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Development of an iodine biofortification technique for fruit crops

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Universität Osnabrück
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Abbreviations and acronyms

% RH	% relative humidity
AMI	Agricultural Market Information Company
BBCH	Federal Biological Research Center, Federal Office of Plant Varieties and the Chemical Industry
BfR	Federal Institute for Risk Assessment
$CaCl_2$	calcium chloride
CH	canopy height
DGE	German Association for Nutrition
DM	dry matter
DNA	deoxyribonucleic acid
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
FM	fresh matter
GF-AAS	graphite furnace atomic absorption spectrometry
I	iodine
ICP-MS	inductively coupled plasma mass spectrometry
IGN	Iodine Global Network
KI	potassium iodide
KIO_3	potassium iodate
KNO_3	potassium nitrate
Na_2SeO_3	sodium selenite
Na_2SeO_4	sodium selenate
$NaIO_3$	sodium iodate
N_{min}	mineral soil nitrogen content
POD	point of deliquescence
POE	point of efflorescence
Se	selenium
TA	titratable acidity
TMAH	tetramethylammonium hydroxide
TSS(C)	total soluble solids (content)
u	atomic mass
UL	tolerable upper intake level
UNICEF	United Nations Children's Fund
VDLUFA	Association of German Agricultural Analytic and Research Institutes
WHO	World Health Organization

Chapter 1
General Introduction

1.1 Background and objectives

Iodine is an essential nutrient for humans which is often inadequately supplied through the diet. Currently, approximately 1.88 billion people are affected by iodine deficiency worldwide (*Andersson* et al., 2012). Despite established prophylactic measures, such as iodization of table salt, the iodine status in the German population has continued to deteriorate in recent years (*Gärtner*, 2016; *Hey* and *Thamm*, 2019). Even a mild deficiency can lead to health impairments. Among other things, an adequate iodine supply is particularly important for the cognitive development of children. Pregnant and lactating women therefore have an increased iodine requirement (*Zimmermann*, 2016; *Velasco* et al., 2018). In view of the fact that almost half of young women in Germany have an iodine deficiency this must be considered as particularly critical (*Johner* et al., 2016). Further options to improve alimentary iodine intake should therefore be examined.

Agronomic biofortification is one approach to increase the iodine content in plant foods (*White* and *Broadley*, 2005). By applying fertilizer in a purposeful way plants can accumulate this trace element, which is often only available to a small extent in the soil, in edible plant parts. However, successful use of this method depends on several factors, such as application technique, timing of treatment and the chemical form of the fertilized iodine. In recent years, iodine biofortification has been studied for vegetables and cereals (*Medrano-Macías* et al., 2016; *Cakmak* et al., 2017; *Gonzali* et al., 2017). Fruits also make an important contribution to a balanced diet. Due to a high production volume and global cultivation, apples, for example, are particularly well suited as a potential vehicle for this approach. Other domestic fruit species, such as the pear or the strawberry, could also be used in Germany.

The general objective of this thesis was to investigate the possibilities of agronomic iodine biofortification of fruit crops. For this purpose, it was necessary to evaluate the extent to which iodine is taken up via the roots, leaves and fruits and further translocated in the plant. Moreover, the specific aims are to identify factors that are decisive for these processes and, based on this, to develop a methodological toolkit suitable for fruit growing.

Structure of the thesis

This thesis is structured as a cumulative work. Individual parts have been published in peer-reviewed journals and reproduced as individual chapters in this dissertation. At the beginning of the chapters, information such as bibliographic data and the individual author contributions are given additionally.

- **Chapter 1** contains a general introduction to the research topic. The problems of insufficient iodine supply in the population are described and the background factors that can contribute to or exacerbate this problem are explained. The advantages and disadvantages of appropriate measures to improve alimentary iodine intake are explained as well as the specific possibilities in the field of biofortification. Furthermore, the research questions, study objectives and corresponding hypotheses are formulated at the end of this chapter.

- In **Chapter 2**, three peer-reviewed publications are included in subchapters.
    - **Chapter 2.1** deals with approaches of iodine biofortification on the strawberry, which was selected as a model plant for soft fruit. In several field trials, the influence of exogenously applied iodine on the iodine content, other quality-relevant fruit parameters and the development of the plants were tested. Furthermore, it was important to evaluate which changes the iodine content in the soil is subject to after soil fertilization and how sustainable such a fertilization measure is.
    - To investigate the uptake and translocation of iodine in pome fruit in a more detailed way, a model experiment was performed in a plastic tunnel with apple trees cultivated in containers (**Chapter 2.2**). After a soil or foliar application, the iodine content in the leaves and fruits was measured. In addition, further data was collected on, among other things, the appearance of the leaves, the iodine content in the growing medium and the distribution of iodine in the fruit. In one experimental variant, a combined application with selenium was carried out. Selenium is also an essential trace element for humans and interacts with iodine.
    - **Chapter 2.3** contains the results of trials with apple and pear trees in an orchard under practical conditions from two trial years. In these trials, the trees were treated with iodine via foliar applications, which proved to be particularly efficient. Furthermore, different combinations with other foliar fertilization measures were tested.
- **Chapter 3** provides a general discussion of the results of all experimental investigations carried out as part of this thesis. Issues such as the suitability of the tested application methods and fruit species or the influence on parameters important for fruit quality, such as the sugar content of the fruits, are discussed here from a superordinate perspective. Furthermore, methodical development to rationalize the iodine extraction in plant matrices is presented and discussed.

1.2 Iodine and its importance in human health

Iodine is a chemical element of the halogens with the element symbol I. The atomic mass is 126.90447 u and the naturally occurring isotope is ^{127}I. It can assume the oxidation states -1, +1, +3, +5 and +7. Iodine was accidentally discovered in 1811 by French chemist Bernard Courtois during the ashing of brown algae. This process produced violet vapors, which gave the element its name after the ancient Greek word for violet (*ioeides*) (*Swain*, 2005).

Iodine is a relatively rare halogen. However, it is ubiquitously distributed in chemical compounds. As sodium iodate ($NaIO_3$), it occurs in Chilean saltpeter at levels of up to 1%. Production volumes of crude iodine were approximately 28,000 t worldwide in 2019, of which 18,000 t were from Chile (*U.S. Geological Survey*, 2020).

Due to its properties, iodine is used in many fields of application: in medicine, for example, in the production of medicines, as an X-ray contrast medium or as a disinfectant. In electrical engineering, it is used in lamps or monitors. In food technology, an iodine-

potassium iodide solution is used to detect starch. To prevent the uptake of radioactive iodine isotopes after a nuclear release, potassium iodide tablets are administered. The aim is to block the uptake of radioactive isotopes in the thyroid gland as a result of the high content of natural ^{127}I (*Hou* and *Ding*, 2009).

For humans and for mammals in general, iodine is an essential trace element. The body needs iodine primarily for normal thyroid function, as it is an integral part of the thyroid hormones triiodothyronine (T3) and thyroxine (T4). These in turn are important for controlling metabolic processes in the body. If these hormones are not present in sufficient quantities due to an underactive thyroid gland, hypothyroidism is present. The thyroid gland attempts to compensate for this deficiency by increasing in size (sturma or goiter) in order to absorb the low iodine present more efficiently. This results in thyroid nodules, which often require medical treatment. Diseases of the thyroid gland result in annual treatment costs of around € 2 billion (*Statistisches Bundesamt*, 2019a).

The European Food Safety Authority (EFSA) recommends a daily intake of 150 µg of iodine for adults. Pregnant and breastfeeding women have a higher requirement and should consume 200 µg according to EFSA. The German Nutrition Society (DGE) gives even somewhat higher values of 230 and 260 µg. For children, intake levels of 70–130 µg are recommended, depending on age (*EFSA*, 2014a; *DGE*, 2015).

As a rule, it is not possible to cover the body's iodine requirements sufficiently through food because most foods contain only low levels of iodine. Milk and dairy products such as cheese and yogurt, for example, are considered to be rich in iodine. In particular, saltwater fish, seafood and, as already mentioned, certain marine algae, contain high levels of iodine (*Fuge* and *Johnson*, 2015), since it occurs dissolved in the oceans in amounts of 50–84 µg L^{-1} (*Schnepfe*, 1972) and marine organisms therefore easily accumulate it. In Germany, sea fish is generally consumed only to a small extent, so it contributes, on average, only about 9% to the iodine supply of the population (*Großklaus*, 2007).

In recent decades, the general status of iodine supply has improved significantly worldwide due to prophylactic measures. While in 1993, 110 countries were still identified as iodine-deficient areas by the World Health Organization (WHO), there are currently only 25 countries (*IGN*, 2019). However, the criteria used by the WHO must be considered when evaluating these data. The classification of iodine supply is based on the determination of the iodine concentration in urine. An iodine concentration in 24-h urine samples between 100–199 µg L^{-1} is considered optimal. The WHO classifies countries whose population has a median urinary iodine concentration of 100 µg L^{-1} as already adequately supplied with iodine. However, this implies that 50% of the people in these countries still have a suboptimal iodine supply. Only if more than 20% of the population is clearly iodine-deficient (< 50 µg I L^{-1} urine) is a country considered to be inadequately supplied with iodine in the aforementioned case (*WHO*, 2007). In view of the symptoms that can already be triggered by a mild iodine deficiency, this classification must be critically questioned. It is estimated that 1.88 billion people worldwide are affected by insufficient iodine intake (*Andersson* et al., 2012). In Germany, the iodine supply of the population has deteriorated in recent years to such an extent that the country must again

be classified as an iodine-deficient area according to WHO criteria (*Gärtner*, 2016). Large differences in iodine supply can also occur in individual population strata and between the genders. Around 58,2% of children and adolescents (0–17 years) do not reach the recommended daily iodine intake (*Hey* and *Thamm*, 2019). Among young women aged 18–29 years, almost one in two (46.8%) is affected. 38.9% of women aged 30–39 years also have a suboptimal iodine intake (*Johner* et al., 2016). In view of the increased iodine requirements of pregnant and lactating women and the great importance of iodine for the development of new life (*Velasco* et al., 2018), this must be considered to be particularly critical.

An excessive supply of iodine is unlikely under normal dietary habits. For this, foods that contain particularly high amounts of iodine would have to be consumed to a greater extent. This may be the case with certain seaweeds, which are frequently consumed as a salad ingredient or sushi, for example (*Romarís-Hortas* et al., 2011). However, it is also possible that iodine enters the body in increased amounts due to specific medications or x-ray contrast media. Higher iodine intake can lead to iodine-induced hyperthyroidism. This can disrupt thyroid hormone synthesis (*Roti* and *Uberti*, 2001; *Leung* and *Braverman*, 2012). Symptoms include weight loss, tachycardia, and muscle weakness. In combination with existing heart disease, the disease can also lead to death (*Zimmermann*, 2008). People who were initially deficient and subsequently had an increased iodine intake that would correspond to the optimal range may also be affected (*Stanbury* et al.,1998; *Delange* et al., 1999). For this reason, monitoring the iodine supply of a population is very important. In the study by *Johner* et al. (2016) no excessive iodine intake was found in the German population. The 95% percentile of iodine intake was 324 µg per day for men and 405 µg per day for women. The tolerable upper iodine intake (UL) was set by *EFSA* (2006) at 600 µg per day for adults. Thus, the determined upper values of the study are still significantly below the tolerable upper limit.

1.3 Measures to address iodine deficiency

Iodization of table salt is the most widespread and successful prophylactic measure to combat iodine deficiency worldwide. It has been recommended by the WHO since 1952 (*WHO*, 1953). In this process, 15–85 mg of iodine per kilogram is added to table salt in the form of potassium iodide or potassium iodate. As a result of this salt iodization, it is estimated that 71% of the world's population has access to iodized salt (*Andersson* et al., 2012). In Germany, iodized salt has a legally specified iodine content of min. 15 and max. 25 mg per kg. Since 1981 only potassium iodate has been used for this purpose. Iodide is slightly cheaper, but the iodate form is chemically more stable. This has made it possible to extend the shelf life of corresponding products, which in turn means advantages for the marketing of the salt (*Habermann* et al., 1978; *BfR*, 2004; *Andersson* et al., 2007; *Charlton* and *Skeaff*, 2011).

Iodized salt is used in about 80% of households in Germany. The use of iodized salt in industrially processed foods is also voluntary and is currently only approximately

30%. The reasons for the low use of iodized salt in industry are explained by *Bissinger* et al. (2018) as follows:

- There is no strong public discussion in favor of fortifying foods with iodized salt and its benefits.
- Awareness campaigns were relatively long ago.
- The industrial companies would have to justify themselves in the face of possible criticism from iodized salt opponents.
- The companies do not see themselves as responsible for implementing health policy goals through iodized salt use.
- Companies have no additional marketing argument from using iodine.

This highlights the need for renewed educational campaigns on the use of iodized salt in food.

The use of table salt as a vehicle for iodine has some advantages: salt is consumed worldwide, salt consumption is hardly subject to seasonal fluctuations, iodization of salt is inexpensive (0.2–0.3 US cents per kg), easy to implement and, furthermore, iodine does not affect the taste or color of salt (*Zimmermann*, 2007). However, there are also disadvantages. The German Nutrition Society (*Strohm* et al., 2016) and the WHO (*WHO*, 2014) recommend reducing salt intake in the diet. A high salt intake increases the risk of high blood pressure and associated cardiovascular diseases, which are the most common cause of death in Germany at approx. 40%. An undesirable side effect of this could be a simultaneous reduction in iodine intake. The iodine content in salt would accordingly have to be adapted to new dietary habits, since iodized salt has so far contributed significantly to the daily iodine supply by an amount of approx. 42% (*Gärtner*, 2016). Another disadvantage is iodine losses during the storage of iodized salt. Warm, humid conditions and unsuitable storage vessels also increase the loss rates here, which can be as high as 38% per year (*Wang* et al., 1999; *Biber* et al., 2002; *Waszkowiak* and *Szymandera-Buszka*, 2008). In addition, high iodine losses also occurred due to common preparation methods, such as cooking in salted boiling water (*Longvah* et al., 2012).

In addition to the use of iodized salt, there are other ways to improve the iodine supply. Iodine tablets as dietary supplements are another alternative (*Gordon* et al., 2009). However, this method involves the risk of over-supply, which can lead to health risks (*Willers* et al., 2015). Another option is iodization of tap water, which has been successfully tested and used in several countries. The availability of tap water is limited, especially in developing countries, so this method can only be used here to a restricted extent. (*Squatrito* et al., 1986; *Regalbuto* et al., 1998; *Andersson* et al., 2007). Due to the fact that often only a small proportion of tap water is used as drinking water, this method must also be critically questioned. Another alternative is the fortification of food (except salt) and beverages. Here, the relevant nutrients are added during the processing of a foodstuff. Currently, however, there is a need for further research on the efficiency and safety of such methods (*Santos* et al., 2019). WHO therefore considers that there is a need to evaluate alternative ways to increase alimentary iodine intake (*WHO*, 2014).

1.4 Biofortification of food crops with iodine

Biofortification of plant foods with nutrients is another approach to improve the supply of essential minerals and vitamins for humans. This method uses agronomic techniques, conventional plant breeding, or modern biotechnologies to selectively enhance food quality during crop cultivation (*Welch* and *Graham*, 2005; *White* and *Broadley*, 2009). In general, the research field of biofortification can be divided into two core areas: 1. genetic and 2. agronomic biofortification.

In the case of genetic biofortification, plants are either selected by conventional breeding methods or modified by genetic engineering techniques so that they are able to increasingly accumulate or synthesize certain nutrients in the crop. A very popular example of this method is 'Golden Rice'. This is a rice variety that synthesizes increased levels of pro-vitamin A through DNA recombination (*Ye* et al., 2000). Vitamin A deficiency particularly affects children. Worldwide, approximately 190 million children under the age of 5 are affected, predominantly in developing countries (*WHO*, 2009). In addition to rice, other staple food crops such as sweet potato, beans, and maize have been considered for genetic biofortification (*Bouis* et al., 2011). Zinc and iron are other mineral nutrients that should be increased in the crop in this way. Currently, more than 140 biofortified varieties of 10 crops in 26 countries in Africa and Asia are available (*Andersson* et al., 2017). However, genetic biofortification also comes with certain disadvantages and limitations. Firstly, breeding methods are extremely time-consuming, so it can take many years before appropriate plants become available and are actually cultivated due to the necessary educational work (*Bouis* et al., 2011). Furthermore, many people have strong concerns about genetically modified plants because they perceive such methods as unnatural and associate them with certain risks (*Frewer* et al., 2013). In the EU and in Germany, there are also legal hurdles that in some cases prohibit the cultivation of genetically modified organisms by law (*European Commission*, 2001). Corresponding breeding methods are initially relatively cost-intensive. However, if breeding is successful, genetic biofortification can be considered very cost-effective, as high expenditures for other health programs can be saved (*Meenakshi* et al., 2010; *Bouis* et al., 2017). However, breeding programs aimed at increasing mineral content in the plant are only successful if there are corresponding plant-available amounts of the element in the soil. For example, a wheat plant that can accumulate zinc at elevated levels cannot be used efficiently in soils that are deficient in zinc (*Cakmak*, 2008).

For iodine, genetic biofortification is probably not very effective, since this element is often present in very low amounts in the soil (mean 5.1 mg/kg soil, median 3.1 mg/kg soil) and the plant-available contents are even lower (*Johnson*, 2003; *Salminen* et al., 2005). Further research is also needed for successful breeding of appropriate plants based on genetic engineering methods (*Gonzali* et al., 2017). *Landini* et al. (2012) succeeded in inhibiting the release of iodine in the form of gaseous methyl iodide (CH_3I) via the leaves of *Arabidopsis thaliana*. For this purpose, the corresponding gene sequence was identified and deactivated. Furthermore, increased uptake was achieved by overexpressing the human sodium-iodide symporter (NIS). This information is important in the context of a

successful breeding program, so that available iodine in the soil can be taken up via the root and accumulated in the crop plant and is not excreted again in the next step.

Agronomic biofortification of food crops is another method that can be used to increase nutrient levels in the plant (*White* and *Broadley*, 2005). *Welch* and *Graham* (2005) describe it as the most important method for finding a short-term solution to the globally widespread problem of nutrient undersupply ("hidden hunger") in humans. In this process, crops are fertilized with the appropriate minerals. The plants can absorb the substances via the roots or leaves as after normal fertilization measures and accumulate them in the plant tissue. The important thing here is to ensure that the substances are administered in a chemical form which, on the one hand, can be absorbed by the plant and, on the other hand, remains in a plant-available form long enough. Furthermore, it is important that within the plant the nutrients are transferred to the harvesting organ. For example, it is of little use if roots and leaves accumulate the nutrients, but the fruits of a plant are consumed.

With regard to biofortification with iodine, the agronomic route has several advantages. A targeted application of iodine addresses the problem of low plant-available iodine quantities in the soil. This makes it possible to save the laborious process of plant breeding and to directly enrich a large number of crops with the element through an adapted fertilization program during production (*Cakmak*, 2008). If enough plant-available iodine is present in the soil, the next step in combination with genetic biofortification could further increase the efficiency of such a measure (*Storcksdieck genannt Bonsmann* and *Hurrell*, 2009). In recent years, work on iodine biofortification of crops has been published (for reviews see e.g. *Medrano-Macías* et al., 2016; *Gonzali* et al., 2017). Vegetables and cereals are the main focus of research in this regard, as they are important staple foods. Accordingly, plants are able to take up iodine through the roots and through the above-ground parts of the plant, and thus techniques such as soil and foliar fertilization, as well as fertigation or hydroponic systems, can be used.

When fertilizing the soil with iodine, potassium iodide (KI) or potassium iodate (KIO_3) is usually used. Iodide (I^-) is the main form absorbed by the roots. Iodate (IO_3^-) must probably first be reduced to iodide for a potential uptake into the plasma membrane (*Muramatsu* et al., 1983; *Kato* et al., 2013). However, iodate has the advantage that it appears to remain in a plant-available form in mineral soils longer than iodide, which is more susceptible to leaching or to being lost in gaseous form (*Yamada* et al., 1999; *Ashworth*, 2009; *Horel* et al., 2014). In line with this, more iodine was detected in plant tissues after soil fertilization with iodate in experiments with various vegetable species (*Dai* et al., 2006; *Lawson* et al., 2015). Another possibility for soil fertilization is the application of iodine-containing organic matter. Iodine-rich algae species that have been previously dried, ground, and homogenized are particularly suitable for this purpose. The organically bound iodine becomes plant-available during the decomposition of the organic matter and can be taken up by crops (*Weng* et al., 2008a, 2008b, 2013). This slowed release can expand the uptake of crops for iodine biofortification, which is very limited for inorganic iodine fertilizers (*Lawson* et al., 2015). However, the algae could possibly contain elevated levels of undesirable heavy metals, which are also released to the fields when fertilizer is applied (*Weng* et al., 2014).

To quantify the efficiency of soil fertilization with iodine, the transfer factor from soil to edible plant parts can be calculated (*Dai* et al., 2004b). In this context, a high transfer factor means a high uptake into the corresponding harvest organ of the plant. Leafy vegetables such as spinach proved to be particularly efficient in this regard, as iodine taken up via the root is transferred directly to the (transpiring) edible plant parts to a high extent with the mass flow (*Zhu* et al., 2003; *Dai* et al., 2004b; *Hong* et al., 2008; *Voogt* et al., 2010). Accordingly, the xylem transport pathway is the primary transport pathway for iodide in plant tissues (*Herrett* et al., 1962; *Blasco* et al., 2008; *Voogt* et al., 2010). Uptake of low molecular organic iodine compounds has also been demonstrated, but their translocation via the xylem into the shoot was marginal (*Halka* et al., 2019a). In contrast, phloem mobility of iodine appears to be low. In this regard, *Humphrey* et al. (2019) used spinach plants to investigate how translocation of iodine occurred in the plant. Using iodine isotopes (^{129}I), it was demonstrated that less than 2% of iodine taken up by leaves was translocated to younger leaves. Therefore, soil fertilization with iodine is not efficient when low transpiring plant parts, such as seeds or fruits, are the harvested product (*Mackowiak* and *Grossl*, 1999; *Hong* et al., 2008; *Tsukada* et al., 2008; *Caffagni* et al., 2012; *Lawson* et al., 2015; *Cakmak* et al., 2017). However, in a pot experiment with cucumbers and eggplants, *Weng* et al. (2008b) also found relatively high levels in the fruits of up to 15 mg per kg of fresh matter (FM), in addition to high iodine levels in the leaves of over 40 mg (kg FM)$^{-1}$. *Kiferle* et al. (2013) observed a similar effect in tomatoes grown in pots in greenhouses. Nevertheless, the limited availability in the soil restricts the sustainability of soil fertilization with iodate or iodide (*Dai* et al., 2004a; *Hong* et al., 2009; *Weng* et al., 2008b, 2014; *Lawson* et al., 2015). To ensure continuous iodine supply in the soil, large-scale irrigation with iodinated water would be another option (*DeLong* et al., 1997; *Ren* et al., 2008). However, this method requires high amounts of iodine, which is thus released into the environment in a relatively undirected form.

Foliar application provides another method. Studies using the iodine isotopes ^{131}I and ^{129}I showed that plants can take up iodine into plant tissues through the leaves (*Oestling* et al., 1989; *Humphrey* et al., 2019). This probably occurs via diffusion through epidermal cells and/or uptake through openings such as stomata and lenticels, as also occurs with other foliar fertilizers (*Eichert* and *Fernández*, 2012). This method is particularly efficient for leafy vegetables, such as lettuce, because the fertilized iodine can reach the edible parts of the plant and can be absorbed without any detour (*Smoleń* et al., 2014). *Lawson* et al. (2015, 2016) were able to recover about one-third of the applied iodine in the crop after foliar fertilization, whereas after soil fertilization under comparable conditions, the efficiency was only about 1%. Foliar applications were also successfully tested on wheat, rice and corn. Soil fertilization proved to be less efficient here as well (*Cakmak* et al., 2017; *Zou* et al., 2019). The significantly lower application rates of foliar fertilization also make it more attractive from an economic point of view. Furthermore, other foliar fertilizers and crop protection products could be applied together with this measure in one operation, if the miscibility of the substances allows a corresponding combined application (*Lawson* et al., 2016).

