Europ.J.Hort.Sci., **69** (2). S. 45–52, 2004, ISSN 1611-4426. © Verlag Eugen Ulmer GmbH & Co. Stuttgart

# Factors Affecting Quality Traits of Onion (*Allium cepa* L.) Bulbs for the Production of Onion Juice Concentrate and Onion Oil

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## Summary

Effects of various agronomic factors on onion (*Allium cepa* L.) raw material for a combined recovery of onion juice concentrate and onion oil were studied in respect to their impact on crucial onion quality traits such as pungency – estimated as total pyruvic acid (PA) – and total soluble solids (TSS). Cultivar differences, year-to-year variability and effects of planting method, bulb size, S and N fertilization, as well as the impact of the type of preceding crop within a crop rotation were investigated in field trials. In a cultivar comparison of 16 cultivars, a weak correlation between TSS and PA was observed ($r=0.64$, $P=0.008$). In a year-to-year variability study PA ranking among six cultivars remained fairly constant over three years. The method of planting had a significant ($P<0.05$) effect on PA. Bulbs directly sown (cvs. 'Sturon' and 'Stuttgarter Riesen') showed increased pungency compared to bulbs grown from sets. Within a batch of onion bulbs (cv. 'Sturon'), grading for bulbs size exhibited no significant difference in PA among three calibres. In a crop rotation onions grown after legumes as preceding crop had significantly higher pungency ($P<0.001$) compared to onions grown after cereals. This effect is assumed to be a result of enhanced mineralisation of organic N and S sources. TSS contents were not significantly affected by any treatment. In fertilization experiments, a combined N (0 and 130 kg N $ha^{-1}$) and S (0, 50 and 100 kg S $ha^{-1}$) application increased pungency and showed a significant NxS interaction for PA ($P=0.032$), but not for bulb-S and bulb-N. No effect on TSS was observed. S application of 100 kg S $ha^{-1}$ *vs.* 0 kg S $ha^{-1}$ had no qualitative impact in terms of relative composition of major onion oil compounds and ratio of the biosynthetic aroma precursors methyl- and 1-propenyl-L-cysteine sulfoxide, but caused a remarkable increase of absolute amounts of volatiles (steam distillate), aroma precursors and industrially produced onion oil.

**Key words.** Fertilization – onion – preceding crop – pungency – pyruvic acid – sulfur

## Zusammenfassung

**Einflussfaktoren auf die Bulbenqualität der Küchenzwiebel (*Allium cepa* L.) bei der Gewinnung von Zwiebelsaftkonzentrat und Zwiebelöl.** Verschiedene agronomische Faktoren bei der Erzeugung von Zwiebelrohware für eine kombinierte Zwiebelsaftkonzentrat- und Zwiebelölgewinnung wurden hinsichtlich ihrer Auswirkungen auf qualitätsbestimmende Merkmale wie lösliche Trockensubstanz (TSS) und Schärfe – angegeben als Pyruvat (PA) – untersucht. Die in Feldversuchen untersuchten Faktoren umfassen Sortenunterschiede, saisonale Schwankungen und Einflüsse durch die Art der Pflanzung, Bulbengröße, S- und N-Düngung, sowie die Art der Vorfrucht innerhalb einer Fruchtfolge. Bei einem Vergleich über 16 Sorten konnte eine schwache Korrelation zwischen TSS und PA beobachtet werden ($r=0{,}64$, $P=0{,}008$). Saisonale Schärfeunterschiede zwischen 6 Sorten ergaben über 3 Jahre keine erheblichen Auswirkungen auf die Rangordnung. Die Art der Pflanzung hatte eine signifikante Auswirkung ($P<0{,}05$) auf die Schärfe der Bulben. Saatzwiebeln (Cvs. 'Sturon' und 'Stuttgarter Riesen') wiesen eine erhöhte Schärfe im Vergleich zu Steckzwiebeln auf. Eine Sortierung von Bulben einer Population in 3 Größenklassen ergab keinen signifikanten Unterschied der Pyruvatgehalte zwischen den Klassen. Verglichen mit Getreide als Vorfrucht erhöhten Leguminosen als Vorfrucht in einer Fruchtfolge die Schärfe von Zwiebeln signifikant ($P<0{,}001$). Dieser Effekt wurde einer verstärkten Mineralisierung von organisch gebundenem N und S zugeschrieben. Signifikante Auswirkungen von Vorfrucht, Bulbengröße oder Art der Pflanzung auf die lösliche Trockensubstanz konnten nicht beobachtet werden. In einem kombinierten N- und S-Düngungsversuch (0 und 130 kg N $ha^{-1}$ bzw. 0, 50 und 100 kg S $ha^{-1}$) wurde eine erhöhte Schärfe und eine Wechselwirkung zwischen N und S ($P=0{,}032$) auf den Pyruvatgehalt festgestellt. Diese Wechselwirkung wurde allerdings nicht zwischen Bulben-S und Bulben-N beobachtet. Ein Vergleich zwischen einer S-Düngung mit 100 kg S $ha^{-1}$ und einer ungedüngten Kontrolle ergab weder qualitative Verschiebungen bei den Hauptaromakomponenten im Zwiebelöl noch eine Veränderung des Verhältnisses der Aromapräkursoren Methyl- und 1-Propenyl-L-Cystein Sulfoxid. Es konnte jedoch eine erhebliche Steigerung an absoluten Gehalten von flüchtigen Aromakomponenten (Wasserdampfdestillat), Aromapräkursoren und industriell gewonnenem Zwiebelöl verzeichnet werden.

