many fruits in the developing stage (34–38 DAF, $n = 37$). Therefore, this variety was grown again in the summer of 2002 and fruits were harvested at an earlier time.

This plant C bore more than twice as many fruits when compared to plant A or B. Again, dependence on node position was not observed (Figure 9); capsaicinoid content increased with age until about 32 DAF. Values for individual fruits (> 26

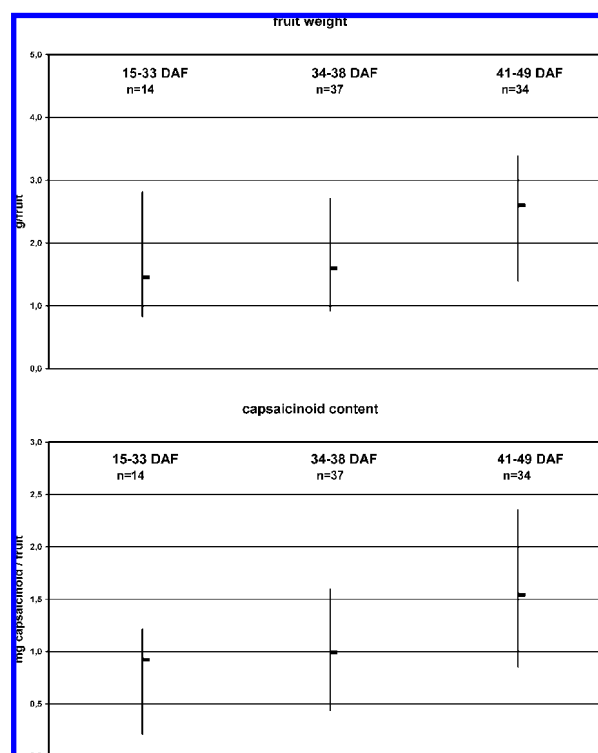


Figure 8. Fruit weight and capsaicinoid content, median and range from the 10th to the 90th percentile. Individual fruits were from two plants, *Capsicum frutescens* var. Hot Siberian.

DAF) from this plant were compared to those from the two plants from 2001 grown in the same greenhouse and conditions.

In the three age groups individual fruit weight in 2002 was lower and capsaicinoid content was comparable to the fruits harvested from the two plants in 2001 (Figure 10). This is seen more clearly in the range (from 10th to the 90th percentile) than in the median. Capsaicinoid accumulation in the placenta

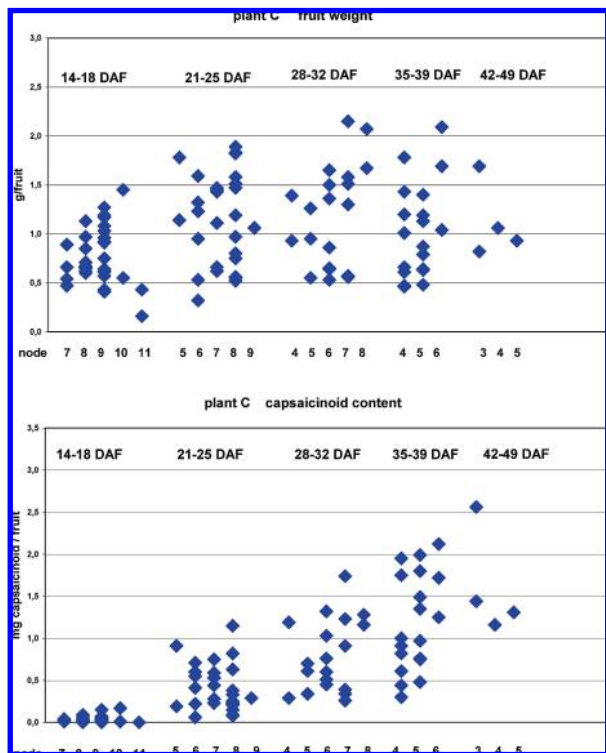


Figure 9. *Capsicum frutescens* var. Hot Siberian: fruit weight and capsaicinoid content from individual fruits. All fruits were from plant C grown in 2002; five age groups, 14–18, 21–25, 28–32, 35–39, and 42–49 DAF; and node positions 4–11.