When foliar application is performed with the same iodine application rate, iodide is often more efficient than iodate. Presumably, iodide can be more easily absorbed into the

plant tissue than iodate due to its lower molecular weight and valence (*Umaly* and *Poel*, 1971; *Mackowiak* and *Grossl*, 1999). Furthermore, potassium iodide has a lower point of deliquescence (POD) than potassium iodate (KI 68.9% rLf, KIO_3 93.8% rLf, *Greenspan*, 1977; *Apelblat* and *Korin*, 1998). This point describes a relative humidity content above which a salt absorbs moisture from the air, for example on a leaf surface and liquefaction occurs. The lower this value is, the faster a substance can be present in a dissolved form after crystallization and be absorbed into the plant tissue (*Schönherr*, 2001). However, this higher enrichment with iodide is also accompanied by higher fluctuations in iodine contents in the crop in practical trials. For this reason, among others, *Lawson* et al. (2016) and *Cakmak* et al. (2017) recommend the use of iodate in foliar applications.

Hydroponic culture represents another approach for agronomic biofortification (*Sambo* et al., 2019). Here, the crop plants are supplied with nutrient solutions detached from the soil, which excludes unfavorable soil properties and possibly present phytopathogenic organisms. This form of culture is technically more complex, but proved to be very efficient in terms of biofortification with iodine (*Blasco* et al., 2008; *Voogt* et al., 2010; *Landini* et al., 2011; *Caffagni* et al., 2012). In corresponding culture methods, iodide was usually more efficient since, as mentioned above, iodate must first be reduced to iodide before uptake by the root (*Zhu* et al., 2003; *Li* et al., 2017). However, this method is only suitable for certain crops and is unsuitable, for example, for large-scale cultivation of cereals or tree fruits.

In consequence of the fact that the iodine is bound in the plant tissue, it is also more stable when, for example, vegetables are cooked for preparation. *Weng* et al. (2014) conducted corresponding experiments on this issue. In the first part of the experiment, conventional celery was cooked with iodized table salt, and in the second part of the experiment iodine-biofortified celery was cooked with iodine-free table salt. After specific times, samples were taken from the celery and the cooking water and analyzed for iodine content. After about 2 minutes, 50% of the iodine was already lost in gaseous form from the cooking water in the first part of the experiment, whereas the celery had absorbed hardly any iodine here. In the second part of the test, approx. 80% of the initial value was still present in the biofortified celery after a cooking time of 10 minutes.

After successfully increasing the iodine content in the harvested product through agronomic biofortification and possible preparation, the next question is whether the increased iodine content in the plant mass is bioavailable to humans. In a human study by *Tonacchera* et al. (2013), subjects consumed biofortified potatoes, carrots, tomatoes, and lettuce with an iodine content of approximately 45 µg $(100\ g\ FM)^{-1}$ over a 2-week period, after which a significant improvement in human iodine supply was measured. Further experiments with test animals and *in vitro* also showed good bioavailability (*Rakoczy* et al., 2016; *Li* et al., 2018; *Cakmak* et al., 2020). *Rakoczy* et al. (2016) conducted an experiment with rats in which the animals received iodine in the form of potassium iodide on the one hand and a comparable amount of iodine via biofortified lettuce on the other. The rats that received the lettuce excreted about 22% less iodine and at the same time accumulated on average 43% more iodine in the organs or muscles. Corresponding biofortified plant-based foods are also interesting for vegetarians and vegans because they provide an alternative to animal-based foods which they avoid and which are currently the

main source of iodine in the diet. (*Remer* et al., 1999; *Krajčovičová-Kudláčková* et al., 2003). After uptake of iodine into plant tissues, it is present mainly in the cytoplasm and to a lower extent in the cell wall or organelles. High uptake rates of iodine bound in this way are better tolerated than uptake of iodine from iodine salt. In experiments with mice, it was shown that weight loss and goiter formation began with administrations of 40 µg of iodine per day from inorganic sources. If the iodine was organically bound in algae, administrations of up to 200 µg per day did not lead to corresponding excess symptoms. Even long-term feeding of the test animals did not lead to corresponding symptoms (*Weng* et al., 2014).

In addition to staple foods such as grains and vegetables, fruit is important for a healthy and balanced diet. Organizations such as the WHO recommend consuming at least 400 g of fruit or vegetables daily. The DGE recommends 5 servings per day, of which 2 should be fruit (about 250 g). An appropriate diet has been shown to reduce the risk of certain cancers and cardiovascular diseases (*WHO*, 1990; *DGE*, 2012). To date, only a few studies on iodine biofortification of fruit crops have been reported (*Gonzali* et al., 2017).

In an experiment by *Li* et al. (2017), strawberry plants were cultivated in a hydroponic system. Iodide and iodate were added to the nutrient solutions in different concentrations. Subsequently, it was possible to measure high concentrations of iodine in all plant parts. In the strawberry fruits, the contents were in a range between 60 and 400 µg (100 g FM)$^{-1}$. Corresponding values would be more than sufficient to improve the iodine supply for humans. Hydroponic systems are currently of no great relevance in commercial strawberry cultivation, which limits the implementation of the experimental results. *Caffagni* et al. (2012) investigated whether iodine biofortification of the tree fruit species nectarines and plums is possible. Here, soil fertilization with potassium iodide at a maximum of 250 g iodine per ha showed no effect on iodine content in the fruits. The iodine content ranged from 0.0 to 0.7 µg I (100 g FM)$^{-1}$. Foliar fertilization with 312.5 g I ha^{-1} significantly increased the iodine content in nectarines to 13.9 µg. However, this value is still too low to improve the iodine supply for humans. For this, a content between 50 and 100 µg I per 100 g fresh mass should be aimed for (*Lawson* et al., 2015). Therefore, further experiments are necessary here to investigate the possibilities of biofortification of fruit crops under practical conditions. A high cultivation volume and a largely global cultivation should also be taken into account when testing corresponding crops, as thus positive trial results could potentially be established in many countries.

1.5 Influence of iodine on the development of plants

Although iodine is not essential for higher plants (*Broadley* et al., 2012), positive effects can be observed in some experiments at low concentrations. As early as the 1950s, *Borst Pauwels* (1961) found a slight increase of biomass in crops such as fodderbeet, barley and tomatoes after a light application of iodide or iodate. Iodate proved to be superior in most cases. In experiments by *Zhu* et al. (2003), *Dai* et al. (2004b), *Weng* et al. (2008c), and *Mao* et al. (2014), similar results were observed in canola, soybean, celery, Chinese cabbage, and water spinach. In other trials with increasing iodine levels,

however, no effect or a negative effect on biomass and yield was observed (*Medrano-Macías* et al., 2016).

In addition to the influence on biomass, it was possible to observe changes in plant constituents after iodine application. Several studies show that moderate iodine biofortification can have a positive effect on the content of valuable substances such as ascorbic acid and flavonoids in plant products (*Blasco* et al., 2008; *Osuna* et al., 2014; *Smoleń* et al., 2015). These bioactive compounds not only play an important role in healthy human nutrition, but may also promote plant tolerance to abiotic and biotic stress (*Medrano-Macías* et al., 2016, 2018; *Habibi* et al., 2018). However, more research on this process is needed (*Dávila-Rangel* et al., 2019).

In addition to health-related aspects, parameters such as the taste of the fruit are also an important purchase criterion for consumers (*Wortmann* et al., 2018). Here, the sugar content is particularly important, as it influences the degree of sweetness and thus the taste of the fruit (*Aprea* et al., 2017; *Charles* et al., 2017). *Li* et al. (2017) found a slight increase in the sugar contents of strawberries at a low iodine supply level. However, higher iodine levels resulted in the opposite effect. Similar changes were observed by *Leyva* et al. (2011) and *Habibi* et al. (2018). In contrast, a decrease in sugar contents was observed in tomatoes even at low concentrations in an experiment by *Kiferle* et al. (2013).

Iodine application can cause other effects in plants besides increasing the iodine content in the plant mass. In the past, iodide was used as a herbicide because relatively low concentrations were sufficient to damage plant tissue (*Herret* et al., 1962). It is suggested that the damaging effect could be caused by intracellular oxidation to elemental iodine, leading to inhibition of photosynthetic processes (*Mynett* and *Wain*, 1971, 1973). Here, iodide seems to be more harmful to plants than iodate at the same concentration. One reason for this may be due to the fact that iodide inhibits the activity of superoxide dismutase, whereas iodate promotes it. This enzyme plays a key role in the defense against reactive oxygen species and thus in the prevention of cell damage (*Blasco* et al., 2011). Therefore, despite the higher iodine contents in plant mass after biofortification with iodide, it is recommended to use iodate rather than iodide (*Mackowiak* and *Grossl*, 1999; *Blasco* et al., 2008; *Lawson* et al., 2015; *Cakmak* et al., 2017).

1.6 Iodine biofortification combined with other foliar fertilization measures

By combining an iodine biofortification with further compounds, several foliar fertilization measures could be bundled in one application. It must be ensured that the individual components are compatible with each other and that no precipitation occurs in the solution. The extent to which a corresponding nutrient cocktail is subsequently compatible with plants and whether the individual components promote or inhibit the uptake of iodine should be checked in preliminary stages.

Apart from iodine, for example, other essential elements for humans are often not consumed in sufficient quantities in the diet. One of these elements is selenium (Se). Worldwide, about one billion people are affected by selenium deficiency (*Jones* et al., 2017). The average selenium intake in Germany is about one third below the DGE recommended intake of 60 µg per day for women and 70 µg for men (*Kipp* et al., 2015; *Steinbrenner* and *Brigelius-Flohé*, 2015). The EFSA recommends an intake of 70 µg per day for both genders (*EFSA*, 2014b). Selenium, like iodine, is essential for normal thyroid function. It is necessary for the biosynthesis of certain enzymes, the so-called selenoproteins, which in turn are important for other metabolic processes (*Schomburg* and *Köhrle*, 2008). Selenium deficiency symptoms do not represent a direct health problem in Germany, but a suboptimal supply appears to exacerbate the course of other diseases (*Schweizer* et al., 2014). Furthermore, the risk of certain cancers seems to increase with suboptimal selenium supply (*Rayman*, 2012; *Hughes* et al., 2015; *Allen* et al., 2016). In addition, a good selenium supply seems to favor the recovery rate after a coronavirus infection. In the course of the COVID-19 pandemic, *Zhang* et al. (2020) and *Moghaddam* et al. (2020) were able to establish initial correlations in this regard.

Like iodine, selenium is present in relatively low amounts in the soil (global median 0.4 mg/kg soil) and is also relatively unevenly distributed, so that there can be strong regional differences in the contents (*Reimann* et al., 2014). Due to climate change and associated biochemical processes in the soil, plant-available selenium contents are likely to decrease further. This will thus also lead to lower contents in crops and further worsen the supply for humans (*Jones* et al., 2017). Since the basic conditions are somewhat similar compared to iodine biofortification, this method is also promising for selenium (*Poblaciones*, 2017). In Finland, where the supply situation for people was particularly bad, widespread selenium fertilization has been carried out since the 1980s. For this purpose, a certain amount of selenium is mixed into the fertilizers. This process has significantly improved the supply of the population (*Alfthan* et al., 2015). In numerous other trials, successful selenium enrichment of crops was achieved via various application methods (*Mimmo* et al., 2017; *Puccinelli* et al., 2017). In some cases, a combined application with iodine has already been performed in this context (*Zhu* et al., 2004; *Smoleń* et al., 2014, 2016a). Since both elements are important for normal thyroid function (*Schomburg* and *Köhrle*, 2008), a combination is already recommended (*Lyons*, 2018).

With regard to the increase in the sugar content of fruits mentioned in chapter 1.5, it has been shown in trials that selenium can have a positive influence here in some respects. For example, *Mimmo* et al. (2017) and *Zhu* et al. (2017) achieved an increase in strawberries and table grapes. In experiments by *Pezzarossa* et al. (2012), increased sugar content was also measured in pears after selenium application, whereas no significant changes occurred in peaches in the same study. Further research is therefore also required at this point in order to be able to exploit positive effects in a targeted manner.

Apart from selenium, a combined application with potassium nitrate (KNO_3) could be beneficial. On the one hand, *Shen* et al. (2016) demonstrated that increased fructose and sucrose contents appeared in fruits after KNO_3 application in 'Kousui' Japanese pears (*Pyrus pyrifola*). On the other hand, in experiments by *Cakmak* et al. (2017), a combined

application of iodate and potassium nitrate was found to improve iodine uptake in cereals. At this point, further research activities are also necessary for clearer statements.

1.7 Research objectives and hypotheses

The main objective of this work was to further explore the approaches to agronomic iodine biofortification of fruit crops. In order to be able to achieve this goal, various experiments were carried out from 2012 to 2018 in protected cultivation and the open field. In fruit crops the ripe fruit usually only represents the harvest organ that is consumed by humans. However, there are significant differences between herbaceous and arboreal fruit species in terms of plant anatomy. Fruit size and surface texture can also vary widely. Consequently, fruit crops that are of great importance in cultivation and consumption were selected for the present studies. Therefore, strawberry, apple and pear trees were chosen. Strawberry plants (*Fragaria x ananassa*) differ significantly from fruit trees in their growth habit, cultivation methods and fruit characteristics. Apple (*Malus domestica*) and pear (*Pyrus communis*) trees are similar in principle, but in detail even these two pome fruit species have specific differences - in the texture of the fruit surface, for example. In order to be able to work out the significance of these differences for iodine biofortification, both pome fruit species were included. The individual research questions, study objectives and hypotheses are listed below:

- **Identification of an appropriate application method**

  Research question: To what extent is iodine accumulated in the fruit of fruit crops after soil or foliar application and which application factors have a significant influence on this?

  Study objectives: Field trials with selected fruit crops (strawberry, apple, and pear) were carried out to show whether berry and tree fruits can be biofortified with iodine at levels sufficient for human consumption.

  Hypotheses:
  - In general, the iodine content of fruits increases with increasing iodine fertilization.
  - Foliar applications, which directly wet the fruit, are clearly superior to soil fertilization.
  - Iodine taken up via the root is primarily translocated to the more transpiring leaves via the xylem transport pathway.
  - The retranslocability of iodine taken up via the leaf is estimated to be low.
  - Foliar-applied iodide accumulates more in fruits than iodate.

- **Influence of fruit type on iodine biofortification**

  Research question: Do soft fruit and tree fruit species differ in their suitability for iodine biofortification?

  Study objectives: Field trials with selected fruit crops (strawberry, apple, and pear) were to show whether berry and tree fruits can be biofortified with iodine at levels

sufficient for human consumption. At the same time, the investigations should provide information on which fruit species specific properties are important for increasing the iodine content in the edible plant parts.

Hypotheses:

- By means of iodine foliar fertilization, the iodine content of fruits can be increased to a level appropriate for human nutrition of 50–100 µg (100 g FM)$^{-1}$.
- The uneven, thin-skinned structure of strawberries favors the uptake of sprayed iodine into the fruit compared to pome fruit species, which develop thicker, relatively plain and waxy fruit peel.
- The greater the time interval between a foliar iodine fertilization and fruit harvest, the lower the increase in iodine content in the fruit (dilution effect due to fruit growth).

- **Plant compatibility of iodine fertilization**

Research question: What is the influence of iodine fertilization on the development of fruit crops in general and fruit in particular?

Study objectives: In the experiments conducted the objective was to determine whether soil-applied and foliar-applied iodine had a detrimental effect on crop development. For this purpose the method was to visually record and score damage to leaves (e.g. chlorosis, necrosis) and fruits caused by iodine. The inclusion of fruit yield and average fruit weight as additional evaluation parameters was also envisaged.

Hypotheses:

- Iodate is better tolerated by plants than iodide at increasing iodine fertilizer rates.
- Leaves accumulate more exogenously applied iodine than fruits and are therefore more likely to show damage after such treatment.

- **Iodine distribution in the fruit and effect of fruit preparation and storage**

Research questions: How is foliar-applied iodine distributed in fruits? Do household processing methods and fruit storage affect the iodine content? Does any particular damage occur during storage of iodine-biofortified pome fruit?

Study objectives: For this purpose it was necessary to examine the pome fruit with regard to iodine content directly after harvesting and after storage for several months in the unwashed, washed and peeled state. In the case of the stored fruit, the external condition also had to be evaluated visually. For strawberries, which can only be stored for a few days, the aim was only to test the effect of washing.

Hypotheses:

- At harvest time the majority of iodine in biofortified pome fruit is located in the fruit peel.
- During storage the iodine penetrates more strongly into the fruit flesh.
- Washing reduces the iodine content only in freshly harvested fruits.

- **Influence of iodine fertilization on the sugar content of fruits**

  Research question: Do iodine fertilization measures affect sugar accumulation in fruits when the leaf tissue is damaged?

  Study objectives: The aim was to determine the soluble solids content in the harvested fruits. This parameter, which is easy to determine, can be used as an indicator for the sugar content and sweetness degree of fruits.

  Hypothesis:
  - As leaf damage increases following iodine fertilization, the total soluble solids content in the fruit decreases.

- **Combination of an iodine application with other foliar fertilization measures**

  Research question: Does a combined application of iodine with sodium selenate and potassium nitrate affect iodine uptake and sugar accumulation in fruit?

  Study objectives: Experimental variants in which solutions with and without these mixing partners are applied should provide information on whether a co-fertilization of iodine with other foliar fertilizer salts is possible.

  Hypotheses:
  - Combined foliar fertilization of iodine with sodium selenate has no effect on the iodine content of the fruits, but increases their selenium content.
  - A combined application of potassium nitrate promotes iodine uptake by the fruits and increases their soluble solids content.

- **Methodological improvement of iodine extraction from plant matrices**

  Research question: Can the workflows for iodine extraction according to *DIN EN 15111* (2007) be optimized to save resources?

  Study objectives: In order to be able to efficiently process the large sample size for iodine analyses, it was necessary to evaluate whether the extraction procedure using tetramethylammonium hydroxide (TMAH) established for the analysis of foodstuffs could be optimized for routine measurements in terms of low time and cleaning requirements.

  Hypothesis:
  - The use of screw-top laboratory bottles and volumetric flasks can be dispensed with if the centrifuge tubes are used directly for weighing and extraction. This allows workflows to be optimized and the cleaning effort to be minimized.

Chapter 2
Scientific publications within the context of this work

2.1 Iodine biofortification of field-grown strawberries – Approaches and their limitations

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Highlights:

- Strong retention of soil-applied iodate in a loamy sand soil.
- As a result, low transfer of iodine from soil to strawberry fruits.
- Translocation of leaf-absorbed iodine to fruits was marginal.
- Only directly sprayed fruits accumulated substantial amounts of iodine.
- Repeated foliar treatments decreased the content of soluble solids in fruits.

Keywords:
agronomic biofortification, iodine, foliar sprays, soil fertilization, strawberry fruits, iodine dynamics in soil

Author contributions:
Christoph Budke: Conceptualization, Investigation, Methodology, Formal analysis, Validation, Visualization, Writing - original draft. **Stephanie thor Straten**: Validation, Resources. **Karl Hermann Mühling**: Review & editing, Resources. **Gabriele Broll**: Review & editing. **Diemo Daum**: Conceptualization, Review & editing, Supervision, Funding acquisition.

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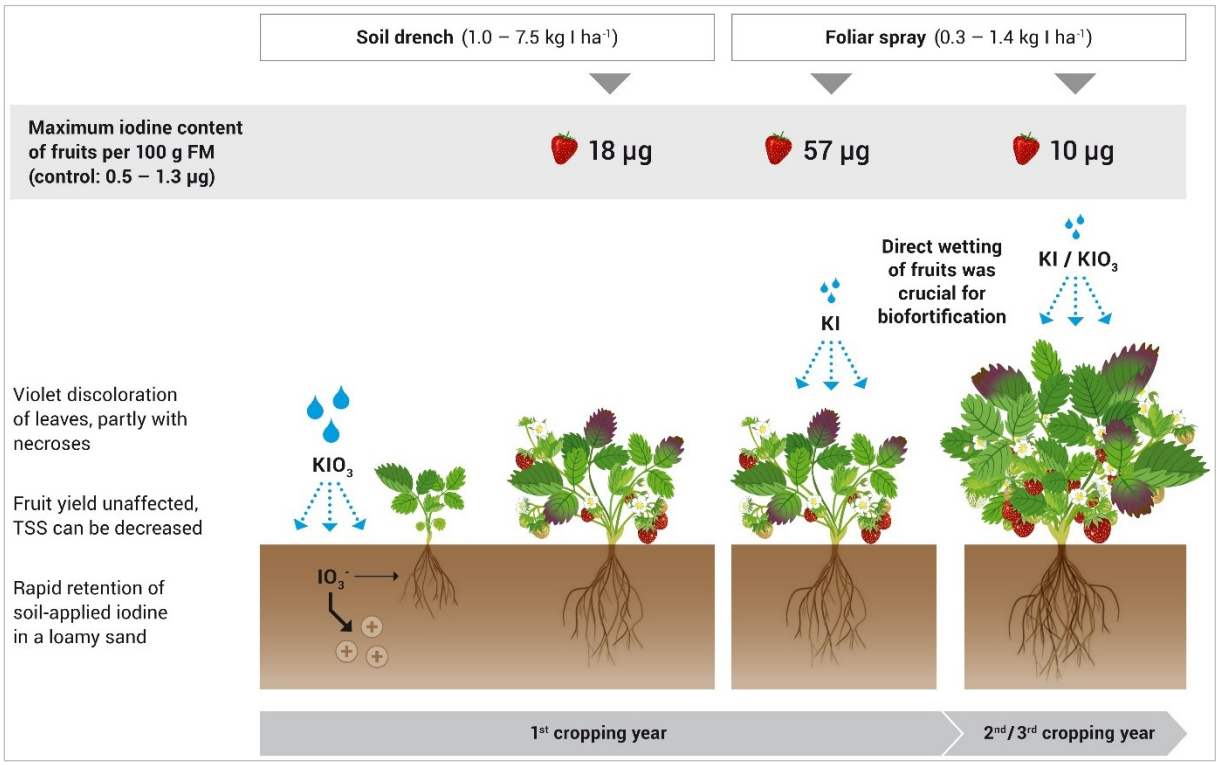
Abstract

The potential of iodine biofortification in strawberry fruits by means of soil and foliar fertilization was investigated in three field experiments and a preliminary phytotoxicity test in the greenhouse. In the main experiment iodine was applied by one-time potassium iodate soil drenches two weeks after planting or, alternatively, by using potassium iodide foliar sprays from the beginning of flowering. Beside the iodine accumulation in fruits, effects on crop yield and quality were determined. The soil fertilization resulted in a relatively low iodine accumulation in strawberry fruits, probably because the concentration of phytoavailable iodine in the soil rapidly decreased after its application. A markedly higher iodine content in fruits was achieved when it was aerially applied, either by a single treatment shortly before harvest or by repeated sprays during the flowering period. Yield, firmness and total acidity concentration of strawberry fruits were not significantly affected by any of the tested iodine applications. However, as a result of repeated foliar sprays the concentration of soluble solids in fruits was slightly diminished. Attempts to substantially increase the iodine content in fruits of strawberry plants cultivated in the second and third year failed, even following frequent sprays. In conclusion the results of this study suggest that only a relatively small proportion of exogenously applied iodine enters the fruits of field-grown strawberries due to its strong retention in soil and low phloem mobility in plants.

Graphical Abstract



Introduction

Iodine (I) is an essential trace element for humans. As an integral constituent of the thyroid hormones iodine plays an important role in the regulation of metabolism and other physiological functions. Insufficient iodine intake may cause manifold diseases including goiter, hyperthyroidism, reproductive failure, and mental retardation (*Pretell* and *Pandav*, 2017; *Zimmermann*, 2012). Due to measures implemented in iodine prophylaxis programs over the past decades, especially the iodization of table salt, severe iodine deficiency is nowadays relatively rare. However, even mild to moderate iodine deficiency is still widespread in many regions of the world and can have adverse health impacts (*Andersson* et al., 2012; *Eastman* and *Li*, 2017). Pregnancy and early childhood are the most critical phases of life in this respect (*Velasco* et al., 2018) because thyroid hormones are closely involved in brain development and maturation (*Berbel* and *Escobar*, 2011).