## Introduction

For the combined production of onion juice concentrate and onion oil, raw material is usually produced by contract growers in the region around the plant site. For growers and processors it is of major importance to know about factors enhancing the desired quality traits of onions. In respect to the aim of production, i.e. efficient onion juice concentration and maximum aroma recovery, high contents of total soluble solids (TSS) and strong pungency are desired. The latter can be conveniently estimated by the concentration of pyruvic acid (PA) after maceration of onions (SCHWIMMER and WESTON 1961). Pyruvic acid, besides being a metabolite of glycolysis, is a by-product of the aroma reaction, which develops as a result of an alliinase mediated cleavage of the aroma precursors *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs; LANCASTER and BOLAND 1990) after cell disruption. As early as the 1940 s investigations about factors influencing the pungency of onions have been conducted (PLATENIUS and KNOTT 1941). Since then numerous reports about factors affecting onion flavour have been published, however, most of them were based on greenhouse experiments, employing artificial substrates or hydroponic cultures and/or cultivars adapted to short day conditions (FREEMAN and MOSSADEGHI 1970; RANDLE 1992; HAMILTON et al. 1997). Factors known to bear an effect on onion flavour are genotype, N and S availability, water-supply, soil type, and growing temperature (RANDLE 1997, 2000; HAMILTON et al. 1998; RANDLE and LANCASTER 2002). The present study was conducted to demonstrate the variability of pungency and TSS of long-day onion cultivars available to growers in Central Europe, and to investigate the effects of N and S fertilization on natural soil as encountered under industrial production conditions. In addition, the impact of factors, which are mainly based on empirical experience of growers, such as type of preceding crop, method of planting and bulb sizes were also included.

## Materials and Methods

*Plant material*

Onions were cultivated on loamy silt soil at the Experimental Station for Horticulture of Hohenheim University, Stuttgart, Germany. Common agricultural practice of fertilization (50 kg N $ha^{-1}$ at sowing, N supplemented to a total of 130 kg N $ha^{-1}$ at initial bulbing) and plant disease control were adopted, except otherwise noted. Onions were cultivated in four rows (30 cm between rows) on a bed (25 plants $m^{-1}$ if directly sown and 20 plants $m^{-1}$ if grown from sets). Unless an experimental plot design was employed, onions were randomly sampled from the two inner rows of a bed. Onion bulbs were harvested manually at 80 % tops down and dried for three weeks in an open shed before cutting off the leaves. Bulbs were stored at ambient temperature and analysed four to six weeks after harvest.

Fertilization experiment: Two levels of N fertilization (0 and 130 kg N $ha^{-1}$) were assigned to main plots and three levels of S fertilization (0, 50, 100 kg S $ha^{-1}$) were assigned to subplots. Sulfur was supplied as gypsum ($CaSO_4 \cdot 2H_2O$) and nitrogen as nitrolime ($NH_4NO_3 + CaCO_3$). Soil was analysed prior to fertilization at time of sowing (15 kg $NO_3$-N $ha^{-1}$, 11 kg $SO_4$-S $ha^{-1}$; 0–30 cm) and beginning of bulbing (72 kg $NO_3$-N $ha^{-1}$, 54 kg $SO_4$-S $ha^{-1}$; 0–60 cm).

*Determination of total pyruvic acid (PA)*

The method was based on that described by SCHWIMMER and WESTON (1961) with some modifications: onion bulbs (10 bulbs per replicate) were cut longitudinally into quarters after dry skin, neck and roots had been removed. Bulb quarters were homogenized (Grindomix 200, Retsch, Germany), filtered and left to stand for 30 min at room temperature. One ml juice was diluted with 4 ml trichloroacetic acid solution (TCA 5 % m/v), vortexed and centrifuged (5300 rpm, 5 min). One hundred µl of the supernatant were pipetted into 2 ml 2,4-dinitrophenylhydrazine (DNPH) reagent (62.5 mg DNPH $l^{-1}$ 1 N HCl). After incubating for 10 min at 37 °C, 5 ml 0.6 N NaOH were added and the absorption of the resulting solution was determined at 420 nm. Sodium pyruvate standards (0–20 µmol $ml^{-1}$) were used for calibration. Since background levels of pyruvic acid of intact onion tissues were shown to be negligible (<0.4 µmol $ml^{-1}$) and constant (YOO and PIKE 2001; RESEMANN, unpublished results), estimation of background pyruvic acid was omitted.