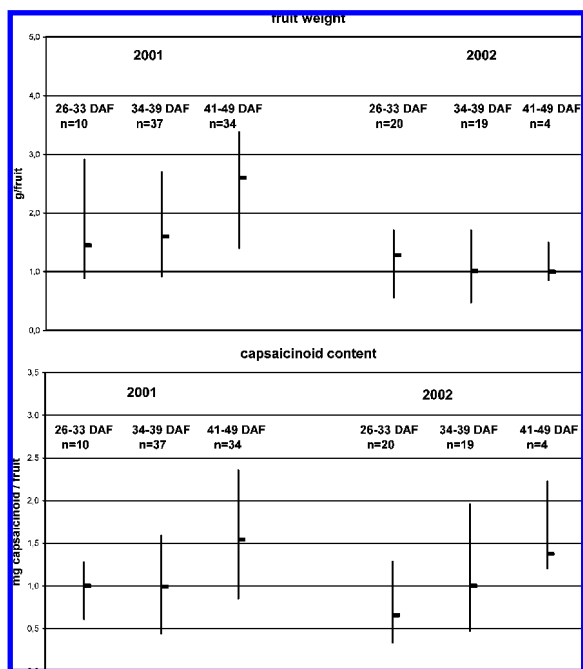


Figure 10. *Capsicum frutescens* var. Hot Siberian: fruit weight and capsaicinoid content, median and range from the 10th to the 90th percentile. Individual fruits were from two plants grown in 2001 and one plant grown in 2002, three age groups.

apparently occurred at a similar rate in both years, whereas growth of the pericarp, determining the fruit weight, was lower in 2002. Considering the concentration (mg/kg), fruits with higher pungency were obtained in 2002.

Capsaicinoid Profiles in the Variety *Capsicum annuum* Var. Cayenne. The pattern of fatty acids is thought not to

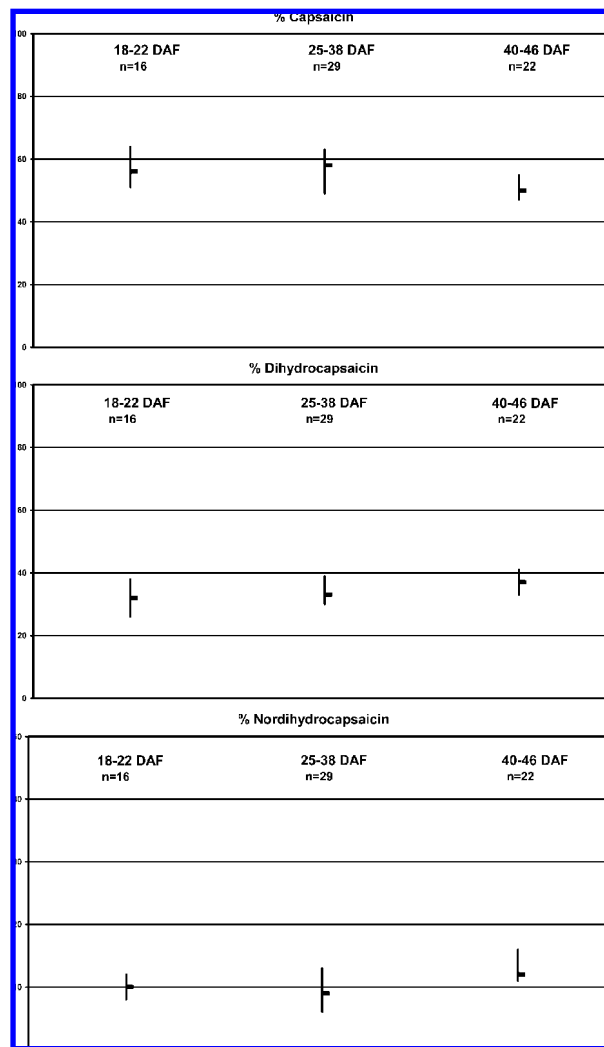


Figure 11. *Capsicum annuum* var. Cayenne: pattern of the three main capsaicinoids expressed as percent capsaicin, dihydrocapsaicin, and nordihydrocapsaicin of their sum (total capsaicinoid). Fruits were from two plants, median and range from the 10th to the 90th percentile.

change during fruit development (2). This was analyzed by looking at the three main capsaicinoids separately and in relation to total capsaicinoid content.

Whereas values for the individual capsaicinoids C, DC, and NDC showed the same large variability as for total capsaicinoid content (data not shown), the relative contribution, expressed as percent, was rather uniform within the three age groups (Figure 11). The fatty acid pattern for the capsaicinoids from the twins and triplets was within the same range (data not shown). In ripe fruits (40–46 DAF) nordihydrocapsaicin contributes slightly more and capsaicin less to the total capsaicinoid content.

Capsaicinoid Profiles in the Variety *Capsicum annuum* Var. DeArbol. As observed for *C. annuum* var. Cayenne, the relative contribution of the three main capsaicinoids was more uniform than total capsaicinoid content (Figure 12).