In Germany about 33% of the children and 32% of the adults are currently suffering from mild to moderate iodine deficiency. More seriously, 46.8% of women aged 18–29 and 38.9% aged 30–39 had an iodine intake below the estimated average requirement (*Gärtner*, 2016; *Johner* et al., 2016). This despite the fact that about 80% of the households in Germany use iodized table salts for cooking and food preparation. However, only about 30% of the commercially available salted foods are produced with iodized salt (*Remer*, 2009; *Bissinger* et al., 2018). Overall a decrease in salt consumption is expected in the next few years since the German Nutrition Society and other institutions recommend a reduction of the salt intake in the population in order to prevent hypertension and related cardiovascular diseases. As an undesired side effect, the iodine intake might decrease as well (*Strohm* et al., 2016). For this reason, alternative strategies to improve iodine supply are becoming increasingly important.

Biofortification of food crops is proposed as a suitable tool to enrich the human diet with iodine and other micronutrients (*White* and *Broadley*, 2009). Two main approaches are being pursued for this purpose: (1) genetic biofortification, which uses conventional plant breeding methods or genetic engineering and (2) agronomic biofortification through supplying the desired micronutrients to plants by means of soil or foliar applications. Further attempts in the greenhouse production rely on hydroponic techniques (*Sambo* et al., 2019). In the case of iodine, the agronomic approach is the method of choice since iodine-deficient soils are the main cause of low iodine content in plant food. This needs to be addressed before breeding strategies can become relevant (*Storcksdieck genannt Bonsmann* and *Hurrell*, 2009). In general, plant breeding is a very time-consuming process whereas adaptations in fertilization procedures can be quite easily implemented in crop production (*Cakmak*, 2008; *Alfthan* et al., 2015).

Previous studies on iodine biofortification mainly focus on vegetable and cereal crops. Leafy vegetables seem to be good candidates for this purpose because root-applied iodine is primarily translocated by mass flow throughout the xylem vessels. Accordingly, iodine preferentially accumulates in high-transpiring shoot organs (*Zhu* et al., 2003; *Hong* et al., 2008; *Voogt* et al., 2010). Foliar iodine sprays have proven to be even more efficient for biofortifying leafy vegetables such as butterhead lettuce (*Smoleń* et al., 2014; *Lawson* et al., 2015). This method was also successfully tested to increase grain iodine in wheat,

rice, maize (*Cakmak* et al., 2017; *Zou* et al., 2019). However, iodine uptake of hydroponically grown tomato plants was higher when iodine was supplied to roots rather than onto the leaves (*Landini* et al., 2011).

For iodine fertilization treatments, most commonly potassium salts containing iodide (I^-) and iodate (IO_3^-) are chosen. In soils, I^- is more susceptible to losses caused by leaching or volatilization processes than IO_3^- (*Yamada* et al., 1999; *Ashworth*, 2009; *Horel* et al., 2014). Correspondingly, a higher iodine enrichment in vegetable crops was observed when applying the oxidized iodine form to soils (*Dai* et al., 2006; *Lawson* et al., 2015). In contrast, by using foliar sprays it was proved that I^- was absorbed better through aboveground plant parts (*Lawson* et al., 2016). However, higher doses of foliar- and root-applied I^- were less tolerated by plants compared to IO_3^- (*Mackowiak* and *Grossl*, 1999; *Blasco* et al., 2008; *Lawson* et al., 2015). Although land plants do not need iodine for normal growth and development, in some studies at low iodine fertilization rates an increase in biomass production was observed (*Zhu* et al., 2003; *Hong* et al., 2008; *Weng* et al., 2008). Furthermore, iodine can stimulate the biosynthesis and accumulation of health-promoting bioactive substances such as phenolic compounds and vitamin C, as particularly found in leafy vegetables (*Blasco* et al., 2008; *Smoleń* et al., 2015). The induction of antioxidants is considered a crucial mechanism for adaptive responses leading to stress tolerance in plants. Therefore, it was hypothesized that iodine biofortification may contribute to mitigate damage from abiotic and biotic stress (*Medrano-Macías* et al., 2016).

To date, only few reports have been published dealing with the effects of exogenously applied iodine on tree fruit and soft fruit crops. In a field experiment with plums and nectarines, it was not possible to increase iodine content in fruits above 15 µg $(100\ g\ FM)^{-1}$ by foliar sprays and it remained negligible following a soil I^- fertilization (*Caffagni* et al., 2012). Markedly higher levels of iodine were found in strawberries cultivated in a hydroponic system when I^- and IO_3^- were added to the nutrient solution. Iodine absorbed by roots was distributed in all parts of the plant. In fruits the iodine content ranged between 60 and 400 µg I $(100\ g\ FM)^{-1}$ (*Li* et al., 2017). Already at the lower accumulation level the biofortified strawberries would significantly contribute to dietary iodine supply considering that a daily iodine intake of 150–200 µg is recommended for adolescents and adults (*EFSA*, 2006; *Andersson* et al., 2007).

Strawberries are highly appreciated for their taste, nutritional value and antioxidant compounds. They are an excellent source of vitamin C, folat, and a wide range of phenolic compounds such as phenolic acids, flavonoids and anthocyanins. These antioxidative substances have important roles in the scavenging of reactive oxygen species, which are related to the occurrence of several diseases (*Giampieri* et al., 2012; *Ariza* et al., 2016). Strawberries are also rich in mineral nutrients, such as manganese, potassium, magnesium, copper and iron. The average native iodine content was reported to be in the range of 2–3 µg $(100\ g)^{-1}$, and thus somewhat higher than in most other fruits species (*Souci* et al., 2016). However, even with this iodine content, strawberries have yet only a marginal impact on the iodine intake of humans.

The strawberry is an economically important fruit crop in many countries across the world and widely consumed fresh or in processed forms, such as jams, juices and fruit pre-

parations (*Giampieri* et al., 2012; *Simpson*, 2018). In Germany, strawberries are the most popular summer soft fruit. In terms of fruit growing area, it is in second place behind apples (*AMI*, 2018; *YouGov*, 2017). The majority of strawberries are produced in open field plantations. However, the share of fruits coming from protected cultivation – mainly plastic tunnels – has risen in the last few years from 4% in 2009 to 16% in 2018 (*Statistisches Bundesamt*, 2019b). Even under these conditions, plants are usually grown in soil or, to a smaller extent, in substrates. Hydroponic systems are still without relevance in German commercial strawberry production.

The objective of the present study was to assess the potential for biofortifying fruits of field-grown strawberries with iodine. We hypothesized that foliar sprays are more efficient than soil drenches for this purpose, as previously observed for vegetables (*Lawson* et al., 2015) and cereals (*Cakmak* et al., 2017). Thus, both approaches were investigated using different doses of potassium iodide (KI) or potassium iodate (KIO_3). Beside the iodine accumulation in fruits, effects on crop yield and quality parameters were taken into consideration. Furthermore, the concentration of $CaCl_2$-extractable soil iodine was monitored during the course of cultivation following a KIO_3 soil fertilization. In a preliminary greenhouse trial, the phytotoxicity of various iodine treatments on strawberry plants was tested to determine appropriate application rates for the subsequent field experiments.

Materials and methods

Plant material and growing conditions

The experiments were carried out with strawberry plants (*Fragaria x ananassa*). For preliminary tests in the greenhouse and the first field trial cv. 'Elsanta' was used. The second and third field trials were conducted with cv. 'Senga-Sengana' and cv. 'Sonata', respectively. In the greenhouse experiment, the plants were cultivated in a peat-based substrate adjusted to pH ($CaCl_2$) 5.5 and base dressed (mg L^{-1}) with 210 N, 230 P_2O_5, 275 K_2O, 115 Mg as well as micronutrients by applying of 1.5 g (L substrate)$^{-1}$ PG-MIX® Low Mo (Yara, YARA GmbH & Co. KG, Dülmen, Germany). The growing media was filled into one-meter-long balcony flower boxes with a volume capacity of 22.5 L. In each container nine bare rooted A^+ frigo plants were placed and cultivated from the middle of March till the end of May 2012. Set points in the greenhouse for heating (day/night) and ventilation were 18 °C/8 °C and 20 °C, respectively. Plants were irrigated manually from the top as needed.

The field experiments were carried out on two sites (Langförden, soil type Stagnosol, first field trial: 52°47'13.0"N 8°13'34.8"E, second field trial: 52°47'17.9"N 8°13'52.4"E, and Bassum, soil type Anthrosol, third field trial: 52°51'10.0"N 8°48'17.1"E) in the north-western region of Lower Saxony, Germany. At both locations the plants were grown in a loamy sand soil adjusted with calcium carbonate to pH ($CaCl_2$) 5.5–6.0 and contained sufficient amounts of P, K and Mg (class C according to the German nutrient-availability classification system (*Kießling* and *Hoffmann*, 2016)). N fertilizer demand was estimated according the N_{min}-method (*Wehrmann* and *Scharpf*, 1986) by subtracting the initial soil mineral nitrogen concentration (NO_3^- + NH_4^+) from the N_{min} target value of 60 kg

N ha^{-1} in the top 30-cm soil layer. N fertilization was realized by a base dressing using calcium ammonium nitrate. In the first field trial A^+ bare rooted frigo plants were planted on May 8, 2012. In the second and third field trial strawberry plants were already grown in the 3rd and 2nd year of cultivation, respectively. The planting distance was 0.25 m x 1.0 m, which corresponds to 40,000 plants per ha. In order to prevent infestation with fungi and pests, pesticides containing the active ingredients cyprodinil, fludioxonil, fenhexamid, azoxy-strobin and lamda-chhylotrin were applied according to recommendations of the regional fruit extension service. Climatic data were collected at local meteorological stations. From May to July 2012 (first two field trials) the daily mean air temperature, total precipitation and number of rain days were 16 °C (max. 24 °C/min. 7 °C), 280 mm, and 46 days, respectively. From May to June 2014 (third field trial) the corresponding weather conditions were 14 °C (max. 18 °C/min. 11 °C), 112 mm and 20 days, respectively. Due to the relatively wet weather conditions in both growing seasons only a few additional irrigation cycles were necessary to ensure the water demand of the plants.

Trial set-up and iodine treatments

All experiments were performed in a completely randomized block design with 4 replications. Each plot had a gross size of 5.5 m x 0.6 m and included 20 plants. Stock solutions for the iodine treatments were prepared in the laboratory with pure potassium salts (KIO_3 and KI both from VWR International GmbH., Bruchsal, Germany). To allow for an accurate and reproducible dosage, the iodine solutions were portioned in polyethylene bottles by an automatic dispenser in volumes and concentrations corresponding to the calculated iodine demand per plot. To improve wetting properties, all solutions used for foliar sprays additionally contained 0.02% (v/v) of the nonionic organosilicone adjuvant Break-Thru® S 240. In the control treatments pure water was sprayed on plants.

In the greenhouse trial KI was applied for both substrate drenches and foliar sprays, when plants have reached the stage of full flowering (BBCH 65). In the substrate treatments 1 L of the diluted stock solution was supplied to each growing container with the help of pouring vessels to set the desired iodine levels of 0, 1.0, 2.5 and 7.5 mg (L substrate)$^{-1}$. These figures corresponded to an iodine supply of 2.5, 6.25 and 18.75 mg per plant, respectively. Care was taken to avoid any contact of the applied solution with the aboveground plant parts. A hand-held spray system served for the foliar treatments. Each plant was sprayed with 25 mL solution containing 0, 0.25, 0.75 and 1.50 g I L^{-1}. These figures corresponded to an iodine supply of 0, 6.25, 18.75 and 37.5 mg per plant, respectively.

In the first field trial, the main experiment of this study, KIO_3 and KI were administered as soil and foliar fertilization, respectively. These forms proved to be particularly efficient for the relevant application method as outlined in the introductory section. The soil drenches were carried out 14 days after planting. The KIO_3 stock solution was diluted with 20 L water for each single plot (3.3 m^{-2}) to achieve the desired iodine soil fertilization rates of 0, 1.0, 2.5 and 7.5 kg I ha^{-1}. The working solutions were uniformly distributed over an area of 30 cm from the left and right side of the plants with the help of watering cans. Again, care was taken to avoid any contact of the applied solution with the aboveground plant parts. Two days after the iodine soil drenches, an additional 15 L of water m^{-2} were

applied to ensure that the administrated iodine reached the intensively rooted soil horizon. The foliar fertilization was carried out with a backpack sprayer. In a total of six treatments different iodine concentrations as well as several dates and numbers of KI sprays were investigated. All spray treatments were conducted with a water amount of 1,000 L ha^{-1}. The first application took place on June 1 at the balloon stage (BBCH 59) with a rate of 0.2 kg I ha^{-1}. In further trial variants 0.1, 0.2 and 0.4 kg I ha^{-1} were applied when about 40% of flowers were open (BBCH 61-65). Another treatment comprised a foliar spray three days before the first picking (July 2, BBCH 85) with 0.2 kg I ha^{-1}. Finally, repeated applications were examined, which occurred in weekly intervals starting at the balloon stage. In this way in total 0.8 kg I ha^{-1} were supplied with four sprays.

In the second field trial, strawberry plants in the 3rd year of cultivation were sprayed once a week with KI using a 3-nozzles fork starting from the balloon stage of the primary flowers (May 4) till full flowering (June 1). Altogether, 0.3 kg I ha^{-1} were applied. In the third field trial, strawberry plants cultivated in the 2nd year were treated with a hand-held sprayer by using both iodine forms, KI and KIO_3. Here, the number of applications was increased to seven. The treatments started as the flowers opened (April 30) and were continued until three days before the last picking occurred (June 24). In total 0.35, 0.70, and 1.40 kg I ha^{-1} were applied as KI and 0.7 kg I ha^{-1} as KIO_3.

Fruit sampling and determination of fruit quality parameters

The harvest period of the field grown strawberry plants extended over three to four weeks. Fruits were usually picked twice a week. In the main experiment the first and last picking dates were on July 5 and July 26, respectively. In the second and third field trial the harvest was already completed on June 29. The total yield and the number of fruits were recorded. From the quotient of these values the average individual fruit weight was determined. For further fruit analyses the produce of each plot and harvest week was pooled into one homogenous sample. For the measurement of the total soluble solids (°Brix) a strawberry juice was produced and centrifuged at 6,000 rpm for 15 min. A digital refractometer was used for the determination. The titratable acidity was measured according to *DIN EN 12147* (1997) by titration with a known molarity solution of sodium hydroxide using phenolphthalein as indicator and expressed as g tartaric acid L^{-1}. The firmness of the strawberry fruits was determined using a Durofel DFT 100 penetrometer with a TipType 50 stamp. The values are expressed as Durofel units ranging from 0 (very soft) to 100 (very firm).

Iodine determination in fruit and soil samples

To imitate a common domestic cleaning process, the freshly harvested strawberries were washed by hand under running deionized water. Afterwards the strawberries were freeze-dried and ground using a 0.2 mm sieve in an ultra-centrifugal rotor mill at 14,000 rpm. After every sample, the removable components of the mill were completely cleaned with deionized water. Bevor chemical digestion, the strawberry powder was dried again overnight in a drying cabinet at 60 °C.

Originally, it was planned to use the alkaline digestion method described by *Kučera* and Krausová (2007), which was successfully adapted by *Lawson* et al. (2015) to determine the iodine content of several vegetable species. After the alkaline digestion with potassium hydroxide (KOH), the iodine was detected spectrophotometrically based on the Sandell-Kolthoff reaction by using a flow injection analysis (FIA) system. However, during the analysis of digestion solutions from strawberry fruits, gas bubbles were formed under acid conditions in the reaction unit of the FIA system. This was due to the release of carbon dioxide (CO_2) from carbonates generated by the combustion of plant- organic carbon. It was not possible to remove the CO_2-bubbles sufficiently by the installation of bubble traps. As a result, iodide detection was interfered with and erroneous measurements occurred. Various efforts to solve this methodical problem, e.g. by intermediate cleaning cycles as recommended by *Matthes* et al. (1978), had failed. Thus, it is assumed that sugary sample matrices are not suitable for the described method.

Therefore, fruit samples were digested a second time using 25% tetramethyl ammonium hydroxide solution (TMAH) and analyzed by inductively coupled plasma mass spectrometry (ICP-MS) according to the standardized method *DIN EN 15111* (2007). For quality assurance during iodine analysis, internal and certified external reference materials (milk powder NIST-1849a) were used. The iodine concentration in soil samples was also analyzed by ICP-MS after extraction with 0.0125 M calcium chloride solution ($CaCl_2$), as described by *Lawson* et al. (2015). Soil samples were taken from treatments with KIO_3 soil drenches in the soil layers of 0–15 cm and 16–30 cm. Two to three hours after the iodine fertilizer application, the first soil samples were taken. The last of six sampling dates occurred two months later. In order to avoid changes in the iodine content after sampling, the soil samples were collected in a cool box and about three hours later frozen at -18 °C until analysis.

Statistical procedures

The data obtained from fruit analyses – iodine content, quality parameters, fruit yield and average fruit weight – were subjected to one-way ANOVA and, if needed, transformed to meet assumptions of normality and homogeneity of variances. Post-hoc comparisons of means were carried out using the Student-Newman-Keuls and Tukey's test at $\alpha = 0.05$. All statistical tests were conducted using the program IBM SPSS® Statistics, version 25.

Results

Iodine-induced symptoms on leaves and fruit yield

In a preliminary greenhouse trial, the phytotoxicity of various iodine treatments on strawberry plants was tested at the stage of full flowering. Substrate drenches with KI were well tolerated by plants up to a dose of 2.5 mg (L substrate)$^{-1}$. Higher levels of root-applied iodine led to slight discolorations and necrotic margins on younger leaves (**Figures 1A – 1C**). When KI was aerially applied, leaf deformations were already observed at lowest dose of 0.25 g I (L spray solution)$^{-1}$. With increasing iodine concentration, the toxicity became more severe, as indicated by pronounced chlorosis and necrosis on leaves (**Figures 1D – 1F**). As a result, the following fruit development was strongly impaired compared to the control (results not shown).

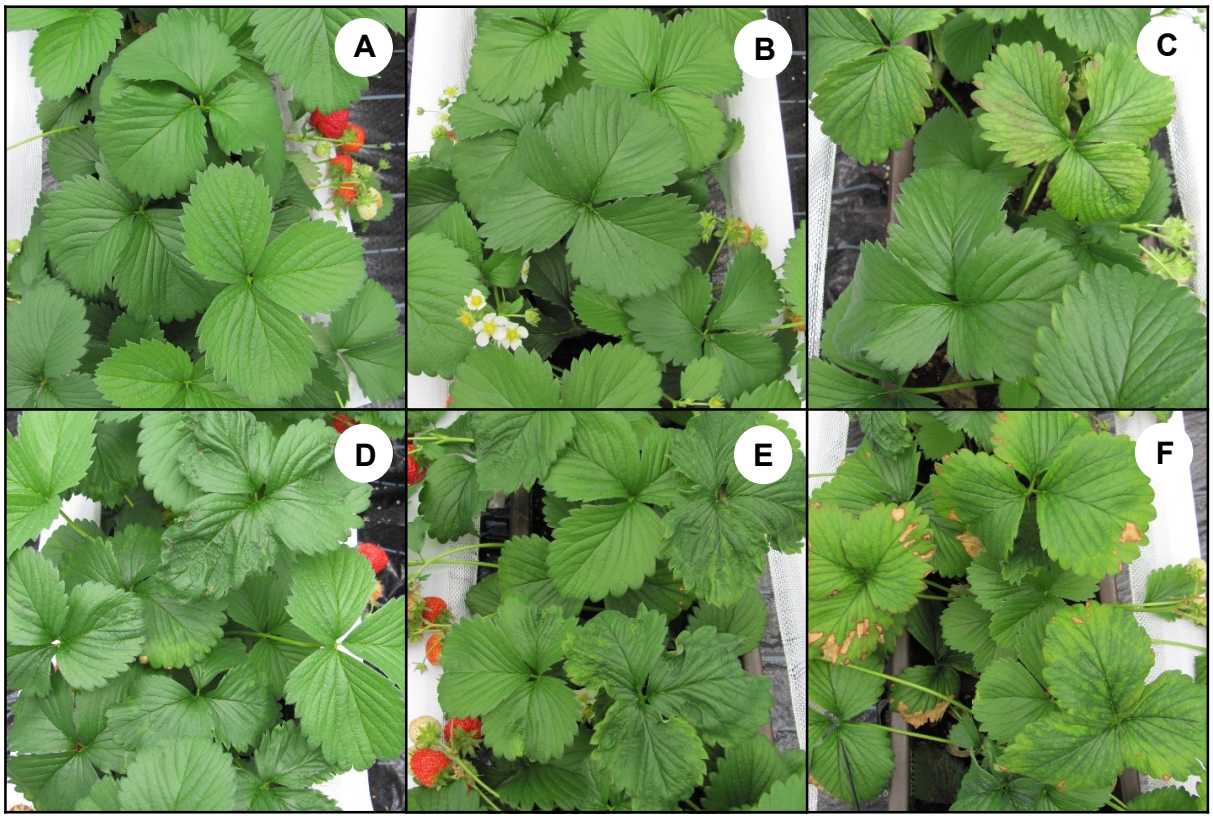


Figure 1: A visual comparison of strawberry plants grown in peat substrate in the greenhouse one week after different iodine treatments. Substrate drenches: (**A**) 1.0, (**B**) 2.5, and (**C**) 7.5 [mg I^--I (L substrate)$^{-1}$]. Foliar application (each plant was sprayed with 25 mL): (**D**) 0.25, (**E**) 0.75, and (**F**) 1.50 [g I^--I (L spray solution)$^{-1}$].

Based on these findings, in field experiments the iodine fertilization rates for soil drenches and single foliar sprays were usually limited to a maximum rate of 7.5 kg I ha^{-1} and 0.2 kg I ha^{-1}, respectively. Nevertheless, even on field-grown strawberry plants iodine-induced foliage anomalies could be observed. Most obvious was the violet discoloration of leaves, as appeared particularly at the highest KIO_3 soil fertilization rate and following repeated KI foliar sprays (**Figure 2**). Furthermore, tips and margins of older leaves became necrotic in these treatments. The intensity of this damage was rated in the first field experiment during the second week of harvest (**Figure 3**). Beside the control treatment,

plants remained unimpaired when grown at the lowest soil and foliar iodine fertilization rate. Above this level, the degree of necrosis clearly rose with increasing iodine supply. In the case of foliar sprays, injuries were more intense when the iodine application was carried out in the picking stage compared to the balloon or flowering stage.

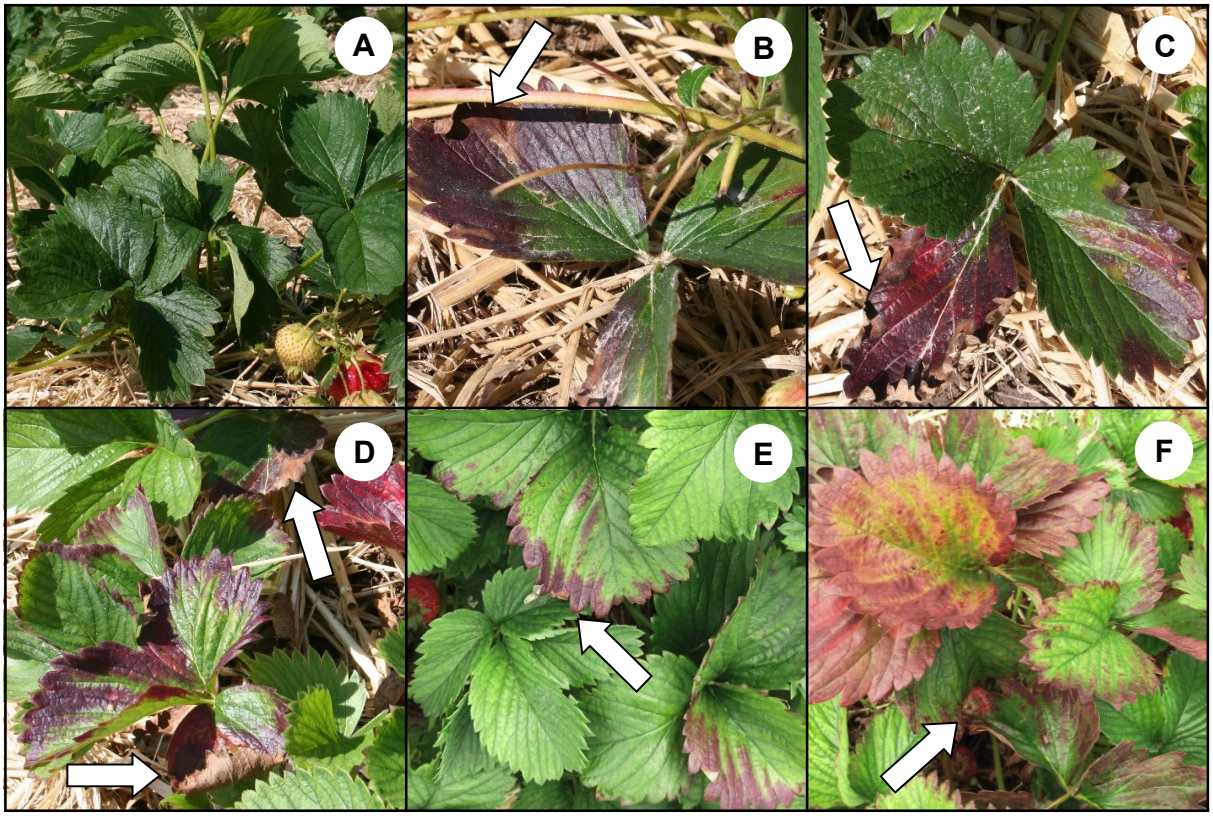


Figure 2: A visual comparison of strawberry leaves grown under field conditions using different iodine application methods, doses and forms. Photos a–d were taken 3 days before the last picking week of plants in the first cropping year; photos e–f were taken immediately before the last picking of plants in the second cropping year. (**A**) control; (**B**) soil drenches: 7.5 kg IO_3^--I ha^{-1}; foliar application: (**C**) 0.4, (**D**) 4 x 0.2, (**E**) 7 x 0.05, and (**F**) 7 x 0.2 [kg I^--I ha^{-1}]. The white arrows indicate necrotic leaf tissue.