*Determination of bulb N and S*

Elemental analysis for bulb S/N-determination was conducted using oven-dried and pulverized samples employing a CE Elantech Flash Elemental Analyzer (NA 2500, Thermo Electron Inc., USA).

*Total soluble solids*

Total soluble solid content of the juice was determined using a digital precision refractometer (RX 5000, Atago, Japan).

*Aroma precursor analysis*

Analyses of the aroma precursors *S*-1-propenyl-L-cysteine sulfoxide and *S*-methyl-L-cysteine sulfoxide were conducted using an HPLC method based on that described by RANDLE et al. (1995) with modifications according to RESEMANN et al. (2004).

*Onion oil analysis*

Semi-quantitative onion oil determination was performed by simultaneous distillation and extraction (SDE) with subsequent gaschromatographic analysis of the volatiles by means of mass spectrometric detection. Two hundred g juice were transferred into a 2 l round-bottom flask and mixed with 800 g phosphate buffer solution (pH 5.6, 0.5 M). Volatiles were extracted into 30 ml dichloromethane in a Likens-Nickerson apparatus for 3 h at boiling temperature. 1 ml internal standard solution (2 mg $ml^{-1}$ diisopropyl disulfide, DiPDS) was added and the extract concentrated to 1 ml using a Snyder-column and a gentle stream of nitrogen. Content of onion oil was estimated by summation of the areas of 16 identified major peaks (alk(en)yl-di- and

trisulfides, 2-methyl-2-pentenal, 3,4-dimethylthiophene) expressed as mg DiPDS $kg^{-1}$ juice.

GC/MS-analysis: Instrumentation consisted of a Varian GC 3400 (1077 split/splitless injector), equipped with a BPX-5 column 30 m, 0.25 mm ID, 0.5 µm (SGE, Darmstadt, Germany), coupled to a Varian Saturn II mass spectrometer. Operating conditions were as follows: Carrier gas He (1 ml $min^{-1}$), 1 µl injection volume, injector 220 °C, transferline 220 °C, run 45 °C/3.33 min, 45–200 °C/58.33 min, 220 °C/3.34 min.

*Statistical analysis*

Statistical analysis was conducted using SigmaSTAT® software V. 2.03 (SPSS Science). For the comparison of means Students t-test and ANOVA procedures, for multiple comparison procedures Fishers LSD ($\alpha$=0.05) test were used.

## Results and Discussion

*Selection of cultivars*

The potential of onion flavour production is mainly determined by genetic factors (LANCASTER and BOLAND 1990). Since both parameters, TSS and pyruvic acid (PA), are of major economic importance, the task finding suitable cultivars does not only concern the range between cultivars but also their absolute contents. To evaluate various genotypes by ranking, 16 cultivars have been grown from seeds and their pungency and TSS were compared (Fig. 1). PA values ranged from 3.6 µmol $ml^{-1}$ (cv. 'Ailsa Craig') to 9.3 µmol $ml^{-1}$ (cv. 'Matador'). Pungency of the latter, a seed shallot, was generally followed by Stuttgarter (cvs. 'Stuttgarter Riesen', 'Jetset', 'Centurion', 'Sturon') and Rijnsburger types (cvs. 'Hystar', 'Saldo'). Both types are characterized by similar high pungency as indicated by pairwise comparison. A weak correlation between TSS and pungency can be observed (r=0.64, P=0.008; Fig. 1). Cv. 'Stardust', a dehydrator-type, and cv. 'Golden Gourmet', a shallot, revealed the highest contents of total soluble solids (16.4 % and 17.0 %, respectively) but moderate pungency (Fig.1). Ignoring the latter two cultivars the correlation coefficient between TSS and pungency will rise to r=0.86 (P<0.001). However, several exceptions to the often assumed phenotypical correlation between TSS and pungency have been reported previously (RANDLE 1992). RANDLE and LANCASTER (2002) stated, that the relationship between pungency and TSS is *per se* a positively biased association, since substances causing pungency contribute to soluble solids. Moreover, carbohydrates and flavour precursors are produced in different biosynthetic pathways, which are likely to perform independently (RANDLE and LANCASTER 2002). GALMARINI et al. (2001), however, found significant positive phenotypic and genetic correlations between TSS and pungency and proposed a linkage or pleiotropy of genes controlling these traits. To screen for suitable cultivars, additional technological and economic factors have to be considered. Although the seed shallot 'Matador', exhibited superior pungency and TSS values, its irregular bulb shape, making it