Results from plants A and B were different. In plant A capsaicin and dihydrocapsaicin both contribute ~42% in developing and ~40% in older fruits; values for nordihydrocapsaicin increased from 12 to 19%. In plant B more capsaicin, >50%, less dihydrocapsaicin, <40%, and less nordihydrocapsaicin, 9–12%, were present. These values are the median for the percent values for all fruits in the age group. The values for the three capsaicinoids add up to 100% only for an individual

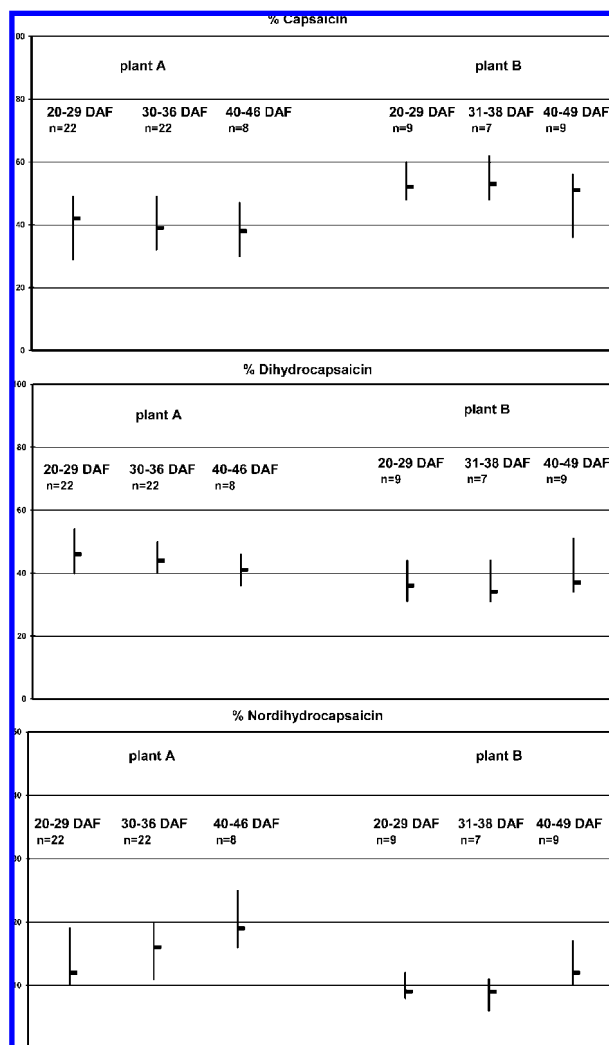


Figure 12. *Capsicum annuum* var. DeArbol: pattern of the three main capsaicinoids expressed as percent capsaicin, dihydrocapsaicin, and nordihydrocapsaicin of their sum (total capsaicinoid). Fruits were from two plants, median and range from the 10th to the 90th percentile.

fruit, not for the three values for the median. Values for the twins and triplets from plant A as well as from plant B fit the corresponding range.

Capsaicinoid Profiles in the Variety *Capsicum frutescens* Var. Hot Siberian. In *C. frutescens* the values for the individual capsaicinoids were variable (data not shown). Their pattern, expressed as relative contribution, was uniform (**Figure 13**). In contrast to the total capsaicinoid content (**Figure 10**), there was a marked difference between fruits from plants A and B, grown in 2001, from those from plant C, grown in 2002. The former exhibited a pattern of 48% capsaicin, 38% dihydrocapsaicin, and 12% nordihydrocapsaicin; the latter, 66% capsaicin, 29% dihydrocapsaicin, and 5% nordihydrocapsaicin. In ripe fruits nordihydrocapsaicin contributes slightly less and capsaicin somewhat more to the total capsaicinoid content when compared to the developing fruits.

DISCUSSION

We could confirm our earlier results (14) that individual fruits from the same plant exhibit variable capsaicinoid contents. For three age groups, (i) young fruits, (ii) developing fruits of medium age, and (iii) older ripe fruits, a consistent dependence of pungency on node position was not observed.