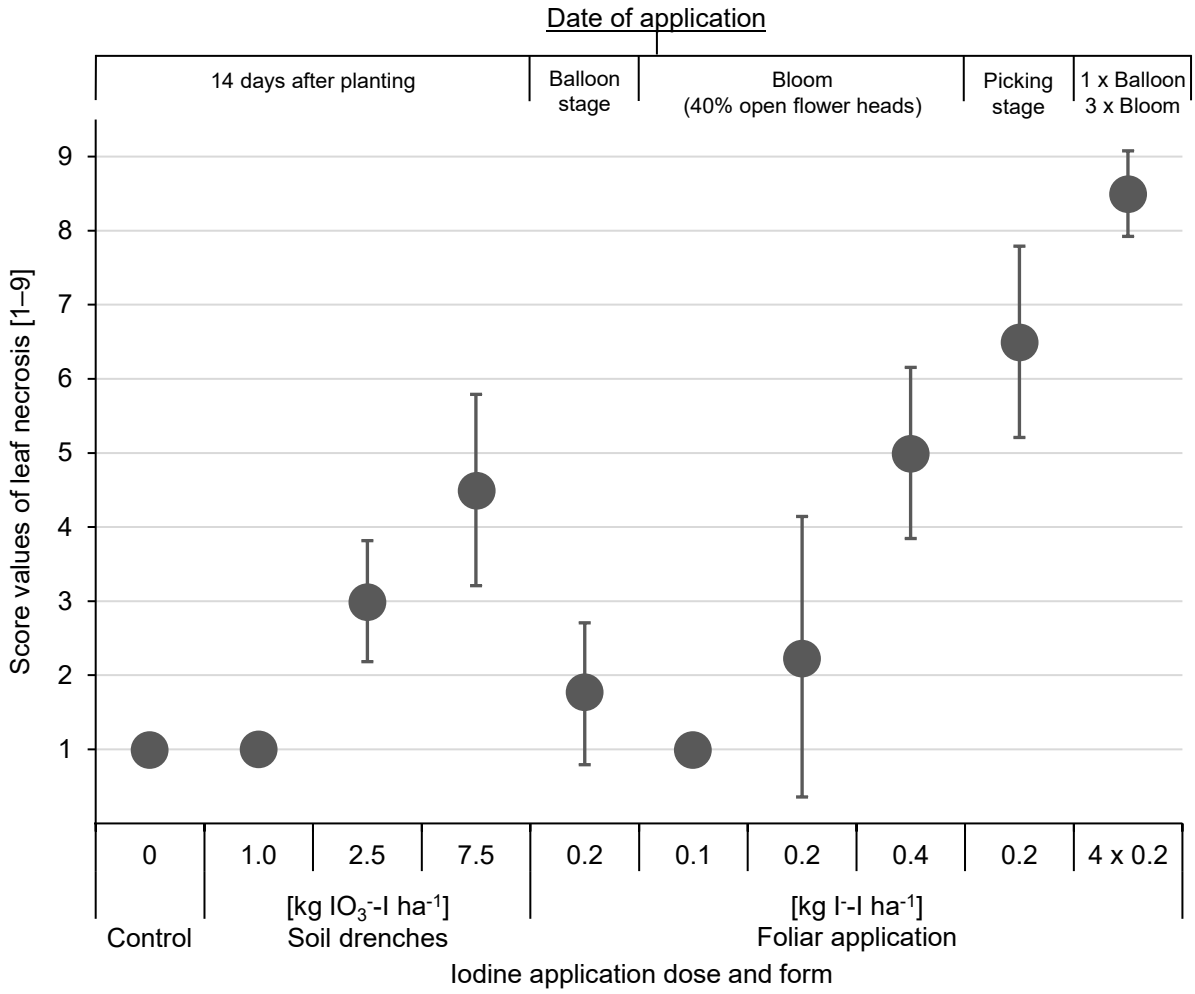


Figure 3: Severity of leaf necroses on field-grown strawberry plants in the second picking week as affected by different iodine treatments in the first cropping year. Means ± standard deviation (*n* = 4), (1 = no damage, 9 = severe damage).

Despite the aforementioned leaf damages, fruit yield achieved over the entire harvest period was not significantly affected by any of the investigated iodine treatments (**Figure 4**). On the whole, the mean yield ranged from 7.3 to 7.8 t ha^{-1}. Apart from the total fruit yield, the average single fruit weight was similar throughout all treatments. The same observations in terms of fruit yield and average fruit single weight were made in the field experiment with strawberries in the second cropping year (results not shown).

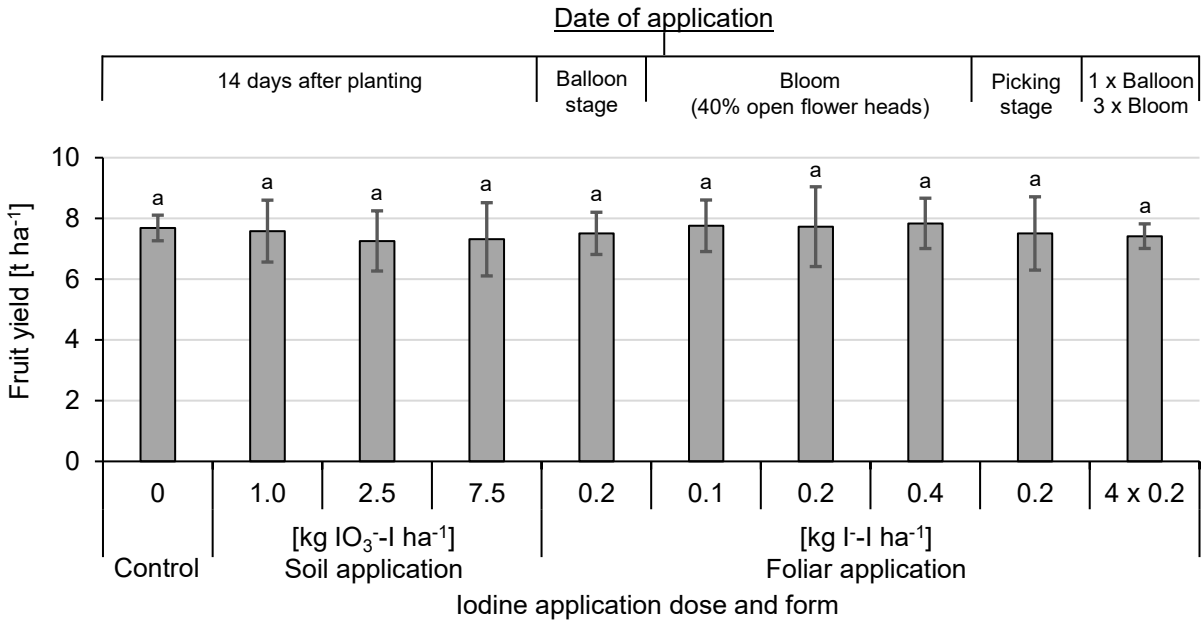


Figure 4: Influence of different iodine treatments on the fruit yield of field-grown strawberries in the first cropping year (sum of all 4 picking weeks). Means ± standard deviation (*n* = 4). Means with same letters do not differ according to Student-Newman-Keuls test at α = 0.05.

Fruit iodine content

Without exogenous supply, the iodine content in fruits of newly planted field-grown strawberries ranged between 0.5 to 1.3 µg I (100 g)$^{-1}$. Following a one-time KIO_3 soil fertilization conducted two weeks after planting, the fruit iodine content increased up to 14 µg I (100 g)$^{-1}$ in the first picking week (**Figure 5**). A much higher iodine accumulation was detected when KI was aerially applied, either by a single treatment shortly before first fruit picking or by repeated sprays during the entire flowering period. With these treatments iodine content approached 50 µg I (100 g)$^{-1}$ or was slightly above, respectively. However, when KI was sprayed once at the balloon or flowering stage, considerably less iodine entered the fruit.

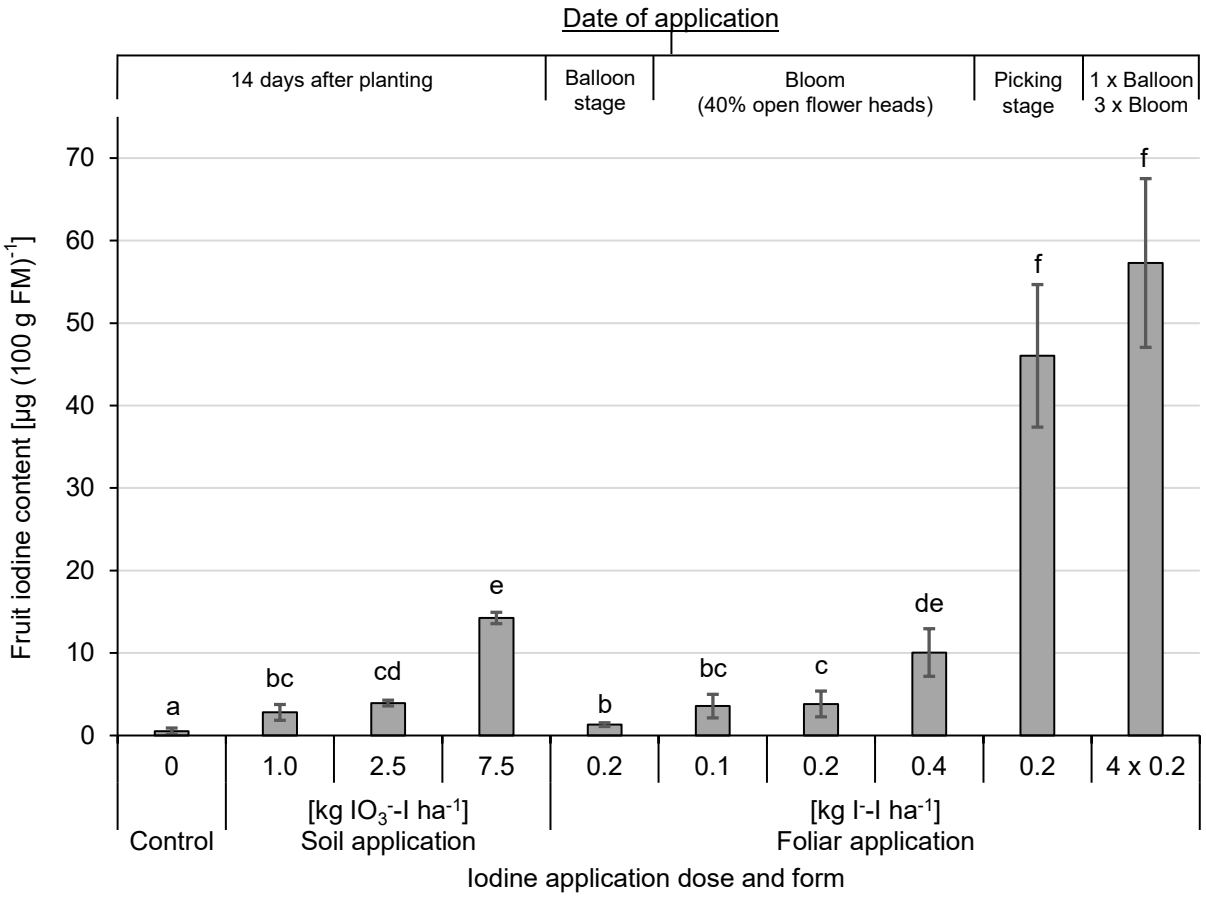


Figure 5: Iodine content in fruits of field-grown strawberries in the first picking week as affected by the iodine supply in the first cropping year. Means ± standard deviation (*n* = 4). Means with same letters do not differ according to Tukey's test at α = 0.05.

Regardless of the iodine application method, time and dose, the iodine content in strawberries decreased steadily during the harvest period. At the last picking date, in most treatments the iodine content was similar to the control (**Table 1**). Overall, the highest level of iodine enrichment was obtained by four-time foliar sprays spread over the entire flowering period. Applying single KI sprays with a dose of 0.2 kg I ha^{-1}, the iodine accumulation in fruits was strongly affected by the time of application. The closer the treatment was to the picking date the more iodine was found in the fruits. This impact was most pronounced in the first week of picking (**Figure 6**).

Table 1: Iodine content in fruits of field-grown strawberries in different picking weeks as affected by the iodine supply in the first cropping year. Means ± standard deviation (*n* = 4). Means within a column (picking week) sharing a letter do not differ according to Tukey's test at α = 0.05.

	Fruit iodine content [µg (100 g FM)$^{-1}$]			
Picking week	**1.**	**2.**	**3.**	**4.**
Treatment				
Control (no iodine)	0.5 ± 0.4^{a}	1.3 ± 0.8^{ab}	1.1 ± 0.9^{a}	0.7 ± 0.3^{a}
Soil drenches [kg IO_3^--I ha^{-1}]				
1.0	2.8 ± 1.0^{bc}	0.9 ± 0.4^{a}	1.1 ± 0.8^{a}	0.6 ± 0.1^{a}
2.5	3.9 ± 0.4^{cd}	3.0 ± 0.6^{abc}	1.5 ± 0.9^{a}	0.7 ± 0.2^{ab}
7.5	14.2 ± 0.7^{e}	8.0 ± 1.7^{cd}	3.6 ± 1.4^{ab}	3.1 ± 2.0^{bc}
Foliar application* [kg I$^-$-I ha^{-1}]				
0.2 (Balloon)	1.3 ± 0.2^{b}	1.0 ± 0.6^{a}	1.2 ± 0.6^{a}	1.0 ± 0.5^{ab}
0.1 (Bloom)	3.6 ± 1.4^{bc}	4.2 ± 0.8^{bcd}	1.5 ± 1.3^{a}	0.8 ± 0.8^{a}
0.2 (Bloom)	3.8 ± 1.6^{c}	2.0 ± 1.3^{ab}	1.2 ± 1.3^{a}	1.0 ± 0.7^{ab}
0.4 (Bloom)	10.1 ± 2.9^{de}	2.2 ± 1.3^{abc}	2.0 ± 1.2^{a}	1.4 ± 1.0^{ab}
0.2 (Picking)	46.0 ± 8.6^{f}	18.4 ± 4.6^{de}	13.4 ± 1.4^{bc}	7.6 ± 1.9^{cd}
4 x 0.2 (1 x Balloon + 3 x Bloom)	57.3 ± 10.2^{f}	37.6 ± 8.8^{e}	25.5 ± 5.1^{c}	14.4 ± 4.2^{d}

* The time of application is indicated in parentheses; in the case of repeated sprays the number of treatments is indicated as well.

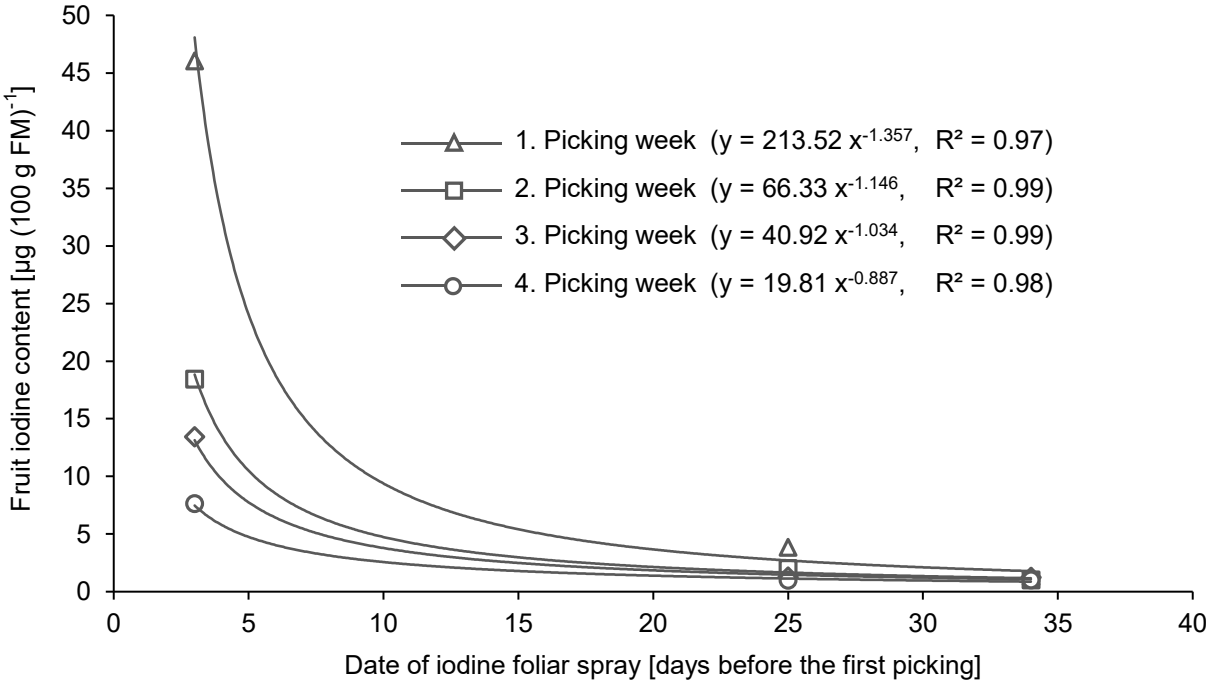


Figure 6: Iodine content in fruits of field-grown strawberries as affected by the application date of a single iodine foliar spray in the first cropping year *(n* = 4). Each of the iodine treatments shown was carried out with 0.2 kg I$^-$-I ha^{-1}.

The native iodine content of fruits harvested from strawberry plants in the second and third year of cultivation was always below 1 µg I (100 g FM)$^{-1}$. Furthermore, even with repeated foliar sprays in the flowering period, the iodine content in fruits did not exceed 10 µg I (100 g FM)$^{-1}$ (**Table 2**). Nevertheless, within this range a clear increase as affected by the applied iodine dose was noticed. At the same fertilization rate, applications with KIO_3 resulted, on average, in a higher iodine enrichment in fruits than when using KI. However, this effect was only statistically significant in the last week of harvest.

Table 2: Iodine content in fruits of strawberries in the second and third cropping year after repeated foliar sprays. Means ± standard deviation (*n* = 4). Means within a column (picking date) sharing a letter do not differ according to Tukey's test at α = 0.05.

Cultivation year	Number of sprays, iodine dose [kg I ha^{-1}] and form		Fruit iodine content [µg (100 g FM)$^{-1}$]		
			Picking date (last spray before picking)		
			Jun. 12 (Jun. 1)	Jun. 19 (Jun. 13)	Jun. 26 (Jun. 21)
Second year	Control		0.5 ± 0.1^{a}	0.5 ± 0.0^{a}	0.6 ± 0.1^{a}
	5–7 x 0.1	IO_3^-	6.0 ± 0.4^{ab}	4.5 ± 0.2^{bc}	7.0 ± 1.0^{c}
	5–7 x 0.05	I^-	2.4 ± 0.3^{a}	2.1 ± 0.4^{ab}	1.9 ± 0.1^{ab}
	5–7 x 0.1	I^-	3.6 ± 0.5^{ab}	3.2 ± 0.6^{ab}	3.1 ± 0.3^{b}
	5–7 x 0.2	I^-	9.3 ± 5.3^{b}	5.3 ± 2.7^{c}	8.2 ± 2.1^{c}
			Jun. 20 (Jun. 1)	Jun. 27 (Jun. 1)	
Third year	Control		0.5 ± 0.3^{a}	0.7 ± 0.3^{a}	
	2 x 0.1 + 2 x 0.5	I^-	9.8 ± 0.7^{b}	8.1 ± 0.9^{b}	

Strawberry fruits were usually washed with deionized water before analyzing. In the last field experiment the influence of this cleaning procedure was examined on fruits which had received six KI or KIO_3 foliar sprays, the last one 6 days before picking. On average, iodine content was 19% lower in washed fruits than in unwashed fruits. The effect increased with increasing fruit iodine content and when applying KIO_3 as compared to KI, with a maximum reduction of 30% and 24%, respectively (results not shown).

Soil iodine concentration

In the field experiment which included KIO_3 soil drenches, the concentration of $CaCl_2$-extractable iodine was monitored during the whole course of strawberry cultivation. A very fast decline in this iodine fraction was observed in the upper 15 cm of the soil. Already on the day of fertilization it was not possible to recover a notable part of fertilized iodine. Three weeks later the detected soil iodine concentration was, even at the highest KIO_3 fertilization, close to the level in the control plots (**Figure 7**). It was not possible to attribute the depletion of iodine in the topsoil to leaching losses since the concentration of $CaCl_2$-extractable iodine in the 16–30 cm soil layer remained nearly constant in the period concerned.

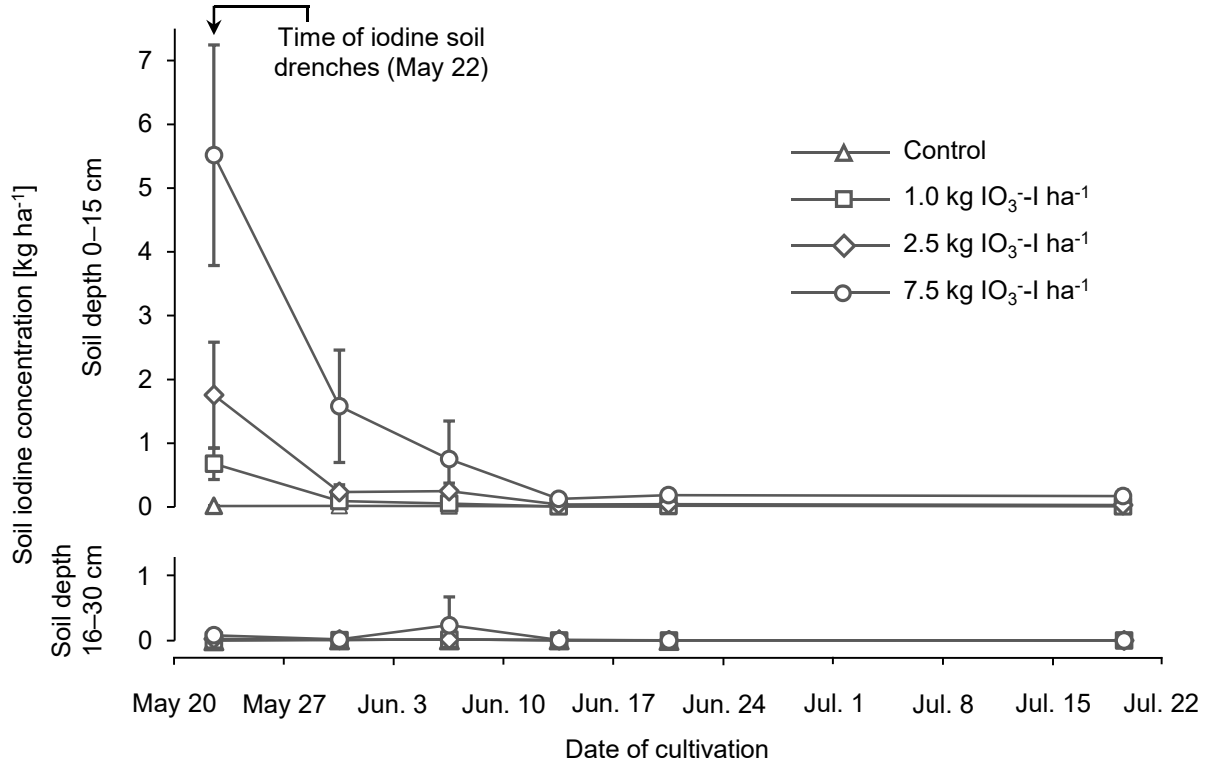


Figure 7: Development of the calcium chloride-extractable iodine concentration in two depths of a loamy sand soil after applying single KIO_3 soil drenches in different doses in the second week of newly planted strawberries. Means ± standard deviation (n = 4).