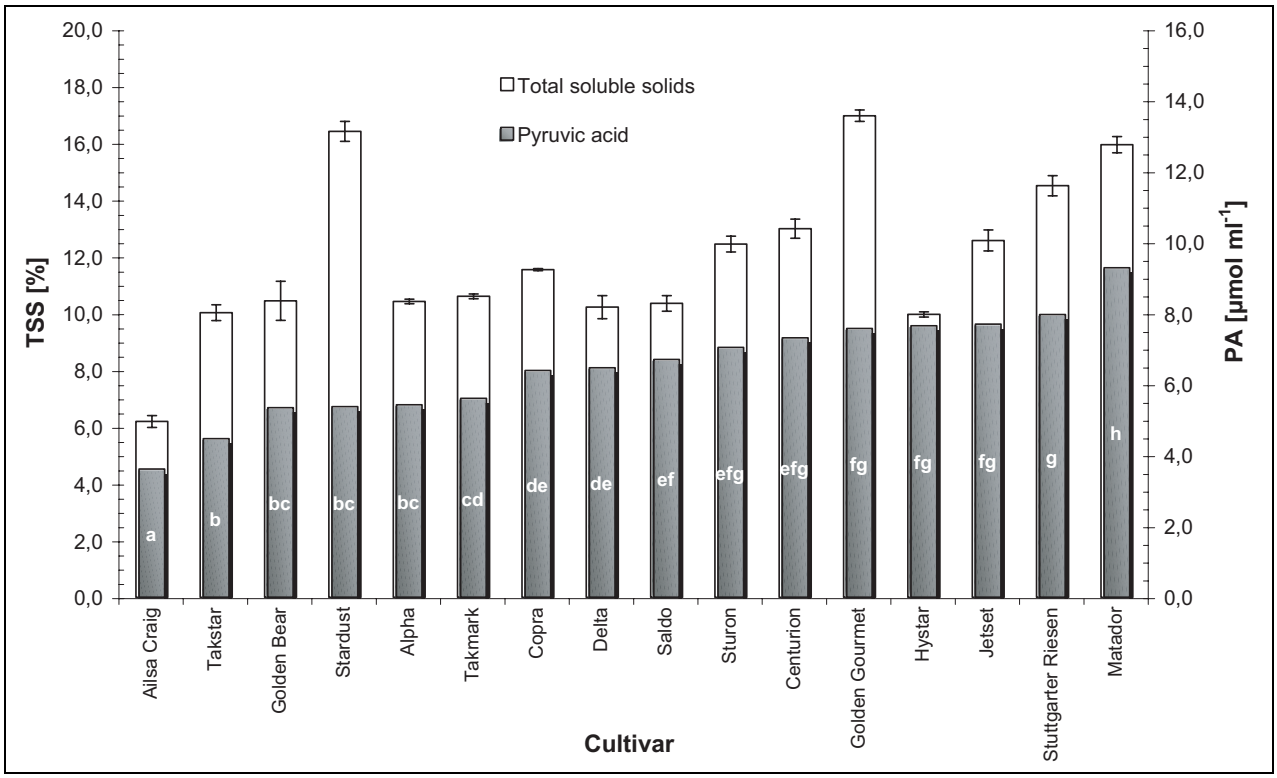



Fig. 1. Pyruvic acid (PA) and total soluble solids (TSS) of 16 cultivars of *Allium cepa* L.. Letters denote classes from pairwise comparisons (Student-Newman-Keuls, P<0.05).

Table 1. Pyruvic acid (PA) and total soluble solids (TSS) of two cultivars (cvs. 'Stuttgarter Riesen', 'Sturon') grown from sets and from direct sowing.

| Cultivar | 'Stuttgarter Riesen' | | 'Sturon' | |
|---|---|---|---|---|
| | Sets | Direct sowing | Sets | Direct sowing |
| **1999** | | | | |
| Harvest | July 19 | August 6 | August 2 | August 12 |
| Mean bulb weight [g] | 93±27 | 63±17 | 135±53 | 68±18 |
| TSS [%] | 13.4±0.01 | 14.5±0.02 | 12.4±0.01 | 13.2±0.14 |
| PA [µmol ml$^{-1}$] | 6.96±0.47 | 7.97±0.52 | 5.32±0.34 | 7.04±0.38 |
| | * (P=0.028) | | *** (P<0.001) | |
| **2000** | | | | |
| Harvest | July 5 | August 11 | July 18 | August 23 |
| Mean bulb weight [g] | 61±13 | 53±9 | 76±15 | 67±11 |
| TSS [%] | 16.1±0.71 | 14.06±0.51 | 13.19±0.16 | 10.25±0.39 |
| PA [µmol ml$^{-1}$] | 6.33±1.08 | 7.81±0.46 | 5.72±0.51 | 7.79±0.52 |
| | * (P=0.045) | | *** (P<0.001) | |

Means ± $s_d$ (n=4)

prone to high soil load, and its high seed costs reduce the suitability of this cultivar for processing. On the other hand, cultivars of the Stuttgarter type combine a high pungency and TSS potential and offer advantages in technological handling for the production of onion oil and onion juice concentrate.