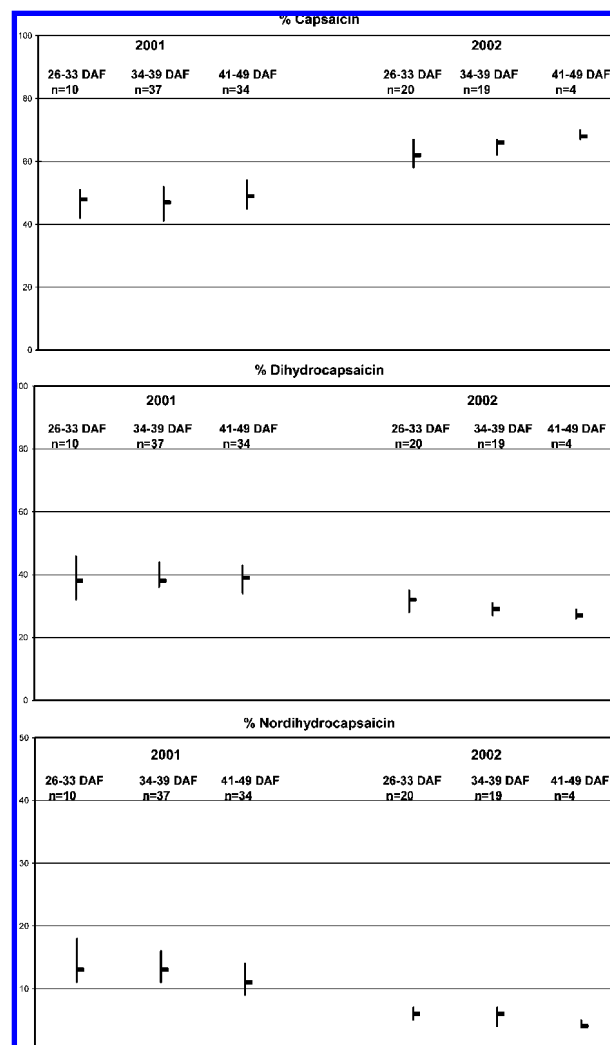


Figure 13. *Capsicum frutescens* var. Hot Siberian: pattern of the three main capsaicinoids expressed as percent capsaicin, dihydrocapsaicin, and nordihydrocapsaicin of their sum (total capsaicinoids). Individual fruits were from two plants grown in 2001 and from only one plant grown in 2002, median and range from the 10th to the 90th percentile.

Our results do not show lower total content (mg/fruit) or concentration (mg/kg) of capsaicinoids for ripe fruits, as discussed in relation to peroxidase-catalyzed metabolism (16). Preliminary observations indicated, instead, that in intact fruits capsaicinoid levels are stable; only a thorough destruction of the cell compartments led to a decrease of capsaicinoids (15).

The range of the values for 80% of the fruits (individuals between the 10th and 90th percentile) can extend from about 0.5 to 2.5 times the value for the median. In particular, for fruits of the same age and the same position on the plant, the capsaicinoid content may differ by an even larger factor (**Tables 2 and 3**). Therefore, a bulked sample of only a few fruits may suffer from sampling error. It may not be representative for an individual plant, or a variety, when used to demonstrate the influence of environmental parameters on capsaicinoid accumulation or the dependence on time.

Influences on the growth of pericarp, and thereby on fruit weight, will confound the analysis of capsaicinoids when bulked material is used to determine concentration values. Fruit weight per se had no significant effect on capsaicinoid concentration (mg/kg) in quantitative trait loci analysis; a low negative correlation ($r = -0.3$) had been detected (12). Fruits had been bulked, and therefore capsaicinoid content per fruit could not

be obtained. Individual fruits were extracted in a comprehensive study on the inheritance of capsaicin and dihydrocapsaicin, but again contents were analyzed as concentration values (mg/kg); details on sampling were not given, and the fruit weight and its possible influence were not considered (10).

When calculated as milligrams of capsaicinoid per kilogram, fruits from *C. frutescens* var. Hot Siberian grown in 2002 had a higher pungency (mg/kg) than those grown in 2001. This was due to different fruit weight, but not to different capsaicinoid accumulation in the placenta, because milligrams per fruit was in the same range in both years (Figure 10). The environmental influence had acted on the growth of the pericarp, which contributed more to the fruit weight in 2001 than in 2002. This observation would have been missed if only concentration values (mg/kg) had been obtained. Proteomic tools should be applied to individual fruits to elucidate the large variability in capsaicinoid content and how the environmental factors affect it.

Irrespective of the total capsaicinoid content, the relative contribution of the three main capsaicinoids, capsaicin, dihydrocapsaicin, and nordihydrocapsaicin, is the same for all fruits from an individual plant throughout the development, leading to a distinct fatty acid pattern for this plant. This is not necessarily characteristic for the variety, as the difference for two plants of *C. annuum* var. DeArbol shows (Figure 12). It had been stated before that capsaicinoid profiles are not useful for taxonomic classification (17), and they were not included as a trait in establishing a core collection (18).

The observed differences between the two plants of *C. frutescens* var. Hot Siberian grown in 2001 and the one grown in 2002 (Figure 13) raise the question about the regulation of the biosynthesis of the capsaicinoids. Whereas total capsaicinoid content may be regulated in the phenylpropanoid pathway with the last step catalyzed by a putative aminotransferase (pAMT) leading to vanillylamine (19, 20), the pattern of the characteristic fatty acids is probably regulated in the fatty acid synthase II complex (21). This would explain the occurrence of the same fatty acids in the esters of vanillyl alcohol (capsinoids) (22) or in coniferyl esters (23), if vanillylamine is not available to capsaicinoid synthase, the enzyme catalyzing the final step in the capsaicinoid biosynthesis.

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