Fruit Quality Parameters

Fruit quality parameters of the newly planted strawberry crop, as investigated in the first field experiment, are compiled in **Table 3** for two picking weeks. Titratable acidity (TA), firmness and average single weight of fruits were not affected by any of the investigated treatments. However, following repeated KI foliar applications, the concentration of total soluble solids (TSS) decreased significantly. As a result, the TSS/TA ratio was reduced as well. In addition, soil drenches at the highest KIO_3 dose did reduce the TSS concentration in fruits in the second picking week.

Table 3: Total soluble solids, titratable acidity expressed as tartaric acid, ratio of soluble solids to titratable acidity, firmness and average single weight of fruits of strawberry plants as affected by different iodine treatments in the first cropping year (n = 4). Means within a column (picking week) sharing a letter do not differ according to Student-Newman-Keuls test at α = 0.05.

	Total soluble solids [°Brix]		Titratable acidity [g tartaric acid L^{-1}]		Ratio of soluble solids to titratable acidity		Firmness [Durofel units]		Average single fruit weight [g FM]	
Picking week	**1.**	**2.**	**1.**	**2.**	**1.**	**2.**	**1.**	**2.**	**1.**	**2.**
Treatment										
Control (no Iodine)	8.5 a	9.0 a	9.5 a	11.0 a	9.0 ab	8.2 a	70.2 a	80.0 a	16.6 a	11.4 a
Soil drenches [kg IO_3-I ha^{-1}]										
1.0	8.7 a	9.1 a	9.6 a	10.9 a	9.1 ab	8.3 a	70.6 a	78.0 a	17.0 a	11.5 a
2.5	8.8 a	9.0 a	9.1 a	11.1 a	9.7 a	8.1 a	69.9 a	78.0 a	16.4 a	11.5 a
7.5	8.3 ab	8.5 bc	8.9 a	11.0 a	9.3 ab	7.7 ab	67.9 a	79.9 a	16.2 a	11.0 a
Foliar application* [kg I-I ha^{-1}]										
0.2 (Balloon)	8.5 a	9.2 a	9.8 a	11.1 a	8.7 ab	8.2 a	69.3 a	80.7 a	15.8 a	10.9 a
0.1 (Bloom)	8.7 a	9.2 a	9.4 a	11.0 a	9.2 ab	8.3 a	70.0 a	81.9 a	16.3 a	10.8 a
0.2 (Bloom)	8.8 a	9.1 a	9.4 a	11.2 a	9.4 ab	8.1 a	68.8 a	81.9 a	15.8 a	10.7 a
0.4 (Bloom)	8.9 a	8.9 a	9.2 a	10.8 a	9.7 b	8.3 a	68.9 a	80.0 a	15.8 a	11.0 a
0.2 (Picking)	8.3 ab	8.7 ab	9.6 a	11.3 a	8.6 ab	7.7 ab	69.8 a	81.1 a	15.8 a	11.7 a
4 x 0.2 (1 x Balloon + 3 x Bloom)	7.8 b	8.2 c	9.2 a	10.8 a	8.4 b	7.6 b	70.2 a	81.5 a	15.3 a	11.4 a

***** The time of application is indicated in parentheses; in the case of repeated sprays the number of treatments is indicated as well.

A detrimental influence of frequent KI sprays on TSS accumulation in fruits was also observed in the field experiment with strawberries cultivated in the second year. The TSS concentration was negatively correlated with the dose of iodine applications (**Figure 8**). On average, about 1.0 °Brix less was found at the highest KI fertilization rate compared to the control treatment. The differences were more pronounced as harvest time progressed.

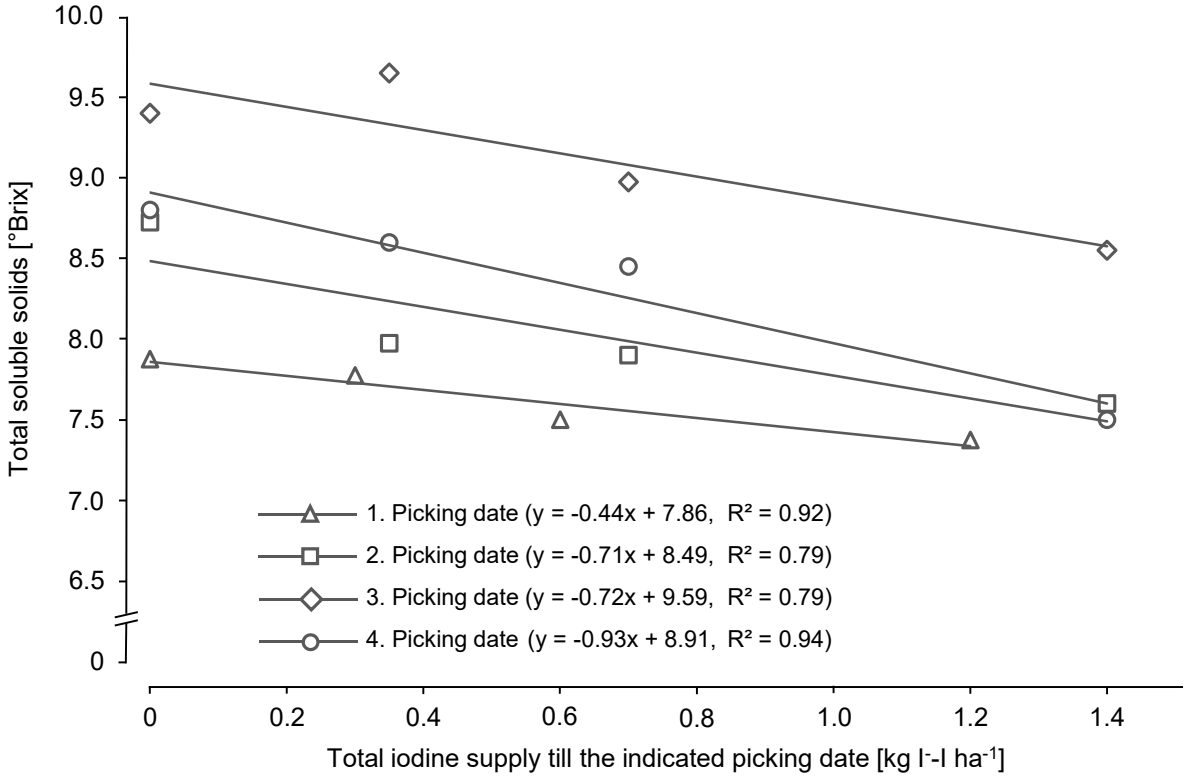


Figure 8: Total soluble solids in fruits of strawberry plants cultivated in the second year as affected by the total amount of iodine applied by foliar KI sprays till the indicated picking date (*n* = 4).

Discussion

Influence of iodine on the development of strawberries

Applying iodine to field-grown strawberries by using single soil drenches and foliar sprays with a dose of 1.0 kg IO_3^--I ha^{-1} and 0.1 I^--I kg ha^{-1}, respectively, did not have any visible implications on the plant development. However, at higher doses and when using repeated foliar sprays, leaves turned violet and become necrotic at the margins. The intensive violet discoloration of the foliage (**Figure 2**) indicates a stimulation in the biosynthesis of anthocyanins, as previously ascertained in leafy vegetables at moderate exogenous iodine supply (*Blasco* et al., 2008). An accumulation of anthocyanins and other flavonoids is well known as an adaptation mechanism of plants to environmental stress such as high ultraviolet radiation, heat and drought (*Petrussa* et al., 2013; *Ruiz-García* and *Gómez-Plaza*, 2013). Phytotoxic symptoms such as chlorosis, necrosis and abscission of leaves were frequently observed in several plant species at excessive I^- supply (*Mackowiak* and *Grossl*, 1999; *Caffagni* et al., 2011; *Landini* et al., 2011). It is assumed that the detrimental effects of I^- on plants may be caused from its intracellular oxidation to elementary iodine (I_2), resulting in the inhibition of photosynthetic processes (*Mynett* and *Wain*, 1971). Furthermore, it has been shown that high doses of I^- may decrease the activity of the superoxide dismutase, which play a crucial role in the defense against reactive oxygen species and thus in prevention of cell damage (*Blasco* et al., 2011).

Remarkably, despite the damage to strawberry leaves, fruit yield was not negatively affected by any treatment of the field experiments. Nevertheless, preliminary greenhouse trials have shown that fruit development will be severely impaired when strawberry plants are sprayed once with solutions containing ≥ 0.75 g I^--I L^{-1} (which corresponds to ≥ 0.75 kg I^--I ha^{-1} when applying 1,000 L ha^{-1}). Considerably lower critical concentration levels were reported for root-applied iodine. Shoot biomass production, including the fruits, of hydroponically cultivated strawberries was already reduced when the supplied nutrient solutions contained ≥ 1.0 mg I L^{-1} (*Li* et al., 2017). In our experiments with IO_3^- soil drenches much higher iodine concentration in the root zone were established. For example, assuming that 7.5 kg IO_3^--I ha^{-1} were initially homogenously infiltrated in the upper 15 cm soil layer, this would result in an average IO_3^--I concentration in the soil solution of approximately 15 mg L^{-1}. However, soil analyses indicated a very rapid decline in phytoavailable soil iodine after the fertilization event (**Figure 7**). Therefore, plants might have grown most of the time with less than 1 mg IO_3^--I L^{-1} in the soil solution. Roots in the deeper soil layer (16–30 cm) were continuously exposed to lower concentrations of dissolved IO_3^-. Furthermore, IO_3^- is known to be less harmful to plants than I^- (*Lawson* et al., 2015; *Li* et al., 2017). Hence, overall, it seems reasonable that fruit yield remained unaffected by the IO_3^- soil drenches administered in this study.

When using foliar sprays, most of the applied iodine gets directly onto aboveground plant organs. Leaves account for the largest share of shoot area and are thus the predominant entry points for aerially applied iodine. This might contribute to the observation that leaf damage was more severe following foliar treatments, even though much lower amounts of iodine were applied per acreage compared to soil drenches (**Figure 3**). Accordingly, previous investigations have demonstrated that iodine accumulation in the leaves of butterhead lettuce was up to 20 times higher after spraying the foliage as compared to the soil fertilization approach, each with an application rate of 1 kg I ha^{-1} (*Lawson* et al., 2015). Since fruit yield of field-grown strawberries remained unaffected even at the highest spray rate used in this study, it is concluded that these iodine doses did not significantly interfere with the flower development and fruit setting of plants. Furthermore, photosynthetic capacity of the foliage must still have been high enough to supply fruits with sufficient assimilates for their growth.

Iodine accumulation in strawberry fruits

The native iodine content in fruits of field-grown strawberries investigated in this study ranged between 0.5–1.3 µg I (100 g FM)$^{-1}$. *Souci* et al. (2016) reported for fresh strawberry fruits somewhat higher values, with a mean of 2.8 µg I (100 g FM)$^{-1}$ and a variation of 0.6–3.0 µg I (100 g FM)$^{-1}$. Fertilizing KIO_3 by one-time soil drenches enhanced the iodine level in fruits, but only to a modest extent. With the highest application rate of 7.5 kg IO_3^--I ha^{-1}, the fruit iodine content increased to a maximum of 14 µg (100 g FM)$^{-1}$ (**Figure 5**). It is likely that the fast decrease in phytoavailable soil iodine has limited the uptake of iodine by plants. However, the iodine accumulation was even markedly lower than previously reported for field vegetables. With the same form, rate and timing of iodine application, as well as under comparable growing conditions the iodine content in the edible parts of butterhead lettuce and kohlrabi increased to 143 and 90 µg (100 g FM)$^{-1}$, respectively (*Lawson* et al., 2015). The differential responses of the crops are clearly linked

to the physiological characteristics of the plant parts concerned. After its uptake by roots iodine moves with the mass flow in the xylem vessels to the shoot, driven by the transpiration of water from leaves. For this reason, most of the iodine in the shoot is usually present in the foliage (*Gonzali* et al., 2017). To a lower extent, iodine is also found in stem parts (such as kohlrabi tubers), presumably stored in the xylem parenchyma cells as known for plant nutrients (*White*, 2012). In contrast, fruits are generally poor in iodine. Most probably this is due to a low mobility of iodine in plants (*Herrett* et al., 1962). The supply of water and solutes to fruits mainly relies on the transport via phloem (*White*, 2012). Accordingly, the iodine content in fruits of hydroponically cultivated strawberry plants was, on average, about 7–9 times lower than in leaves when I^- and IO_3^- was added to the supplied nutrient solution, respectively (*Li* et al., 2017). Likewise, in several soil fertilization experiments a very low translocation of iodine from roots to fruits and seeds was detected (*Hong* et al., 2008; *Tsukada* et al., 2008; *Caffagni* et al., 2012; *Cakmak* et al., 2017). The transport of root-absorbed iodine to fruits is probably largely limited to the xylem sap influx occurring in the early stage of fruit development, as reported for other phloem-immobile elements such as calcium (*Hocking* et al., 2016).

Compared to KIO_3 soil drenches, foliar sprays with KI resulted in a significantly higher iodine accumulation in fruits of newly planted strawberries. Using repeated treatments during the flowering period, each with 0.2 kg I^--I ha^{-1}, the iodine content rose up to 57 µg (100 g FM)$^{-1}$ (**Figure 5**). Slightly lower iodine levels in fruits were achieved with a single treatment conducted a few days before the harvesting started. Thus, aerial iodine applications in strawberries were more effective than those found in a field experiment with nectarines and plums. Here it was not possible to increase iodine content in fruits to above 15 µg (100 g FM)$^{-1}$ when spraying on four dates about 0.3 kg I^--I ha^{-1} in total (*Caffagni* et al., 2012). However, due to the distinctly larger canopy of fruit trees compared to strawberries, at similar iodine application rates a lower iodine accumulation in tree fruits can be expected.

The timing of foliar KI treatments was a crucial factor for enriching strawberries with iodine. The larger the time gap between spray date and harvest date, the less iodine was found in the fruits. In addition, the fruit iodine content steadily decreased during the harvest period. When applications ceased before first picking, in the last harvest week on average only one-fifth of the iodine content was detected in fruits than at the beginning of the harvest (**Table 1**). Single sprays timed during the balloon and flowering stage did not even differ in fruit iodine content from the control treatment on the last picking date. Therefore, it is assumed that only a small proportion of the iodine absorbed by leaves is translocated to the developing fruits, if at all. In line with these findings, a recent study on spinach has shown that less than 2% of radioactively labelled iodine (129 I^-) applied to a single leaf was transferred through the phloem to younger leaves (*Humphrey* et al., 2019). By contrast, investigations on tomato and cereal plants indicate a translocation of leaf-absorbed iodine in fruits and seeds, respectively, to an extent allowing a sufficient level of iodine biofortification (*Landini* et al., 2011; *Cakmak* et al., 2017; *Zou* et al., 2019). So far it is unclear whether these contradictory results may reflect plant genotypic differences in iodine remobilization and phloem mobility or whether they are based on other reasons.

Contrary to the results obtained with newly planted strawberry plants, foliar KI sprays in the second and third year of crop cultivation were not successful in terms of iodine accumulation in fruits. Even when treatments were repeated seven times and continued until the last week of harvest, iodine content in fruits remained below 10 µg I $(100\ g\ FM)^{-1}$ (**Table 2**). Most probably this was due to morphological changes in the plant structure. In the years after planting, the strawberry crop forms a much denser canopy. Thus it is more difficult to wet the fruits sufficiently with the spray mist, even when using a 3-nozzle fork. The later spray system is specially constructed for uniform spraying and penetration of row crops (*Lechler*, 2017) and was applied in the second field trial. Once again, the findings clearly indicate that leaf-absorbed iodine did not significantly contribute to the iodine enrichment of strawberry fruits.

At the same dose, KIO_3 was superior to KI in increasing fruit iodine content. In contrast, by leaf spraying of butterhead lettuce KI has proven to be more efficient in this respect. This was explained, among other things, by the smaller size of I^- ions and the higher hygroscopicity of KI salts as compared to IO_3^- ions and KIO_3 salts, respectively (*Lawson* et al., 2016). Apparently, these aspects had negligible relevance for the uptake of iodine in strawberry fruits. However, in the present study both iodine forms were only compared in one field experiment and at one application rate. Moreover, the observed differences were statistically significant in only one of three picking weeks. Previous studies indicate that the impact of the iodine form on the uptake of foliar applied iodine by plants may vary under different growing conditions (*Lawson* et al., 2016; *Cakmak* et al., 2017). Thus, further investigations are needed to better understand these interdependencies.

Washing of strawberries with deionized water did reduce the fruit iodine content by a maximum of 24% and 30% when the last KI and KIO_3 foliar spray took place six days before harvest, respectively. Thus, most of the iodine intercepted by fruits was already absorbed or at least strongly fixed in the fruit cuticular waxes. A high cuticular sorption of leaf-applied I^- was observed in leaves of broad bean (*Shaw* et al., 2007). In butterhead lettuce iodine content of washed and unwashed leaves did not differ one day after treated with a KI solution. By comparison, in KIO_3 treatments about 50% less iodine was detected following the same washing procedure (*Lawson* et al., 2016). Thus, incorporation of iodine into leaves and fruits seems to proceed more slowly with IO_3^- than with I^-.

Overall, it was proved that the efficiency of the investigated iodine fertilization strategies was very low in strawberries. A maximum of 0.01% and 1.07% of the soil-applied and foliar-applied iodine was found in the fruits, respectively. A much higher proportion of the supplied iodine was presumably present in the canopy due to a higher xylem influx in the high-transpiring leaves and the larger surface area of the foliage (*Li* et al., 2017). Accordingly, in leafy vegetables up to one-third of the iodine fertilizer amount entered the edible plant parts when applying foliar sprays about one week before harvest (*Lawson* et al., 2016). However, even there a soil treatment was less efficient, since not more than approximately 1.0% of the iodine drenched into the soil shortly before crop planting finally reached the shoot of the vegetables (*Lawson* et al., 2015).

Soil iodine dynamics and fertilization strategies

The availability of soil applied IO_3^- for root uptake was limited to a few weeks, as indicated by the rapid decline in the $CaCl_2$-extractable iodine concentration in the 0–15 cm soil layer (**Figure 7**). Notable migration of IO_3^- in the deeper layers of the loamy sand soil investigated in this study was not observed, even though heavy rainfalls occurred in the weeks after the iodine soil drench. This confirms previous findings showing that iodine leaching in oxic soil, where IO_3^- usually represents the dominant inorganic iodine form, is very limited (*Ashworth*, 2009). The iodine depletion in the topsoil was most probably due to the sorption of IO_3^- on sesquioxides (*Fuge*, 2013). This process occurs very quickly (within hours or days) and increases with decreasing pH (*Sheppard* et al., 1995; *Shetaya* et al., 2012). Furthermore, iodine can be volatized from soils after microbial transformation to organic iodine compounds such as methyl iodide. However, this process was found to be more important in the presence of I^- and in waterlogged soils (*Amachi*, 2008; *Fuge*, 2013).

In the present field experiment, the KIO_3 soil drench was realized four weeks before the first picking. Possibly the soil-to-fruit iodine transfer could be enhanced by using one or several applications during the fruit development phase. Fertigation could be another method to improve the phytoavailability of iodine in soil. In pot experiments with soil-grown spinach this approach resulted in a significantly higher iodine enrichment in the edible plant parts than a pre-sowing treatment (*Smoleń* et al., 2016b). Considering the fact that drip irrigation systems are already quite common in Germany, for example, in both open field and protected strawberry cultivation, this approach could quite easily be implemented. Alternatively, it was proposed to develop fertilizers that release iodine slowly (*Gonzali* et al., 2017). An organic fertilizer based on iodine algae was successfully tested for this purpose in several vegetables (*Weng* et al., 2013, 2014). However, iodine accumulation was again much higher in the edible parts of leafy vegetables than in that of fruit vegetables. Thus, further experiments are needed to assess the potential of soil fertilization techniques for biofortifying field-grown strawberries with iodine.

Impact of iodine on fruit quality parameters

Fruit firmness, average single fruit weight and titratable acidity (TA) concentration of fruits were not affected by foliar or soil iodine treatments. However, following repeated KI foliar applications and KIO_3 soil drenches at the highest dose, the concentration of total soluble solids (TSS) decreased up to 1.0 °Brix. As a result, the TSS/TA ratio dropped as well. These changes can adversely affect the organoleptic quality of strawberry fruits since it is positively correlated with both the TSS concentration and the TSS/TA ratio (*Krüger*, 2012). Most probably the altered fruit composition was related to the observed leaves damages which might have diminished the photosynthetic activity and thus the assimilate supply to fruits. In accordance with these findings, *Li* et al. (2017) observed a decrease in soluble sugar content in hydroponically cultivated strawberries when the I^- and IO_3^- concentration in the supplied nutrient solution exceeded 1.0 mg I L^{-1}. Notably, at a concentration of 0.5 mg I L^{-1}, sugar accumulation in fruits was markedly increased, especially when I^- was applied. Similar dose-dependent relationships were also found for the vitamin C content of the strawberries (*Li* et al., 2017). Other bioactive compounds can

also be positively affected by a moderate exogenous iodine supply, e.g. the total content of phenols, flavonoids and anthocyanins, as reported for lettuce (*Blasco* et al., 2008). Violet leaf discolorations, as observed in this study following foliar and soil iodine treatments, indicate a stimulation in the biosynthesis of anthocyanins in strawberry plants. Thus, further research is suggested to explore the impact of iodine on the content of phytochemicals in fruits. These compounds not only substantially contribute to the health value of fruit produce but also may improve the tolerance of crops against abiotic and biotic stress (*Medrano-Macías* et al., 2016; *Habibi* et al., 2018).

Conclusions

The results of this study show that, in principle, field-grown strawberries can be biofortified with iodine by using foliar sprays. When applied once with a dose of up to 0.4 kg I ha^{-1} shortly before harvest, fruit yield and quality were not adversely affected. However, it is crucial for the success of this approach that the spray mist directly reaches the surface of fruits. The translocation of leaf-absorbed iodine to fruits appears to be negligibly small. A sufficient iodine accumulation in fruits at a level of about 50 µg (100 g FM)$^{-1}$ only seems to be achievable in newly planted strawberry fields in the first picking week. To maintain this level over the entire harvest period, treatments have to be repeated more often. This procedure is difficult to realize in commercial strawberry production. Moreover, by doing so, fruit sugar content can decrease due to leaf damage and thus organoleptic quality will be impaired. In the second and third year of cultivation, it is even more challenging to enrich strawberries with iodine. At this stage of plant development, fruits are largely hidden by a dense canopy and thus hardly hit by aerial applications.

Single soil drenches with KIO_3 applied two weeks after planting were less suited for increasing iodine content in strawberry fruits. The uptake of iodine by roots during the stages of flowering and fruit setting was limited by the very fast decline in the concentration of phytoavailable soil iodine. Further investigations are required to find out whether the efficiency of the soil iodine fertilization approach can be improved by a later application date, using the fertigation technique, or by applying slow iodine-releasing fertilizers.