*Quality of onion bulbs grown by different planting methods (sets or direct sowing)*

The choice of direct sowing or growing from sets is mainly determined by the available day length and the demand for early or late harvest. However, in relation to the effect of the method of planting on flavour intensity, contradicting anecdotal evidence from growers exists. Therefore, two cultivars (cvs. 'Stuttgarter Riesen' and 'Sturon') were examined over two years, grown from sets and by direct sowing, respectively (Table 1). Whereas TSS showed inconsistent behaviour between 1999 and 2000, PA was significantly higher for bulbs of directly sown onions. Although for each planting method harvest was done at 80 % tops down, bulb fresh weights differed significantly between bulbs from sets and direct sowing. In these experiments, bulb size was negatively correlated to pungency. As expected, bulbs grown from sets of the respective cultivar reached maturity up to 2 weeks earlier. The higher pungency of directly sown onions may be ascribed, therefore, to their longer growth period.

*Year-to-year variability of PA and TSS (1999, 2000, 2001)*

To investigate year-to-year variation in pungency and TSS, six cultivars were compared over three years (Table 2). Generally, the ranking of pungency among these cultivars remained fairly constant throughout the study period. However, a shift towards higher pungency from 2000 to 2001 was noticable. As pungency has been shown to be affected by environmental factors (RANDLE and LANCASTER 2002), yearly fluctuations in pungency are not unexpected. PLATENIUS and KNOTT (1941) and RANDLE et al. (1993) suggested that increasing temperatures increase sulfur utilization and pungency of onion bulbs. Moreover, water supply (FREEMAN and MOSSADEGHI 1973) and solar radiation (RANDLE and LANCASTER 2002) may influence pungency of onion bulbs. On the other hand, TSS was highest in 1999 compared to succeeding years. Year-to-year differences up to almost 2 % TSS within one cultivar revealed a surprisingly high variability, considering the assumption that genetic rather than environmental factors should influence TSS (SIMON 1995; HAMILTON et al. 1998; MCCALLUM et al. 2001).

*Bulb size*

The relationship between pungency and bulb size is a matter of controversial debate among growers. It is evident, that bulb size is determined by many factors, e.g. available space, water-supply, growing temperature, nutrition etc. The possibility to select for more pungent processing material by grading onions for size would be an issue of practical relevance. Bulbs of cv. 'Sturon' randomized and calibrated into three fractions (40 bulbs each) did not show significant differences of PA contents (ANOVA, P=0.068) among onion size fractions and no correlation (r=0.31) between PA and bulb size could be detected (Table 3). This is in contrast to SHEHATA et al. (1982) who found increasing PA contents with decreasing bulb size. As disclosed by the effect of the preceding crop (see below) bigger bulbs had a higher level of pungency than smaller ones. Presumably, if size differences were determined by soil fertility, as may be the case in crop rotation experiments, size would be positively correlated with pungency, considering that high N and high S supply enhance pungency (see below RANDLE 1992; RANDLE 2000; COOLONG and RANDLE 2003). On the other hand, if size variation was a feature of natural variation within a bulb population of a cultivar, pungency would not be correlated to size. More detailed investigations are needed to gain deeper insight into the relationship between bulb size and pungency.

Table 2. Year-to-year variability of six onion cultivars in pyruvic acid (PA) and total soluble solids (TSS).

| Cultivar | 'Copra' | 'Matador' | 'Saldo' | 'Stuttgarter Riesen' | 'Sturon' | 'Golden Bear' |
|---|---|---|---|---|---|---|
| | Pyruvic acid [µmol ml$^{-1}$] | | | | | |
| 1999 | 6.39±0.45 | 9.29±0.49 | 6.71±0.48 | 7.97±0.53 | 7.04±0.38 | 5.35±1.05 |
| 2000 | 6.39±0.17 | 9.18±018 | 7.17±0.21 | 7.26±0.34 | 6.69±0.27 | 6.22±0.17 |
| 2001 | 7.12±0.19 | 10.57±0.54 | 8.24±0.31 | 8.81±0.49 | 8.46±0.48 | 7.13±0.54 |
| $LSD_{0.05}$ | 0.480 | 0.687 | 0.566 | 0.736 | 0.614 | 1.104 |
| | Total soluble solids [%] | | | | | |
| 1999 | 11.54±0.03 | 15.95±0.27 | 10.35±0.28 | 14.50±0.35 | 12.44±0.28 | 10.43±0.67 |
| 2000 | 9.54±0.33 | 15.18±0.35 | 9.42±0.38 | 13.81±0.22 | 10.25±0.40 | 9.07±0.22 |
| 2001 | 9.64±0.26 | 14.96±0.41 | 9.85±0.24 | 13.00±0.22 | 10.62±0.25 | 8.55±0.14 |
| $LSD_{0.05}$ | 0.390 | 0.552 | 0.484 | 0.434 | 0.502 | 0.663 |

Means ± $s_d$ (n=4)

Table 3. Effect of bulb size on pyruvic acid (PA) (cv. 'Sturon', direct sowing).

| Grade | Pyruvic acid [µmol ml$^{-1}$] | Mean weight [g] | Calibre [mm] |
|---|---|---|---|
| Small | 7.64±0.21 | 102±11.8 | 50–70 |
| Medium | 7.29±0.31 | 166±23.7 | 70–80 |
| Large | 7.72±0.18 | 271±37.9 | 80–100 |
| Difference (ANOVA) | n.s. (P=0.068) | *** (P<0.001) | |
| Correlation r | 0.31 | | |

Means ± $s_d$ (n=4)

Table 4. Effect of S-fertilization (0,50,100 kg S ha$^{-1}$) on PA, bulb S, bulb N and TSS of onion bulbs (cv. 'Stuttgarter Riesen') at two N-fertilization levels (0,130 kg N ha$^{-1}$).

| N/S-levels | | PA | Bulb-S | Bulb-N | TSS |
|---|---|---|---|---|---|
| N[kg ha$^{-1}$] | S [kg ha$^{-1}$] | [µmol ml$^{-1}$] | [% dw] | [% dw] | [%] |
| 0 | 0 | 7.17±0.16 | 0.42±0.04 | 1.28±0.12 | 14.17±0.40 |
| | 50 | 7.73±0.43 | 0.46±0.01 | 1.40±0.05 | 13.77±0.21 |
| | 100 | 7.53±0.20 | 0.44±0.00 | 1.30±0.03 | 13.97±0.15 |
| 130 | 0 | 7.41±0.14 | 0.49±0.00 | 1.89±0.10 | 13.97±0.46 |
| | 50 | 8.20±0.21 | 0.56±0.02 | 1.93±0.10 | 13.47±0.55 |
| | 100 | 8.57±0.17 | 0.58±0.04 | 1.98±0.09 | 13.73±0.23 |
| | S-levels | *** | *** | n.s. | n.s. |
| | N-levels | *** | *** | *** | n.s. |
| | S x N | ** | n.s. | n.s. | n.s. |

Means ± $s_d$ (n=4; bulb-S and bulb-N n=3)
*,**,*** significant at 0.05, 0.01 and 0.001 levels; n.s. no significance at P=0.05 (ANOVA)

*Sulfur and nitrogen fertilization*

Beside genetic factors, onion pungency is mainly influenced by the sulfate availability in the soil (FREEMAN and MOSSADEGHI 1970; RANDLE 1992). Moreover, GAMIELY et al. (1991), BUFLER et al. (1999) and RANDLE (2000) presented data suggesting a role of nitrogen fertilization in onion flavour development. In a field trial on sulfur deficient soils SACHDEV et al. (1991) found significant interactions of N and S for several parameters like yield of bulbs, leaves and total dry matter. However, effects on pungency were not investigated. In the present study, to evaluate the effect of S and N fertilization, treatments were laid out in a split-plot design with four replications. Both, nitrogen and sulfur fertilization increased pungency (Table 4). The interaction between S and N was significant for PA at P=0.032. Changes in pungency at 50 and 100 kg S ha$^{-1}$ were not significantly different indicating saturation with S applied at approximately 50 kg S ha$^{-1}$. PA showed a non-significant tendency to rise with increasing N application even at low S levels (0 kg S ha$^{-1}$). The data in table 4 indicate, that even at S levels of 29 kg S ha$^{-1}$ (data not shown) in the upper soil (0–30 cm) supplementa-