2.2 Iodine uptake and translocation in apple trees grown under protected cultivation

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Abstract

Background and Aims: Agronomic biofortification of food crops with iodine may improve the dietary intake of this trace element, which is essential for human development and health. So far, little is known about the suitability of this technique in pome fruits. The objectives of this study were (1) to investigate uptake and translocation of exogenously applied iodine in apple trees, (2) to identify possible strategies of iodine biofortification for this type of fruit and (3) to evaluate interactions between foliar applied iodine and selenium. Methods: Apple trees were cultivated in a plastic tunnel for two growing seasons. Iodine was applied via leaves or substrate. During the 2nd year, simultaneous foliar application of iodine and selenium were tested as well. At harvest time, iodine and selenium content in leaves and fruits were determined. The phytoavailable iodine concentration in the growing medium was analyzed following an extraction with calcium chloride. In addition, the dynamics of iodine applied as potassium iodide and iodate in a peat-based substrate was investigated in an incubation experiment without plants. Results: The iodine concentration in washed apples increased more than 100-fold, valuing around 50 µg (100 g FM)$^{-1}$ by foliar application of iodine as compared to the control treatment. However, this level was only achieved in fruits which were directly wetted by the spray solution. The translocation of leaf-absorbed iodine to fruits was negligible. Following a substrate fertilization, the fruit iodine content remained rather low due to a strong retention of iodine in the growing medium. When using foliar sprays, the addition of selenium did not affect the iodine enrichment of the apple fruits. Conclusions: Foliar fertilization of iodine seems to be a promising method to biofortify apples with iodine. The level of I achieved in apple fruits by means of foliar fertilization can significantly contribute to the daily I intake requirement of humans.

Introduction

Higher plants do not need iodine (I) for their growth and development. Nevertheless, they are able to absorb this element in various forms by roots and aboveground organs. Ion channels and chloride transporters, driven by ATP-dependent proton pumps, allow I to enter the symplast (*White* and *Broadley*, 2009; *Medrano-Macías* et al., 2016). Iodide (I^-) is usually the main form of I that is absorbed by roots. If iodate (IO_3^-) is also present in the growing medium, it probably passes the plasma membrane only after reduction to I^- (*Kato* et al., 2013). This assumption is supported by findings that I in roots and shoot is predominantly present as I^-, even if plants were solely supplied with IO_3^- (*Muramatsu* et al., 1983). Roots can also take up low-molecular organic I compounds. However, these compounds are scarcely translocated to the shoot (*Halka* et al., 2019a). The long-distance transport of I in plants takes place primarily in the xylem, while the mobility of I in the phloem is assumed to be low (*Gonzali* et al., 2017; *Humphrey* et al., 2019). Therefore, I content in leaves is normally considerably higher than in low-transpiring plant parts, such as fruits and seeds, which are mainly supplied by the phloem (*Hong* et al., 2008; *Tsukada* et al., 2008). Nevertheless, I fertilization experiments with cucumbers, eggplants and tomatoes showed a significant accumulation of root-absorbed I in fruits (*Weng* et al., 2008b; *Kiferle* et al., 2013).

Studies with radioactive I isotopes (^{131}I, ^{129}I) demonstrated that leaves can absorb and accumulate exogenously supplied I (*Oestling* et al., 1989; *Humphrey* et al., 2019). Similar to foliar-applied plant nutrients, I might penetrate into leaves either via the stomata or by permeating the cuticle of epidermal cells (*Eichert* and *Fernández*, 2012). The aerial pathway was revealed to be an efficient approach for biofortifying vegetables, especially leafy vegetables (*Smoleń* et al., 2014; *Lawson* et al., 2015). Likewise, in several cereal species the grain I content was significantly enhanced following foliar I sprays during heading and early milk stages, whereas a soil I fertilization did not have any effect (*Cakmak* et al., 2017). This suggests that cereal plants are able to translocate appreciable amounts of leaf-absorbed I to seeds or either that portions of I, wetting directly the head, accumulate into the grains by diffusion.

While the uptake and transport of I in plants has already been extensively investigated in vegetables and cereals, there are only a few reports dealing with berry and tree fruit crops. In strawberries hydroponically cultivated in a nutrient solution with the addition of I^- and IO_3^-, the I content increased in all parts of the plants, but with a noticeable gradation in the order: roots > leaves > stems > fruits (*Li* et al., 2017). The I content in the fruit exceeded 60 µg (100 g FM)$^{-1}$ and thus was sufficiently high to contribute significantly to the I requirement of an adult human of 150–200 µg day^{-1} (*Andersson* et al., 2007; *EFSA*, 2006). In field-grown strawberries, soil fertilization with potassium iodate (KIO_3) in doses of up to 7.5 kg I ha^{-1} resulted in a relatively low I enrichment in fruits. By contrast, a single foliar spray of 0.2 kg I ha^{-1} using potassium iodide (KI) enhanced the I content to almost 50 µg (100 g FM)$^{-1}$ in fruits which were well wetted by the spray mist (*Budke* et al., 2020a). However, the results of this study indicated a relatively low transfer of I from leaf to fruit. In field trials conducted with plums and nectarines, repeated foliar KI sprays increased the fruit I content to a maximum of 9 and 14 µg (100 g FM)$^{-1}$, respectively (*Caffagni* et al., 2012).

With a production of over 83 million tons, the apple ranks third worldwide among the most important types of fruits, after watermelon and banana (*FAO*, 2019). In Germany, apples are cultivated on approximately 34,000 hectares, which represents about 59% of the total fruit-growing area (*Statistisches Bundesamt*, 2018; 2019c). With a per capita consumption of around 20 kg year^{-1}, apples are the most consumed fruits of the country (*BMEL*, 2019). Hence, the apple seems to be an interesting target crop for I biofortification. This approach could support existing measures to enhance the dietary I intake, such as the use of iodized table salt. Almost two billion people worldwide suffer from an insufficient supply of I (*Andersson* et al., 2012). Along with selenium (Se), I is essential for normal functioning of the thyroid gland, which plays a major role in the metabolism, growth and development of humans (*Schomburg* and *Köhrle*, 2008). Both elements are often not adequately supplied by the diet because their uptake in food plants is limited by a low availability in soils. Therefore, a simultaneous biofortification of food crops with these mineral elements is proposed as a strategy to prevent widespread thyroid disorders (*Lyons*, 2018). However, this approach and possible interactions in the uptake of I and Se by plants have so far only been investigated on a few crops (*Smoleń* et al., 2014; *Zou* et al., 2019).

Little is known about the suitability of agronomic I biofortification strategies for pome fruits. In a field experiment, *Szwonek* (2009) investigated the influence of a organo-mineral foliar fertilizer, containing two different I concentration levels (1.5–2.4 and 3.0–4.8 mg L^{-1}), on the fruit development and quality of apples. Two applications at the flowering stage resulted in bigger and more uniform fruit sizes as well as a higher content of soluble dry matter in fruits. However, it is questionable whether these effects were attributed to I, when considering the low I concentration in the foliar fertilizer. Furthermore, no information about the iodine content in leaves and fruits were presented in that investigation.

The main objective of the present study was to investigate the uptake and translocation of exogenously applied I in apple trees in order to identify possible strategies for biofortifying fruits with this trace element. Based on the results of our previous study on strawberries (*Budke* et al., 2020a), we hypothesized that, to achieve the desired I enrichment in the range of 50–100 µg (100 g FM)$^{-1}$, foliar sprays are superior to a substrate fertilization. Furthermore, it was assumed that the efficacy of aerial applications depends on the direct wetting of fruits with the spray solution. In one experimental year a combined foliar application of I and Se was tested as well.

Material and methods

Design of the apple tree experiments

The experiments were initiated in 2015 in the horticultural trial station of Osnabrück University of Applied Sciences, Germany, using three-year old and approximately two-meter tall apple trees of the variety 'Golden Delicious' (M 26 rootstock). The plants were grown in 10 L and 20 L containers during the first and second year of trials, respectively. The pots were filled with a peat-based substrate [40% white peat, 40% black peat, 20% clay, pH ($CaCl_2$) 5.3–5.5]. From May to October, the trees were placed at a spacing of 1 x 1 m in a plastic tunnel to protect them against rain and hail (**Figure 1a**). From November to March the trees were moved to an outdoor area.

Figure 1a: Experimental setup in the plastic tunnel (July 27, 2016, 65 days before harvest). **b:** Application of the iodine solution through the substrate. **c:** Unripened apple fruits in plastic bags during the application of the spray solution. **d:** Example of an apple with an iodine content of about 50 µg 100 g FM^{-1} following a foliar spray in 2016 without fruit damage.

In May, the trees were fertilized with the following products: the water-soluble fertilizer FERTY® 3 green (15+10+15+2 + micro-nutrients) at a rate of 0.75 g (L substrate)$^{-1}$, the slow-release fertilizer Osmocote® Exact Standard (15+9+12+2 + micro-nutrients) at a rate of 4 g (L substrate)$^{-1}$, and the micro-nutrient fertilizer Radigen® at a rate of 0.1 g (L substrate)$^{-1}$. The watering of the plants was done by drip-irrigation according to demand. The following crop protection compounds were used during the cultivation of the plants: Bellis® (128 g kg^{-1} pyraclostrobin + 252 g kg^{-1} boscalid), Dantop® (500 g kg^{-1} clothianidin), Fortress™ 250 (250 g L^{-1} quinoxyfen), Scala® (400 g L^{-1} pyrimethanil), Score® (250 g L^{-1} difenoconazol), Vegas® (51.3 g L^{-1} cyflufenamid) and Vertimec® (18 g L^{-1} abamectin). In June, the number of fruits per tree was thinned out to a maximum of 10 to achieve a uniform fruit load.

Application of iodine

Both substrate and foliar fertilization techniques were evaluated. Substrate fertilization was carried out in both years in the same manner: KI and KIO_3 solutions were applied once [1 x 7.5 mg I (L substrate)$^{-1}$], twice [2 x 7.5 mg I (L substrate)$^{-1}$] or three times [3 x 2.5 mg I (L substrate^{-1})], starting in the calendar weeks (CW) 28 and continuing in

CW 32 and 36, respectively (**Figure 1b**). Saucers were used to avoid leaching of the applied I from the root zone.

For foliar fertilization only KI solutions were used, since preliminary trials revealed that application of this form of I resulted in a higher I content in apples compared to treatments with KIO_3. Two foliar applications of each I treatment were performed, first in CW 28 and second in CW 33. In 2015, three concentrations of I with 0.25, 0.5 and 0.75 g I L^{-1} were tested while in 2016 only 0.75 g I L^{-1} was applied. About 100 mL of solution per tree was applied to the canopy between 50 and 150 cm tree height (hand-held spray system, model Easy-Sprayer Plus, Lehnartz GmbH, Remscheid, Germany). This amount of solution ensured complete wetting of the leaves without runoff. In order to investigate the translocation of I from leaves to fruits, fruits were shielded using plastic bags during the application to prevent direct contact with the spray solution (**Figure 1c**). However, during the second year of trial, an additional treatment without shielding was included for direct application of I solution on the fruits. The nonionic organosilicon spray-adjuvant Break-Thru® S 240 (Alzchem AG, Trostberg, Germany) was added to the I solutions [0.02% (v/v)] to improve the wetting of the leaves. The foliar applications were carried out between 6:00 and 8:00 o'clock in the evening to prolong the wetting time. The control treatment without I was carried out with deionised water (3 µS cm^{-1}) and the above-mentioned adjuvant.

Data collection, sampling and sample preparation

During the cultivation period, the appearance of fruits and leaves was evaluated every 14 days. Leaf damage by I toxicity was rated on a scale of 1 (no damage) to 9 (very severe damage). **Figure 2** shows leaves up to grade 5, the maximum degree of leaf damage observed in this study.

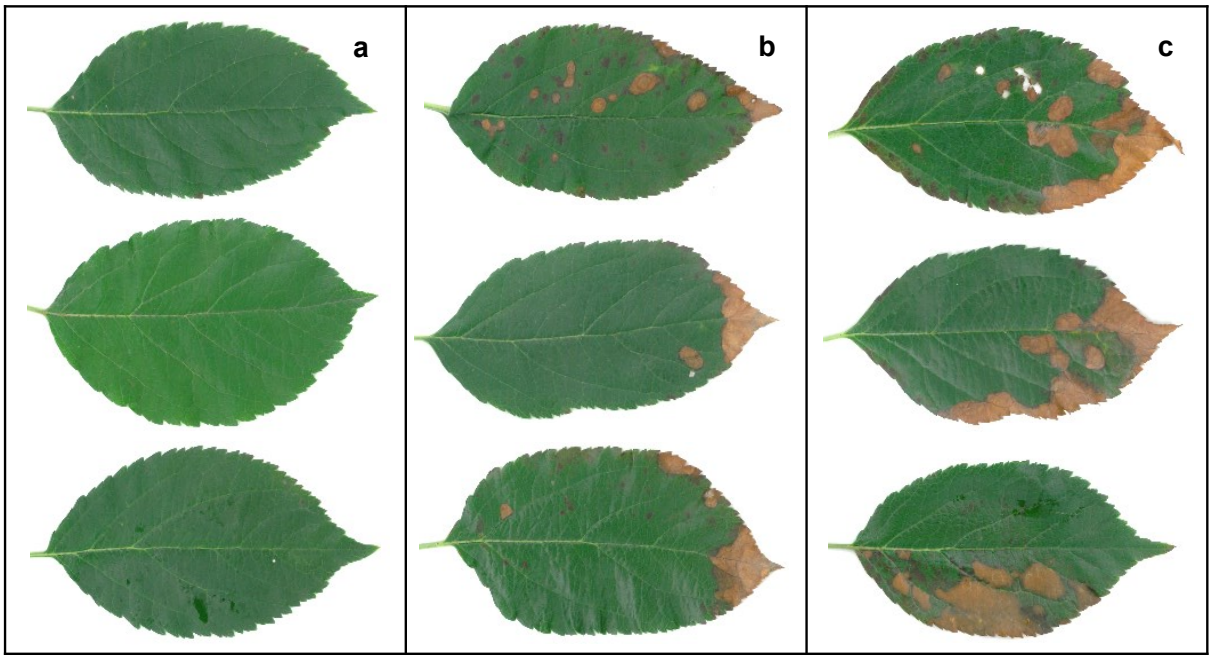

Figure 2: Images of scanned apple leaves of the variety 'Golden Delicious' with varying degrees of damage following exogenous iodine supply. **a:** No damage = score value 1, **b:** Slight damage = score value 3, **c:** Medium damage = score value 5.

The apples were harvested on October 5th in 2015 and on September 30th in 2016. The number of apples and the total weight of fruits per tree were determined and average fruit weight was calculated. One week after harvest, the apples were photographed to document their external appearance. The fruits were analyzed for I contents in order to investigate the uptake and distribution of I in "unwashed", "washed" or "peeled" apples. Deionized water was used to wash the fruits. Each fruit was divided vertically into 8 segments and the core cylinder using an apple divider. Two opposite segments of each apple were used for I analysis. The skin of peeled fruit pieces was discarded. The I content of the fruit skin was estimated by calculating the difference between unwashed and peeled fruit segments. From the core cylinder, only the middle third was used for the analysis. The fresh weight of all fruit parts was recorded in each working step in order to enable a calculation of the total I amount in the different parts of the fruit (peel, flesh, and core).

The I content of leaves collected on the date of fruit harvesting was analyzed both without washing and after washing with deionized water. In 2016, only washed leaves were analyzed but these were differentiated into younger and older ones. In the case of foliar fertilization treatments, the younger leaves were those developed after I application while the older ones had already received such treatment. Leaves as well as fruit pieces were dried at 60 °C until constant weight reaching using a forced air oven (model TUH 75/100, Heraeus Holding GmbH, Hanau, Germany). The dry matter content was calculated as the quotient of sample weight before and after drying. The dried samples were ground in an ultra-centrifugal mill (model ZM 200, RETSCH GmbH, Haan, Germany) at 14,000 rpm to a particle size of ≤ 0.5 mm and stored in plastic cups. The removable components of the mill were washed with deionized water after grinding of each sample.

Iodine determination in plant material

I determination of the plant digests was carried out according to the *DIN EN 15111* (2007) method. Briefly, 1 g of dried plant material was digested with 25% tetramethylammonium hydroxide solution (TMAH) and subsequently analyzed for I using inductively coupled plasma mass spectrometry (ICP-MS, model Agilent 7700x, Agilent Technologies Inc., Santa Clara, CA, United States). Certified reference materials [ERM-BB422 fish muscle and NIST-1849a infant/adult nutritional powder (milk)] as well as fruit powder from own trials which had previously been analyzed by an accredited laboratory (LUFA Nord-West, Hameln, Germany) were used for the analytical quality control.

Iodine determination in substrate and design of the substrate incubation experiment

I in the substrate was extracted with a 0.0125 *M* $CaCl_2$ solution according to a method used previously for soil samples by *Lawson* et al. (2015). The analysis by means of ICP-MS (see methods above) indicated that $CaCl_2$-extractable I concentration in the growing medium of the apple trees rapidly decreased following an I fertilization. Already 26 days after I was applied at the highest fertilization rate of 7.5 mg I (L substrate)$^{-1}$, less than 0.3 mg I (L substrate)$^{-1}$ were detected. Hence, a much tighter sampling scheme was adopted in order to monitor the dynamics of phytoavailable I in the peat-based growing medium. For this purpose, a model experiment was carried out without plants: 32 g of sieved (5 mm) substrate was filled into 500 mL polyethylene bottles, wetted with 8 mL of

deionized water (control treatment), KI or KIO_3 solution containing 2.5 mg I and subsequently incubated in a climatic chamber (model HPP 110, Memmert GmbH + Co. KG, Schwabach, Germany) at constant temperature of 20 °C and 90% relative humidity for a period of 3 weeks. The moisture was kept at a constant level by weighing and adding deionized water accordingly. After an incubation period of 2 h as well as 1, 3, 7 and 21 days, substrate samples were collected for analysis. This experiment was conducted with 4 replicates per treatment.

Combined foliar fertilization of iodine and selenium

A combination of I and Se foliar fertilization was tested in 2016. In this experiment both leaves and fruits were wetted using a spray solution containing 0.75 g I L^{-1} and 25 mg Se L^{-1} in the form of KI and sodium selenite (Na_2SeO_3, Alfa Aesar by Thermo Fisher Scientific - Thermo Fisher GmbH, Kandel, Germany), respectively. The plant parameters described above were also investigated in this treatment. Furthermore, the Se content of the fruit parts and the leaves was determined. For comparison, samples from apple trees not fertilized with Se were analyzed for this element as well. Se determination was carried out according to *DIN EN 13805* (2014). Briefly, 0.5 g of the ground plant material was digested by microwave-pressure digestion in quartz glass vessels with 65% nitric acid at 190 °C under high pressure (model ETHOS plus, MLS GmbH, Leutkirch, Germany). Se concentration was determined using a graphite furnace atomic absorption spectrometer (GF-AAS, Thermo Scientific - SOLAAR M Series AA Spectrometer, Thermo Fisher Scientific Inc., Waltham, MA, United States). Certified reference material and external comparative measurements were used for analytical quality control (refer to section 2.4). In samples with very low Se concentrations (< 2.5 µg L^{-1}), the hydride technique according to *DIN 38405-23* (1994) served as detection method.

Statistical analysis

The fertilization experiments with apple trees were conducted in a randomized block design with three replications. The data obtained from I and Se analysis were subjected to one-way ANOVA and, if needed, logarithmized to meet assumptions of normality and homogeneity of variances. The Tukey HSD test was used for multiple mean comparisons. The statistical analyses were performed with the program IBM SPSS® Statistics, version 25.

Results

Iodine toxicity symptoms on apple trees

Iodine fertilization of apples trees via substrate drenches and foliar sprays did not adversely affect the fruit quality. Even after directly spraying the fruits twice at the highest dose of 0.75 g I L^{-1}, no changes in the appearance occurred, that could impair the marketability of the produce (**Figure 1d**). In contrast, the foliage showed clear necrosis, particularly at the leaf tips and margins, in response to I treatments (**Figure 2**). However, the intensity of leaf damage was in general lower with substrate fertilization than with foliar

fertilization. In the case of substrate fertilization, both I forms (I^- and IO_3^-) led to slight to moderate necrosis of similar magnitude (**Figure 3**). An increase of the fertilization dose from 7.5 to 15 g I (L substrate)$^{-1}$ did not enhance the damage. Splitting 7.5 g I (L substrate)$^{-1}$ into three drenches tended to slightly reduce the degree of leaf necrosis as compared to single application of this dose.

The intensity of leaf damage increased linearly with increasing dose of aerially applied I. At the highest I level, up to one third of the leaf area was necrotic. During both trial years, similar magnitudes of damage were recorded.

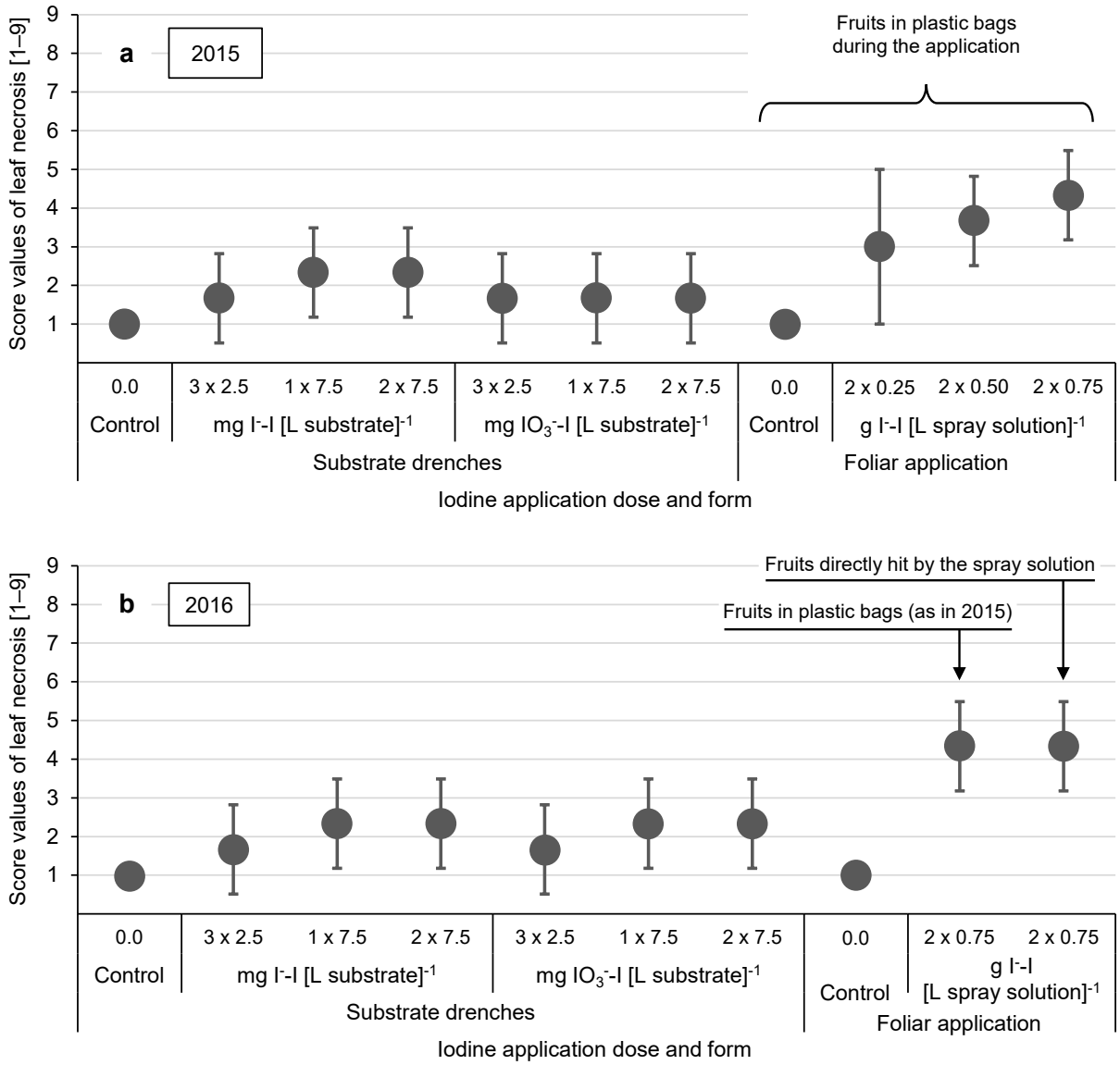

Figure 3: Score values of leaf necrosis of apple leaves (1 = no damage, 9 = severe damage) as affected by the dose, form and method of iodine application during the growing season 2015 (**a**) and 2016 (**b**). Values are means ± standard deviation (*n* = 3).

Development of fruit weight

Leaf damage associated with I fertilization did not affect the growth of apple fruits. The average individual fruit weight was at the same level in any treatment (results not shown) and ranged between 210 g and 250 g in the first and second trial year, respectively. The relatively large fruit sizes reflected both the optimal growth conditions in the plastic tunnel and the relatively low fruit set of the young apple trees. The total number of fruits per tree varied between 5 and 10, irrespective of kind and level of I fertilization. Due to this natural variability and the limited number of trees in the trial variants (n = 3), it was not possible to obtain any statistically valid data on the effects of the treatments on the total fruit yield.

Iodine content of fruits

The mean I content of apples in the control group (**Figure 4**) was 0.4 µg I (100 g FM)$^{-1}$. Substrate fertilization with KIO_3 did not significantly increase fruit I content, while application of KI enhanced I content significantly. At the highest KI fertilization rate, I content in fruits in the growing season 2015 was 3.2 µg I (100 g FM)$^{-1}$. In 2016, I enrichment was only about half as high. Doubling the dose of I in substrate drenches, either as KI or KIO_3, did not enhance I content of apples in any of the experimental seasons.

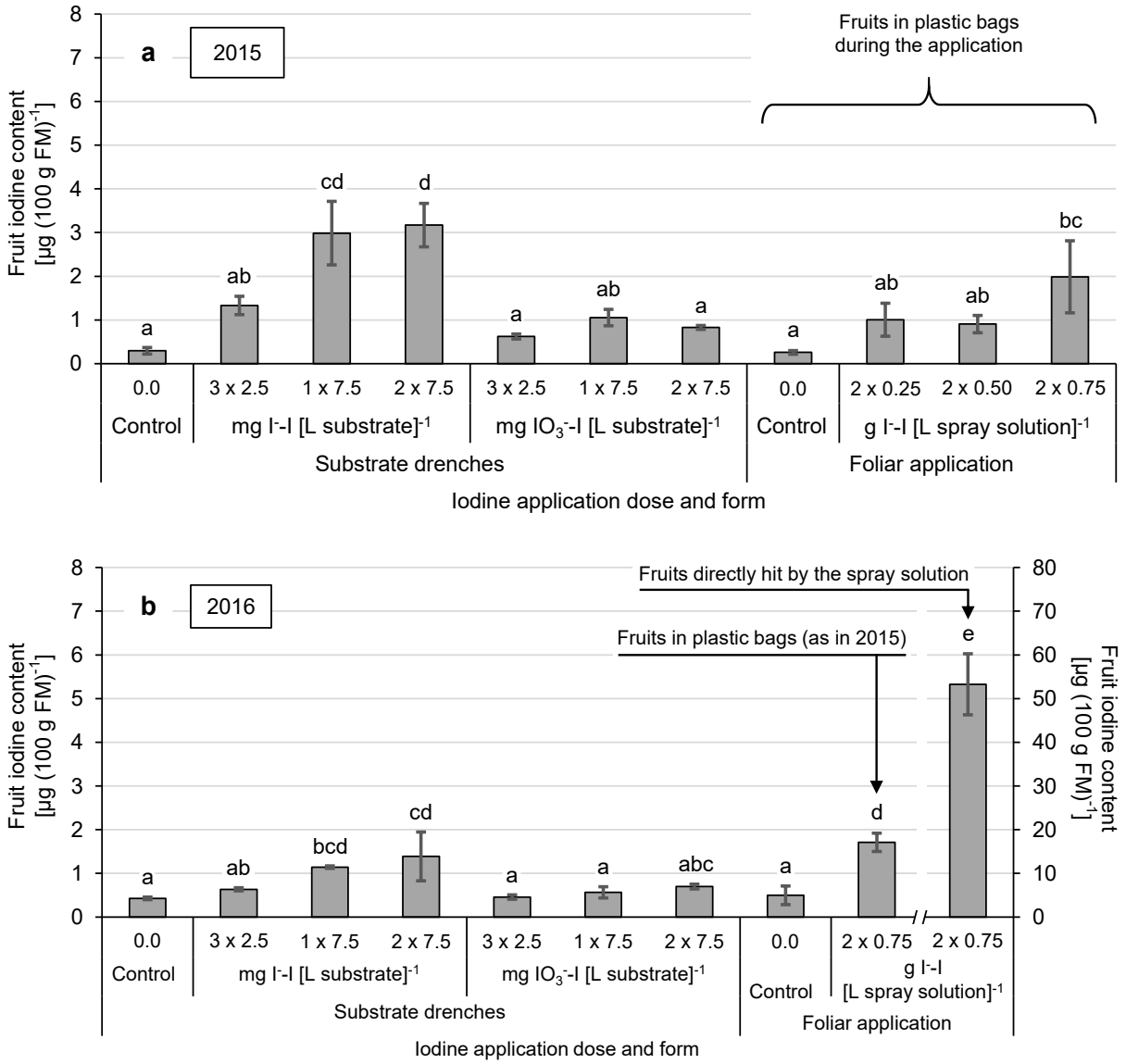

Figure 4: Iodine content in washed apples as affected by the dose, form and method of iodine application during the growing season 2015 (**a**) and 2016 (**b**). Values are means ± standard deviation (*n* = 3). Means with same letters do not differ according to Tukey-HSD test at α = 0.05.

Fruits shielded by plastic bags during foliar I application also remained quite low in I content. However, when the spray solution was applied to both leaves and fruits (**Figure 4b**), a significantly higher I level was achieved, up to 53 µg I (100 g FM)$^{-1}$ at the highest I dose.

The I distribution within the apple fruits is shown in **Figure 5**. When the fruits were wetted by foliar I sprays, about half of the total fruit I amount was found in the peel. The rest was mainly located in the fruit flesh and only a small proportion in the fruit core. Using foliar applications excluding fruits or following substrate drenches, most of the I entered the fruit flesh. The highest I amount in the fruit core was observed following a substrate fertilization with I^-.

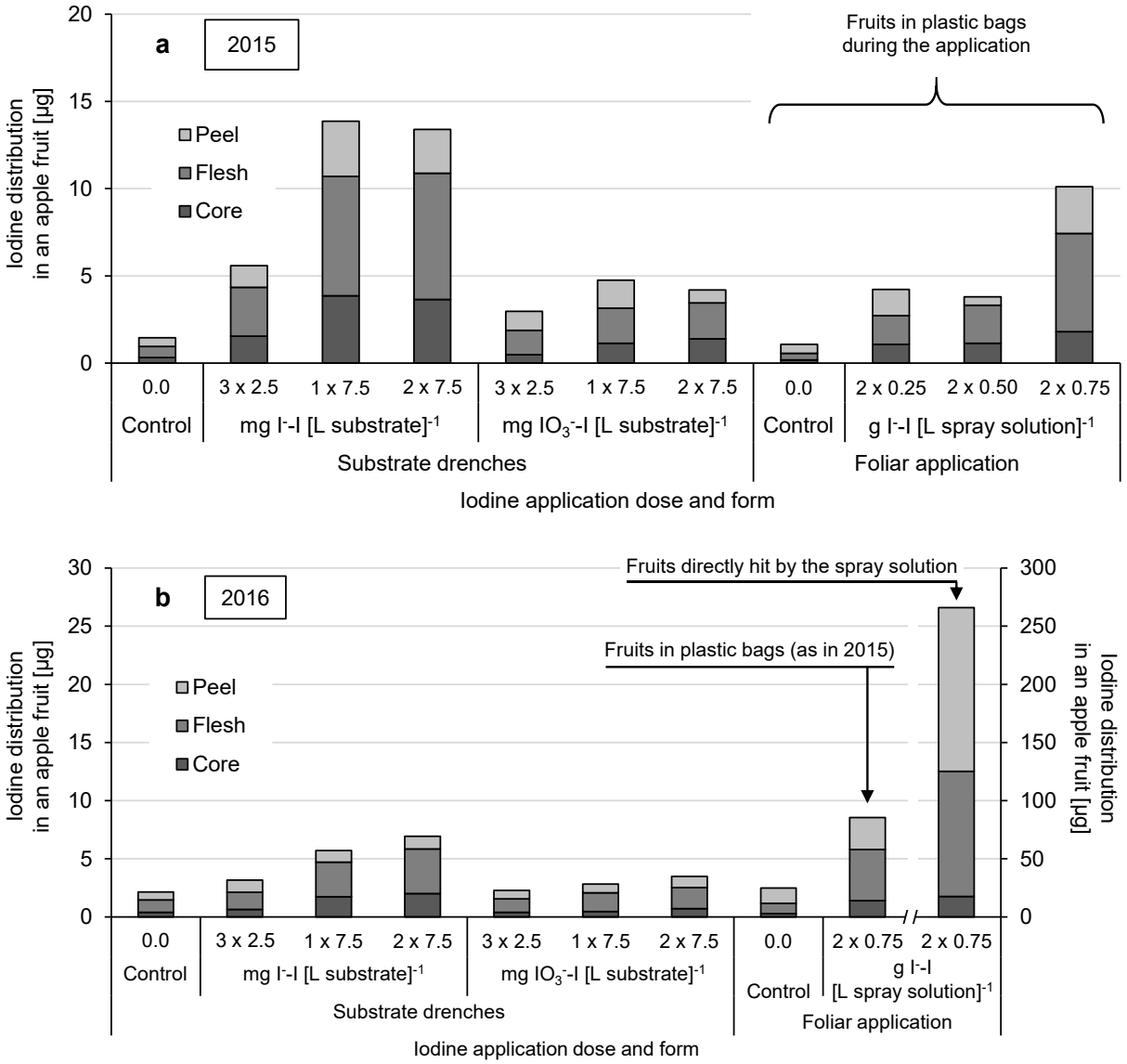

Figure 5: Iodine distribution in washed apple fruits as affected by the dose, form and method of iodine application during the growing season 2015 (**a**) and 2016 (**b**). Values are means (n = 3).

Washing reduced the I content in directly sprayed apples by 8% on average (**Figure 6a**). A decline of about 50% was observed when the fruits were peeled (**Figure 6b**). In general, I losses due to washing and peeling increased with increasing fruit I content.

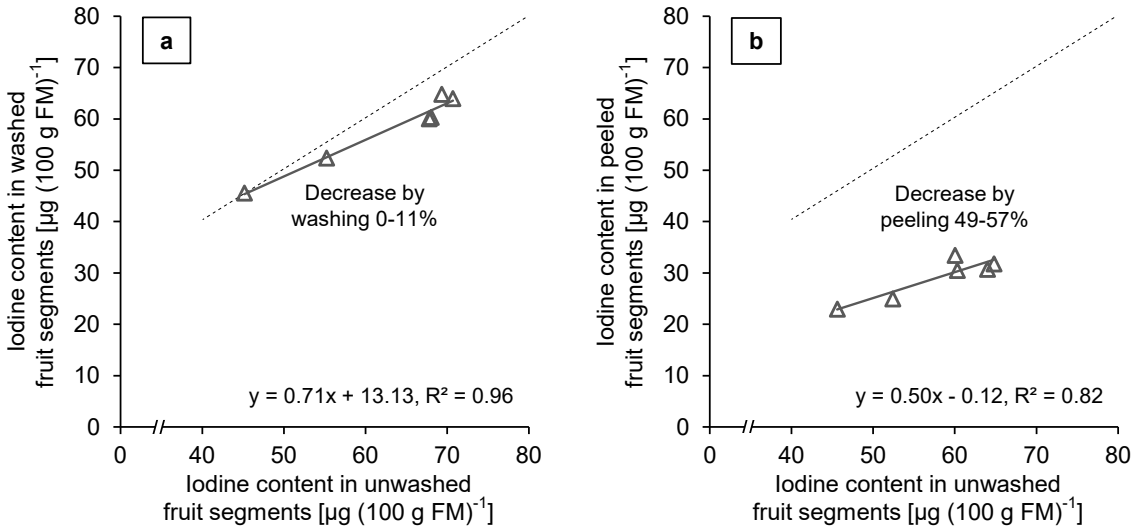

Figure 6: Correlation between the iodine content in unwashed and washed apples (**a**) as well as unwashed and peeled apples (**b**) sprayed with 2 x 0.75 g I^--I L^{-1} in the year 2016 (*n* = 6. The dotted line indicates the angle bisector.

Iodine content of leaves

The accumulation of I in leaves was much higher than in fruits. As a result of substrate and foliar I treatments, I content increased up to 982 µg and 3,748 µg per 100 g FM, respectively. Only minor differences between unwashed and washed leaves were detected. On average, washing reduced the leaf I content by 3.5% and 4.0% when apple trees received a substrate drench or a foliar spray, respectively (**Figures 7a, b**). Like fruits, the washing losses tended to increase with increasing I content in the leaves.

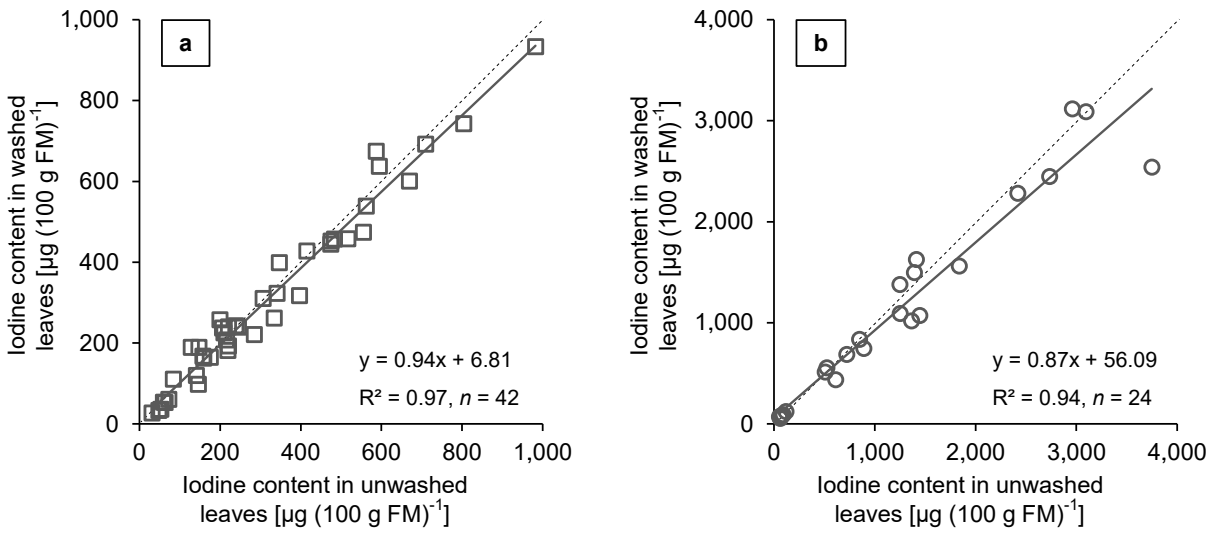

Figure 7: Correlation between the iodine content in unwashed and washed leaves in the year 2015. **a:** substrate drenches, **b:** foliar application. The dotted line indicates the angle bisector.

A comparison between washed old and young leaves revealed a consistently higher I content in older leaves (**Figure 8**). The highest I level was detected in older leaves of foliar-sprayed apples trees. Conversely, young leaves of these trees did not differ from the control group in their I content. As already observed in fruits, substrate drenches with KI resulted in a higher I accumulation in the leaf tissue than those with KIO_3.

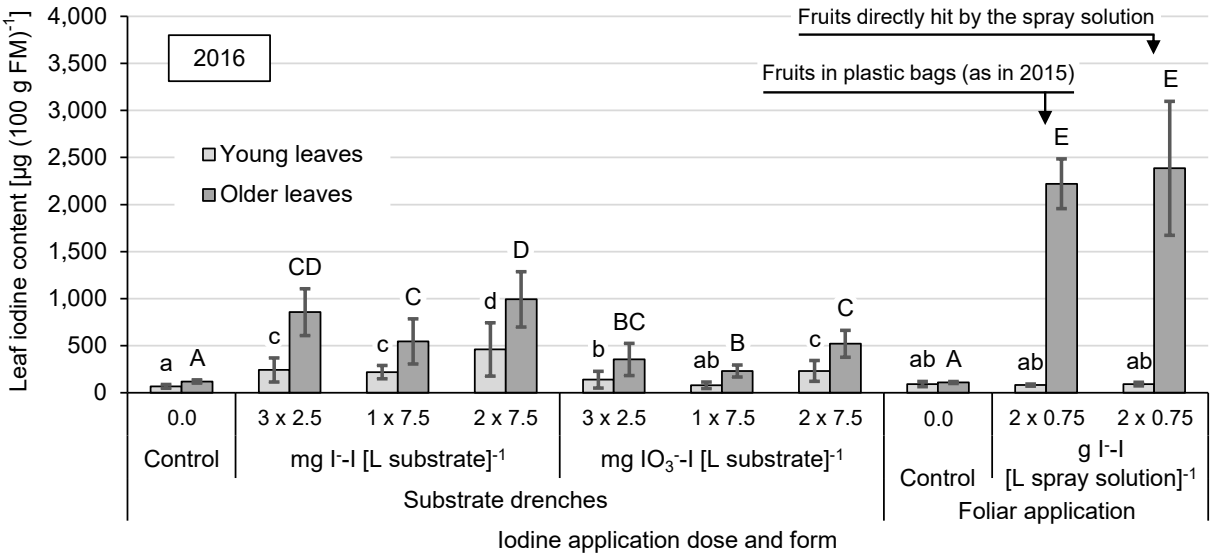

Figure 8: Iodine content in old and young washed leaves as affected by the dose, form and method of I application during the year 2016. Younger leaves emerged after foliar sprays had been carried out. Values are means ± standard deviation (*n* = 3). Means of the same age group with same letters do not differ significantly according to Tukey-HSD test at α = 0.05.

Iodine concentration in the substrate

To characterize the supply of phytoavailable I in the root zone of apple trees following a substrate fertilization with KI and KIO_3, the $CaCl_2$-extractable I concentration in the growing medium was determined. On the first sampling date, 26 days after the first I application, the I concentration was already below 0.3 mg (L substrate)$^{-1}$ and thus close to the control treatment (results not shown). Obviously, the applied I was rapidly converted into a form that is not extractable with $CaCl_2$. A model experiment conducted without plants confirmed strong retention of I by the peat substrate. After just a few hours it was possible to recover only approximately three quarters of the applied IO_3^- with the $CaCl_2$ extraction method. For I^-, the decrease in extractable I was somewhat slower than IO_3^-. Nevertheless, after three days from I^- application, most of the supplied I was no longer detectable in the $CaCl_2$ extracts (**Figure 9**).

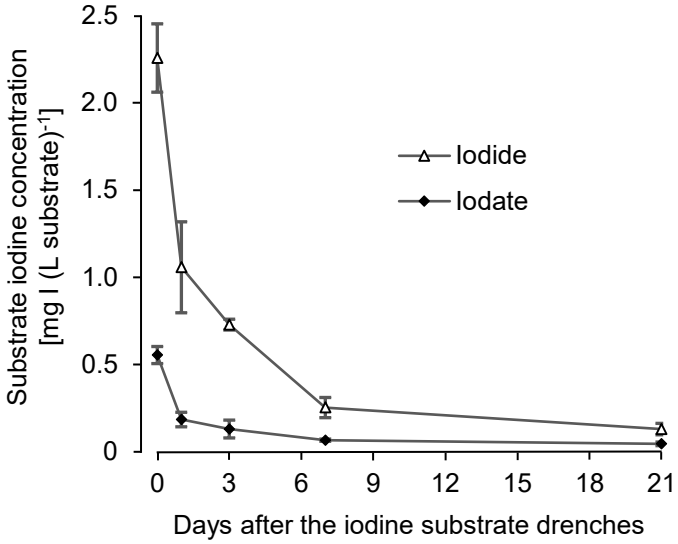

Figure 9: $CaCl_2$-extractable iodine concentration in peat substrate at different times after incubation with KI or KIO_3 at the rate of 2.5 mg I (L substrate)$^{-1}$**.** Values are means ± standard deviation (*n* = 4).

Combined iodine and selenium foliar sprays

Combined I and Se foliar sprays to wet both leaves and fruits had no harmful impacts on the appearance of apple fruits. However, similar leaf damage was observed to that observed with sole I application (**Table 1**). The average single fruit weight did not differ significantly from the control group. While I content of leaves and fruits with combined foliar fertilization of I and Se did not differ from that achieved with straight I foliar fertilization. However, Se supply increased Se content in fruits from 0.2 to 2.9 µg Se (100 g FM)$^{-1}$. Washing and peeling of fruits decreased the Se content by about 10% and 35%, respectively. As reported for I, older leaves accumulated most of foliar applied Se, while young leaves, i.e. those which emerged after foliar fertilization of Se, had the same Se content as that obtained for control treatment (**Table 1**).

Table 1: Apple leaf and fruit parameters as affected by combined iodine and selenium foliar applications in the growing season 2016.

		Treatment	Control		2 x 0.75 g I^--I [L spray solution]$^{-1}$		2 x 0.75 g I^--I and 2 x 25 mg SeO_3^{2-}-Se [L spray solution]$^{-1}$	
Score values of leaf necrosis [1–9]			1.0 ± 0.0		4.3 ± 1.2		4.3 ± 1.2	
Average single fruit weight [g]			259.4 ± 18.5	a	236.5 ± 19.2	a	240.7 ± 26.8	a
Selenium content [µg (100 g FM)$^{-1}$]	**fruits**	**unwashed**	0.2 ± 0.0	a	0.3 ± 0.1	a	2.9 ± 0.2	b
		washed	0.2 ± 0.0	a	0.2 ± 0.0	a	2.6 ± 0.2	b
		peeled	0.2 ± 0.0	a	0.2 ± 0.1	a	1.9 ± 0.3	b
	leaves	**younger leaves**	1.6 ± 0.3	a	1.4 ± 0.1	a	1.2 ± 0.2	a
		older leaves	1.4 ± 0.1	a	1.8 ± 0.1	a	213.4 ± 88.5	b
Iodine content [µg (100 g FM)$^{-1}$]	**fruits**	**unwashed**	0.4 ± 0.1	a	62.7 ± 10.8	b	55.8 ± 5.9	b
		washed	0.5 ± 0.2	a	57.9 ± 7.7	b	51.4 ± 6.4	b
		peeled	0.2 ± 0.1	a	29.1 ± 4.5	b	28.6 ± 1.9	b
	leaves	**younger leaves**	92.3 ± 27.9	a	92.2 ± 19.2	a	74.3 ± 3.4	a
		older leaves	109.0 ± 10.5	a	2.384.9 ± 712.0	b	2.574.4 ± 640.1	b

Discussion

Phytotoxic effects of iodine

Iodine fertilization caused necrosis on the leaves of the apple trees. The damage was more pronounced following foliar sprays compared to substrate drenches. While using the latter method, IO_3^- was slightly better tolerated by plants than I^- (**Figure 3**). The intensity of leaf necrosis was related to I content of the leaves (**Figure 8**). Similar phytotoxic symptoms have been reported on the leaves of I-fertilized tomato plants (*Kiferle* et al., 2013). A reduced activity of the superoxide dismutase is assumed to be involved in I phytotoxicity. This enzyme plays a key role in the defense against reactive oxygen species and thus in the prevention of cell damage (*Blasco* et al., 2011).

In contrast to leaves, apple fruits were not adversely affected by any of the I treatments investigated in this study. This is a basic prerequisite for using this approach in commercial fruit cultivation. Thus, doses of I fertilizers applied in this study seem suitable for the biofortification of apples. However, further field experiments in apple orchards are still required to examine whether the observed leaf damage has a detrimental impact on fruit yield and quality-relevant fruit parameters such as sugar content and fruit flesh firmness. It was not possible to investigate such effects in this study due to the small number of trees and few fruits per tree. In a previous field trial conducted with strawberries,

leaf necrosis appeared after repeated foliar I sprays and finally resulted in a slight reduction of the soluble dry matter of the strawberry fruits (*Budke* et al., 2020a). Corresponding undesired side effects must be taken into account, particularly if the photosynthetically active leaf area is strongly reduced and, as a consequence, the assimilate export into still growing fruits is impaired. If I spraying in apple cultivation takes place relatively late in the season, for example two weeks before harvest, leaf damage is unlikely to have a negative effect on the fruit sugar content. Whether such a fertilization strategy leads to a sufficient I enrichment in apple fruits has to be clarified in subsequent field experiments.