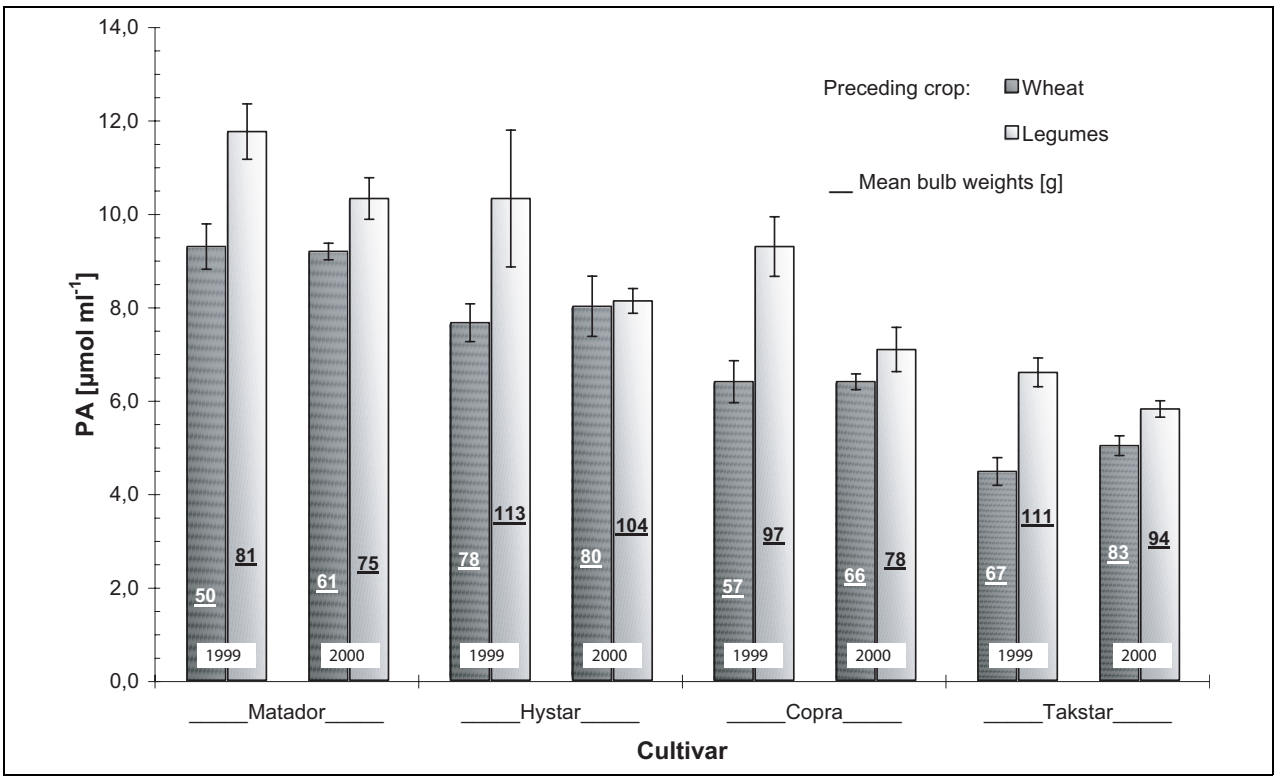



Fig. 2. Effect of legumes (clover mix) and cereals (winter wheat) as preceding crop on pyruvic acid content of onions. Underlined numbers represent mean bulb weights [g].

ry S fertilization still enhances flavour intensity on a loamy silt soil. Availability of N seems to influence S uptake, thus affecting S allocation into the flavour pathway as indicated by a high correlation of bulb S with PA (Table 4). Similar conclusions have been drawn by RANDLE (2000) employing hydroponic solutions for growing onions. In addition, RANDLE (2000) and COOLONG and RANDLE (2003) reported a direct effect of N fertilization on the synthesis of onion flavour precursors in hydroponically grown onions. Since S metabolism seems to be tied to nitrate metabolism in plants (ANDERSON 1990), it may be expected that uptake of S and synthesis of onion flavour precursors is stimulated by increasing N fertilization. Our observations are also corroborated by the results in 1999 (data not shown). The content of bulb S paralleled the trend of PA with significant differences between N and S levels ($P<0.001$ and $P=0.002$, respectively). However, this interaction was not significant ($P=0.065$). Bulb S and PA displayed a strong correlation ($r=0.92$, $P<0.01$). In agreement with SACHDEV et al. (1991) there was no significant interaction ($P=0.39$) between bulb-N and bulb-S. TSS was not significantly affected by different fertilization levels.

*Effect of preceding crops*

To the best of our knowledge, no information exists about the influence of preceding crops within a crop rotation on onion flavour intensity. Within an established rotation (cereals-cereals-vegetables-cereals-legumes-vegetables), two preceding crops, *viz* legumes (clover mix) and cereals (winter wheat), were compared in respect to their effect on PA and TSS in onions. In 1999, onions succeeding cereals were fertilized according to common farming practice (50 kg N $ha^{-1}$ at sowing, supplemented to a total of 130 kg N $ha^{-1}$ at beginning of bulbing; nitrolime as source of N), whereas onions succeeding legumes received only starter fertilizer (50 kg N $ha^{-1}$) at sowing. A comparison of the $N_{min}$ and $S_{min}$ contents of both onion plots for 1999 is given in table 5. In investigations on the effect of a preceding crop conducted in 1999 and 2000, onions succeeding legumes exhibited significantly higher PA values than onions following wheat (1999 and 2000: $P<0.001$; Fig. 2). Moreover, onions following legumes showed significantly increased bulb fresh weights ($P<0.001$) ranging from 13 % to 146 %. In this experiment, a positive correlation between bulb size and pungency was noticed. In 1999 a significantly enhanced pungency was valid for all 16 cultivars under investigation (data not shown). The observed increase in pungency may be, at least in part, associated with an enhanced mineralisation of organic N and S reservoirs in the case of legumes as preceding crop, resulting in improved S and N fertility. The more dynamic mineralisation in the soil by legumes as a preceding crop is obvious, when comparing N and S levels (0–30 cm) at time of sowing and N and S levels (0–60 cm) at time of initial bulbing. A nine fold increase in soil N was noticed with legumes as a preceding crop, whereas wheat as a preceding crop caused only a fourfold increase in soil N at the time of sampling. A difference in mineralisation between both fields is also suggested for S, however, this effect was less pronounced (Table 5). According to the results of the fertilization experiment (Table 4), an N x S interaction may be assumed, leading to an enhanced sulfur utilization and

Table 5. Soil analysis at different developmental stages in 1999.

| Stage [week] | Layer [cm] | Soil analysis 1999 | | | |
|---|---|---|---|---|---|
| | | $NO_3$–N [kg ha$^{-1}$] | | $SO_4^{2-}$S [kg ha$^{-1}$] | |
| | | L | C | L | C |
| Sowing (12) | 0–30 | 18 | 11 | 12 | 10 |
| Bulbing (25) | 0–30 | 95 | 32 | 12 | 6 |
| | 30–60 | 72 | 21 | 18 | 11 |
| Harvest (35) | 0–30 | 55 | 32 | 12 | 7 |
| | 30–60 | 67 | 43 | 15 | 11 |

L = field with preceding crop: Legumes
C = field with preceding crop: Cereals
$NH_4^+$-N<2 kg ha$^{-1}$

Table 6. Flavour related parameters at two sulfur fertilization levels (0 kg S ha$^{-1}$ *vs.* 100 kg S ha$^{-1}$).

| Parameter | S fertilization level | | significance |
|---|---|---|---|
| | 0 kg S ha$^{-1}$ | 100 kg S ha$^{-1}$ | |
| Pyruvic acid [µmol ml$^{-1}$] | 6.40±0.35 | 7.30±0.29 | *** |
| ACSO [µmol ml$^{-1}$] | 6.19±0.20 | 7.63±0.16 | *** |
| Distillate [mg DiPDS kg$^{-1}$] | 47.7±4.5 | 62.2±5.5 | *** |
| TSS [%] | 11.3±0.12 | 10.9±0.25 | n.s. (P=0.053) |

Means ± $s_d$ (n=4)
*** significant at 0.001 level

consequently to more pungency at higher N fertility. Thus, this effect of the preceding crop may be explained on the basis of the mineralisation rate influencing N and S nutrition of the onion plant. This observation was made in both years, though levels and dynamics of mineralisation were lower in 2000 (data not shown). TSS showed no significant response to the type of preceding crop neither in 1999 (P=0.469) nor in 2000 (P=0.874).

*Qualitative and quantitative effects of sulfur fertilization on onion flavour and precursors*

In Germany, atmospheric immissions of S have been reduced more than fivefold since the 1970s (BLANKENBURG and MÜLLER 2002). Thus, for particular crops S became a nutrient to be supplied by fertilization. To evaluate qualitative and quantitative effects of sulfur applied at high rates on flavour and precursor compounds, onions (cv. 'Stuttgarter Riesen') were fertilized with 100 kg S ha$^{-1}$ (as $MgSO_4$) and compared to a control in a completely randomised block design with four replicates. Different parameters associated with biosynthesis and flavour development of onions are listed in Table 6. In agreement with previous S fertilization experiments, a significant increase in PA was found, which is reflected by an increase of ACSO contents. However, there was no significant change in the ratio of the ACSOs *S*-methyl-L-cysteine sulfoxide (MCSO) and *S*-1-propenyl-L-cysteine sulfoxide (1-PeCSO), between S-fertilized onions and control (RESEMANN and CARLE 2003). A shift in ACSO ratio towards 1-PeCSO at higher S availability, as reported by RANDLE (1997) employing hydroponic solutions, could not be observed.

Comparing steam distillates from S-fertilized bulbs and control bulbs, the amount of volatiles of the S-fertilized bulbs was significantly higher (Table 6). However, when considering 16 identified major compounds of the distillates with respect to their relative peak areas, no significant difference between S-fertilized and control bulbs was found (RESEMANN and CARLE 2003). From these results it is concluded, that an increase in flavour precursors and PA following S fertilization is not accompanied by a qualitative change in the composition of major compounds of the distilled product onion oil. Total soluble solids of cv. 'Sturon' were not significantly affected by high rates of applied sulfur (P=0.053). However, the extent TSS responds to increased S levels is supposed to be cultivar dependent (RANDLE et al. 1995; ABBEY et al. 2002). A variability in TSS was observed over the years, but appears not to be directly related to fertilization measures.

*Onion oil yield improvement in industrial scale production*

A trial on industrial scale was conducted to estimate the effect of sulfur fertilization (50 and 100 kg S ha$^{-1}$) on onion oil yield. Onion oil yields from batches (ca. 100 tons each) of S-fertilized onions were compared to a control without S fertilization. In contrast to the control and considering the data of previous years implementing no S fertilization, an over 90 % increase in the average onion oil yield was achieved by S fertilization. Again, there was no difference in yield of onion oil between fertilization with 50 kg S ha$^{-1}$ and 100 kg S ha$^{-1}$ (data not shown). As for PA contents (Table 4), a saturation of the effect of S seems to be reached at 50 kg applied S

per ha. This huge effect of S fertilization on onion oil yield sheds light on the neglected S supply of soils over decades (BLANKENBURG and MÜLLER 2002). Reduced sulfur fertility, as a result of decreasing atmospheric S input, soil leaching, and limited mineralisation of organic S and N, although causing no deficiency symptoms, already diminished industrial onion oil yields to an hitherto underestimated extent. Therefore, in conclusion from our results, this reduction in onion oil yield could be counteracted by selection of adequate cultivars, direct sowing and appropriate measures for S fertilization.

## Acknowledgements

The authors would like to thank Dr. W. Fexer and Dr. A. Walter, Symrise GmbH & Co KG for financial support, and E. Müssig for technical assistance.

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Received August 14, 2003 / Accepted January 20, 2004

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