Iodine uptake and translocation in apple trees

In this study the effect of exogenously applied I on apple trees was investigated by means of pot experiments in a plastic tunnel. The chosen set-up allowed tracking of the uptake and translocation of I in plants under rain-protected conditions. Thus, unintended washing off and displacement of applied I from leaves to untreated fruits was prevented. Furthermore, leaching of I out of the root zone was avoided. Under the given conditions of cultivation, I content in leaves and fruits of the control treatments ranged between 49–120 µg $(100\ g\ FM)^{-1}$ and 0.3–0.5 µg $(100\ g\ FM)^{-1}$, respectively. Similar levels of I were detected in apple trees grown under open field conditions in Osnabrück, Germany (*Budke*, unpublished results). *Souci* et al. (2016) and *Mello* et al. (2013) reported a native I content ranging between 0.8 to 1.8 µg $(100\ g\ FM)^{-1}$ of apple fruits.

By applying I via substrate drenches, I content in leaves of apple trees increased up to 15-fold. In comparison, I enrichment in the fruits was significantly lower with a maximum value of 3.2 µg $(100\ g\ FM)^{-1}$. In other plant species such as tomatoes, plums and strawberries similar differences in the I level between leaves and fruits have been reported (*Landini* et al., 2011; *Caffagni* et al., 2012; *Li* et al., 2017). The results indicate that root-absorbed I follows mainly the transpiration stream, and thus leaves accumulate more I than fruits. Even after foliar sprays, fruit I content remained relatively low if only the leaves were treated (**Figure 4**). In doing so, the increase observed was six fold on average. When both leaves and fruits were sprayed, I content in washed fruits increased more than 100 times the value found for control treatment. These differences between the treatments demonstrate that only a very small proportion of the foliar-applied I was translocated from leaves to fruits. Similarly, in field trials conducted with strawberries foliar I fertilization only led to a significant enrichment of I in fruits which were well exposed to the spray mist (*Budke* et al., 2020a).

The above results indicate a limited phloem mobility of I in plants. *Humphrey* et al. (2019) found, in a study on spinach plants, that less than 2% of the radioiodine (^{129}I) applied to a single leaf was translocated via the phloem into younger leaves. In accordance with this, I content of young apple leaves remained low even if the older leaves had absorbed higher amounts of I (**Figure 8**). In contrast to these results, investigations on tomatoes and cereal plants showed that leaf-applied iodine is transferred to the fruits and seeds in quantities adequate for iodine biofortification (*Landini* et al., 2011; *Cakmak* et al., 2017; *Zou* et al., 2019). These contradictions may be attributed to genotypic differences in the remobilization and phloem mobility of I.

By treating an apple tree twice with a total amount of 0.15 g I, the I content in directly sprayed fruits was increased to about 50 µg (100 g FM)$^{-1}$. Taking into account the canopy application area (0.5–1.5 m tree height) and the plant spacing (1 x 1 m) chosen in this experiment, 0.15 g I tree^{-1} corresponds to a fertilization rate of 1.5 kg I ha^{-1} per meter of canopy height. Assuming common tree heights in orchards to be 2–2.5 m, overall 3.0–3.75 kg I ha^{-1} would be needed. This suggests that the dose-response relationship of foliar I sprays in apples is less pronounced than previously found in other fruit species. For example, in the case of field grown plums and nectarines, aerial applications with about 0.3 kg I ha^{-1} enhanced I content to 9 and 14 µg I (100 g FM)$^{-1}$, respectively (*Caffagni* et al., 2012). In strawberries, a single spray with 0.2 kg I ha^{-1} was sufficient to reach a comparable I level to the one detected in apples (*Budke* et al., 2020a). Several aspects might contribute to these crop-specific differences. In general, it can be assumed that I fertilizer demand increases with increasing plant and fruit size. The surface area to volume ratio declines with increasing fruit size. Thus, at the same uptake rate of solutes per cm^2 of peel, the increase in concentration is lower in large fruits. Furthermore, the different surface topology as well as thickness and composition of the cuticle of fruit peels must be taken into account. The uneven texture of strawberries with numerous, slightly recessed achenes is more likely to promote the absorption of I than the waxy, smooth surfaces present in the above-mentioned tree fruit types. Apple fruits have a relatively thick and waxy cuticle. For example, the cuticle of 'Golden Delicious' apples contains significantly more cuticular waxes (approx. 900 µg cm^{-2}) than that of nectarines and plums (200–300 µg cm^{-2}) as well as strawberries (approx. 20 µg cm^{-2}) (*Belding* et al., 1998; *Riccio* et al., 2006; *Knoche*, 2015; *Huang*, 2017). The hydrophobic coatings hamper the penetration of ions into fruits after a spray treatment.

Although foliar sprays were much more efficient compared to substrate drenches, a relatively small proportion of the aerially fertilized I reached the fruits. If applied to a common apple orchard with a fruit yield of 40 t ha^{-1} and fruit iodine content of 50 µg (100 g FM)$^{-1}$, it implies that not more than 0.6% of the sprayed I is transferred to fruits. However, in practice this proportion could be even lower for the following two reasons. Firstly, fruits on larger apple trees are partly covered by the foliage and thus less exposed to the spray. Secondly, a part of the I applied to fruits can be washed off by rain. To what extent such factors affect the I enrichment in apples needs to be clarified in further experiments under field conditions. When cultivating strawberries in the open field, up to 1.1% of the sprayed I was found in the fruits (*Budke* et al., 2020a). A considerably higher effectiveness of foliar I treatments was observed in leafy vegetables. In lettuce, for example, up to one third of the applied I amount entered the harvested produce, which comprised most of the aboveground plant part (*Lawson* et al., 2015).

When I was applied to the growing medium or to the leaves of apple trees, it was located mainly in the fruit flesh. If both leaves and fruits have been sprayed, about half of the I was detected in the apple peel and the remaining amount was translocated to the flesh with only a small amount reaching the fruit core (**Figure 5**). The distribution of I on the cellular level of apple fruits was not investigated in our study. Studies on I-biofortified vegetables have shown that root-absorbed I was mainly accumulated in the cytoplasm, both in roots themselves and in shoots (*Weng* et al., 2014). In the case of aerial applications, I can be strongly adsorbed on cuticular waxes (*Shaw* et al., 2007).

Accordingly, washing of I-sprayed apples under flowing water reduced fruit I content by only about 8%. Peeling of apple fruits resulted in significantly higher I losses (**Figure 6**). The consumption of I-biofortified apples with peel is therefore recommended, also to intake other fruit ingredients which are valuable for human nutrition, especially polyphenols such as flavonoids (*Drogoudi* et al., 2008). The I content of apple leaves was hardly affected by washing with water, even when I was foliar-applied (**Figure 7**). This indicates, besides the relatively high accumulation of I in the foliage, that I penetrates leaves more easily than fruits.

Iodine dynamics in the substrate

In the peat substrate used for cultivating apple trees, the concentration of $CaCl_2$-extractable I rapidly decreased after the I fertilization event. A model experiment conducted with the same substrate but without plants revealed that most of the supplied I was fixed within one day and that this retention was faster for IO_3^- than I^- (**Figure 9**). This explains the higher I uptake from I^--treated substrate than from the corresponding dose of IO_3^-. Due to the very fast fixation of I in the growing medium, even a splitting of the I fertilization into 2 or 3 applications could not enhance leaves or fruit I content compared to a single application (**Figures 4 and 8**). A continuous supply of I by fertigation can improve the availability and thus the uptake of I by plants, as observed in an investigation with spinach (*Smoleń* et al., 2016b). Nevertheless, the relatively low translocation rate of root-absorbed I to fruits (*Hong* et al., 2008, *Tsukada* et al., 2008) might further limit the efficacy of the substrate fertilization technique.

A strong retention of I was also detected in soils rich in organic carbon. Thus within a few hours after application, $^{129}I^-$ and $^{129}IO_3^-$ were converted to a great extent into water-insoluble forms (*Bowley*, 2013). Subsequently, the I fixation rate slowed down and after about three weeks almost the entire amount of added I was depleted, similar to the findings for the peat substrate investigated in this study. The fixation of I in organic soils was attributed to an adsorption on humic substances. IO_3^- interacts more rapidly with these soil constituents than I^- (*Bowley*, 2013). In mineral soils, iron and manganese oxides are important I sorbents, especially for IO_3^-. However, in such soils I^- is usually fixed more quickly by processes not fully elucidated yet (*Shetaya* et al., 2012). Hence, when using the soil fertilization approach, IO_3^- is considered more appropriate for I biofortification of field-grown crops (*Dai* et al., 2006; *Lawson* et al., 2015).

Once fixed in soils, I does not have any residual fertilization effect in subsequent cropping seasons (*Lawson* et al., 2015). Likewise, in the peat substrate, a stable I sorption occurred as indicated by I enrichment in apple trees during the investigation period. The fruit I content determined in 2016 was lower than in 2015 despite fact that the same amounts of I fertilizer were applied by substrate drenches in both years (**Figure 4**). This decrease was partly due to the higher individual fruit weight in 2016, resulting in a dilution of the absorbed I. In 2017, when the apple trees continued to be cultivated without renewed I fertilization, plant analyses on a random basis did not indicate any differences in the fruit I content between previously I-treated trees and untreated ones. The same was observed for the leaves, with the exception of plants which had received a substrate drench with I^- in the two preceding years. In this case I content was about twice as high as in the control

group (results not shown). Thus, the results also indicate that sprouting apple trees retranslocate only small amounts of I from the wood to the leaves and that a retranslocation to the fruit is completely absent. This supports the assumption that I is hardly mobile in the phloem of apple trees.

Combined iodine and selenium foliar fertilization

In one growing season, it was also examined whether apple fruits can be simultaneously enriched with I and Se by a combined foliar fertilization. Iodine application combined with selenite (SeO_3^{2-}) did not affect I content in fruits as compared to I application alone, but led to a significant increase in the Se content (**Table 1**). This indicates that I^- does not compete with SeO_3^{2-} in its uptake into the fruit. Furthermore, none of the other plant parameters such as I content in leaves, degree of leaf necrosis, and average individual fruit weight were affected by Se. Hence, a simultaneous biofortification of apples with I and Se by foliar fertilization seems, in principle, to be possible. This approach also proved to be suitable for other food crops such as lettuce and wheat (*Smoleń* et al., 2019; *Zou* et al., 2019).

Washing and peeling of apples reduced the Se content by about 10% and 35%, respectively. Therefore, it can be assumed that a large part of the absorbed Se entered the fruit flesh. Se thus seems to penetrate the fruit peel somewhat easier than I. However, in general, the uptake and translocation pattern of both trace elements was quite similar. As already reported for I, Se was not translocated from older to younger leaves. The transfer of Se from leaves to fruits was not separately investigated in this study. In various plants, Se is proved to be phloem-mobile. The long-distance transport is less associated with SeO_3^{2-} but mainly relies on selenate (SeO_4^{2-}) and Se-containing amino acids such as selenomethionine (*Poggi* et al., 2000; *Boldrin* et al., 2013; *White*, 2016). Accordingly, the Se content in protein-rich crops such as peas and cereals can be significantly increased by Se fertilization (*Poblaciones* et al., 2013; *Galinha* et al., 2015; *Lima Lessa* et al., 2019). In view of the relatively low Se enrichment in apples following a foliar Se application, we assume that the translocation of Se is quite restricted in this type of fruit which contains less protein but is rich in sugar. However, this hypothesis has still to be examined in further investigations.

Conclusion

Foliar fertilization of apple trees proved to be more suitable for increasing the I content in fruits than substrate fertilization. Rapid fixation of applied I in the growing medium as well as low phloem mobility of I in plants are limiting factors for I accumulation in fruits via the root pathway. By direct spraying of I on apple fruits, enrichment of up to 50 µg I $(100\ g\ FM)^{-1}$ has been achieved. The consumption of such an apple of average size with peel but without core result in an intake of about 75 µg I. This would permit coverage of a little more than a third of the daily intake requirement of 200 µg I, as recommended for adults by the European Food Safety Authority (*EFSA*, 2006). On the other hand, I intake following consumption of such I-biofortified apple fruits is not expected to exceed the

tolerable upper intake level of 600 µg I per day (*EFSA*, 2006), even if apples are consumed frequently.

In the experiments presented, favorable conditions for the I uptake via the fruit surface existed. Due to the small tree size, all apples were well covered by the foliar sprays. At the same time, wash-off effects were prevented by cultivating the plants in a plastic tunnel. In contrast, in fully developed apple plantations fruits located inside the canopy are partially covered by foliage. Furthermore, under field conditions, precipitation may remove I from the fruit before it is absorbed. To investigate such effects further field experiments in an apple orchard are necessary. With regard to combined I and Se sprays, higher doses of Se should be tested because the enrichment level of this trace element was still relatively low at 2,6 µg $(100\ g\ FM)^{-1}$, which covers about 4% of the adequate daily Se intake for adults (*EFSA*, 2014b). In this respect, there is probably still room for improvement, since Se did not have any additional adverse effects on the leaves and fruits of apple trees by the dose tested.

2.3 Iodine biofortification of apples and pears in an orchard using foliar sprays of different composition

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pome fruit, agronomic biofortification, foliar fertilization, iodide, iodate, selenium, potassium nitrate, total soluble solids

Author contributions:
Christoph Budke and **Diemo Daum** conceived and designed the field experiments; **Christoph Budke** conducted the field experiments and analytical investigations; **Diemo Daum** and **Karl Hermann Mühling** supervised the analytical investigations; **Diemo Daum, Katja Hora, Karl Hermann Mühling** and **Werner Dierend** provided resources to conduct the field experiments and analytical investigations; **Christoph Budke** analyzed the data. **Hans-Georg Schön** supervised the statistical data analysis; **Christoph Budke** wrote the manuscript. All authors contributed to manuscript revision, reading, and approved the submitted version.

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Abstract

Many people across the world suffer from iodine (I) deficiency and related diseases. The I content in plant-based foods is particularly low, but can be enhanced by agronomic biofortification. Therefore, in this study two field experiments were conducted under orchard conditions to assess the potential of I biofortification of apples and pears by foliar fertilization. The fruit trees were sprayed at various times during the growing season with solutions containing I in different concentrations and forms. In addition, tests were carried out to establish whether the effect of I sprays can be improved by co-application of potassium nitrate (KNO_3) and sodium selenate (Na_2SeO_4). Iodine accumulation in apple and pear fruits was dose-dependent, with a stronger response to potassium iodide (KI) than potassium iodate (KIO_3). In freshly harvested apple and pear fruits, 51% and 75% of the biofortified I was localized in the fruit peel, respectively. The remaining I was translocated into the fruit flesh, with a maximum of 3% reaching the core. Washing apples and pears with running deionized water reduced their I content by 14%. To achieve the targeted accumulation level of 50–100 µg I per 100 g fresh mass in washed and unpeeled fruits, foliar fertilization of 1.5 kg I per hectare and meter canopy height was required when KIO_3 was applied. The addition of KNO_3 and Na_2SeO_4 to I-containing spray solutions did not affect the I content in fruits. However, the application of KNO_3 increased the total soluble solids content of the fruits by up to 1.0 °Brix compared to the control, and Na_2SeO_4 in the spray solution increased the fruit selenium (Se) content. Iodine sprays caused leaf necrosis, but without affecting the development and marketing quality of the fruits. Even after three months of cold storage, no adverse effects of I fertilization on general fruit characteristics were observed, however, I content of apples decreased by 20%.

Introduction

Iodine is an integral component of thyroid hormones, which control various metabolic processes in the human body. Globally, around two billion people are insufficiently supplied with this essential trace element (*Andersson* et al., 2012). The associated health disorders range from mild, unspecific symptoms such as listlessness to severe neurological developmental disorders. Iodine deficiency is considered to be the most common single cause of preventable brain damage and intellectual disability in children worldwide (*Benoist* et al., 2009; *Redman* et al., 2016). Even a mild to moderate I deficiency during pregnancy and in the first years of life can lead to children not being able to fully exploit their cognitive development potential (*Velasco* et al., 2018; Bath 2019). The problem of I deficiency exists in both developing and industrialized countries. In Europe, about 44% of the population is inadequately supplied with I, despite its wealth and its high standards of health care (*Zimmermann*, 2017). The widespread occurrence of I deficiency is due to the fact that the native I content in food is usually very low. Food crops such as fruits, vegetables and cereals usually contain no more than about 1.0 µg of I per 100 g of fresh mass, since soils are low in phytoavailable I, and therefore the absorption of this trace element by plants is quite limited (*Fuge*, 2013; *Milagres* et al., 2020).

An option for increasing the I content of food crops is therefore to fertilize the soil with I-containing salts. Various studies show that this measure actually has an effect, but

requires relatively high amounts of I fertilizer (*Ren* et al., 2008; *Weng* et al., 2014). This is due to the relatively rapid fixation of I in the soil when applied as iodide (I^-) or iodate (IO_3^-). In addition, these inorganic I forms can be converted by soil microorganisms into gaseous compounds such as methyl iodide, which are emitted into the atmosphere (*Ashworth*, 2009; *Shetaya* et al., 2012; *Fuge*, 2013). While leafy and root vegetables respond relatively well to I soil fertilization, only little I reaches the edible plant parts of fruit vegetables and cereals using this method (*Hong* et al., 2008; *Cakmak* et al., 2017). Compared to soil fertilization, foliar applications proved to be much more efficient. For example, it was possible to biofortify lettuce adequately with I if the plants were sprayed with 0.5 kg I ha^{-1} one week before harvest. With soil drenches, a 15-fold higher I fertilizer quantity was required for the same I enrichment in this leafy vegetable (*Lawson* et al., 2015). Also in experiments carried out with strawberries and cereals, foliar sprays proved to be superior to soil fertilization in order to increase the I content in the fruits and grains, respectively (*Cakmak* et al., 2017; *Budke* et al., 2020a).

In this study apples and pears were selected as target crops for I biofortification via foliar sprays. These fruits have several characteristics that make them particularly suitable for improving the dietary I intake in I deficiency areas. First of all, apple and pear are among the ten most important fruit species in the world with a production of 86 million tons and 24 million tons, respectively (*FAO*, 2020). Fruits can be stored for a long time - pears for a few months, while apples from domestic production can be offered in food stores throughout the year. Pome fruits are usually eaten with the peel, while other fruits that are regularly consumed in larger quantities, such as bananas and citrus fruits, are peeled. This is important because a previous study on apples showed that more than half of the foliar-sprayed I is localized in the fruit peel. Nevertheless, I in the peel is hardly affected by washing of the fruit under running water - this reduced the fruit I content by only 8% (*Budke* et al., 2020b). Thus, when fresh pome fruits are consumed, most of the biofortified I usually becomes nutritionally effective. In contrast, processed foods, such as potatoes, vegetables, and cereals, may experience significant I losses through cooking, peeling, or extraction compared to harvested produce. Even then, however, enough I remains in the biofortified plant-based foods to substantially increase dietary supply of this micronutrient (*Caffagni* et al., 2012; *Gonnella* et al., 2019; *Cakmak* et al., 2020). Loss of I from iodized table salt during food preparation can be much higher. When cooking vegetables or potatoes with iodized table salt, only very little amounts of the I dissolved in the cooking water enters the prepared food (*Comandini* et al., 2013; *Weng* et al., 2014).

The inorganic I^- and IO_3^- forms, which are mainly used for the biofortification of food plants, are characterized by a high bioavailability (> 95%) in the human organism (*Hou*, 2009). After the incorporation of I into plant tissue, it is mainly present in the cytoplasm, and to a smaller extent in the cell wall or the organelles (*Weng* et al., 2014). Iodine can be incorporated into various organic compounds such as proteins, lipids, polysaccharides and polyphenols (*Millard*, 1988; *Hou*, 2009), and occurs naturally in the form of triiodothyronines or other iodo-tyrosins in lettuce and tomato plants even if they are not receiving exogenous I (*Halka* et al., 2019b; *Sularz* et al., 2020). Recently, in a study on proteomics in *Arabidopsis thaliana* (L.), I has been found to be organified in many important regulatory proteins of the plant, pointing to a nutritional role of I for plants at concentrations which are generally much lower than the I levels applied for purpose of

biofortification (*Kiferle* et al., 2020). So far, little is known about which of these organic I species play a major role in I-fertilized plants. Nevertheless, several studies conducted *in vitro* and as clinical trials indicate that biofortified I remains largely bioavailable in plant foods (*Tonacchera* et al., 2013; *Li* et al., 2018; *Cakmak* et al., 2020).

Previous work showed that it is possible to biofortify apples with I via foliar fertilization in an order of magnitude appropriate for improving the dietary I intake. However, this required that the supplied KI-containing solution was applied directly to the fruits. No significant translocation of I from the leaves to the fruits was observed, although the I content in the leaves rose up to over 2,000 µg (100 g FM)$^{-1}$ as a result of the treatment. Thus it was concluded that leaf-absorbed I in apple trees is hardly translocated via the phloem (*Budke* et al., 2020b). The aforementioned study was performed on apple trees cultivated in a plastic tunnel. The trees were protected from precipitation and temporarily exposed to a microclimate with higher humidity. These conditions may have favored the absorption of the sprayed I into the fruit. Therefore, the present study was designed to evaluate the efficacy of I biofortification under field conditions in an apple and pear orchard.

Regarding the effect of the I form – I^- versus IO_3^- – on I accumulation in plants, different results are reported in the literature. In some cases, foliar-applied I^- proved to be more easily absorbable, while in other experiments no consistent differences between the two I forms could be observed (*Lawson* et al., 2015, 2016; *Cakmak* et al., 2017). At higher fertilization rates, however, IO_3^- is generally better tolerated by plants than I^- (*Dávila-Rangel* et al., 2019). Therefore, we examined in our field experiments how treatments with both I species affected the development and external appearance of leaves and fruits.

Various additives can be used to improve the effect of foliar fertilization. Surfactants contribute to improve wetting of the sprayed above-ground plant parts (*Fernández* et al., 2013). They are particularly important for the treatment of pome fruit, as the fruits form a epicuticular wax layer during their development which is much thicker than that of leaves (*Fernandes* et al., 1964). The hydrophobic coatings impair the penetration of ionic solutes into the fruit. In I fertilization experiments with wheat, apart from a wetting agent, the addition of KNO_3 to the spray solution had a positive effect on the absorption and translocation of the trace element in the plant. The I content in grains was 1.5–2.3 times higher when I was sprayed with a wetting agent or KNO_3 than when I was applied alone (*Cakmak* et al., 2017). In the present study all foliar sprays were supplied with a surfactant additionally. Furthermore, the effect of co-fertilization of KNO_3 on the I accumulation in apples and pears was investigated.

In addition to I, Se plays an important role in normal thyroid function (*Schomburg* and *Köhrle*, 2008). In many European countries and other regions of the world, the native Se content in plant-based foods is very low and therefore an insufficient dietary intake of Se is also widespread (*Rayman*, 2008; *Peters* et al., 2016). Again, as with I, the original reason for this is the low phytoavailability of the trace element in soils (*Poňavič* and *Scheib*, 2014; *dos Reis* et al., 2017). Simultaneous biofortification of food crops with I and Se is therefore considered to be a useful strategy for the prevention of thyroid diseases (*Lyons*, 2018).

Biofortified fruit can be marketed with nutritional claims such as "rich in iodine". The willingness of customers to buy such products is even greater when other quality characteristics such as the taste of the fruit are also appealing (*Wortmann* et al., 2018). The sugar content affects the degree of sweetness and thus the taste of the fruits (*Aprea* et al., 2017; *Charles* et al., 2017). Both I and Se are known to influence the allocation of photoassimilates in plants. Studies on strawberries have shown that, depending on amount, form and application technique, I fertilization can have beneficial and inhibitory effects on the accumulation of soluble solids in the fruits, which are mainly composed of sugars (*Li* et al., 2017; *Budke* et al., 2020a). Spraying of pear trees with sodium selenate (Na_2SeO_4) resulted in a significant increase in the total soluble solids content of the fruits (*Pezzarossa* et al., 2012). In addition, foliar applications with KNO_3 can enhance fructose and sucrose content, as was observed in 'Kousui' Japanese pears (*Pyrus pyrifola*) (*Shen* et al., 2016). Therefore, we also included a combination treatment consisting of I with Na_2SeO_4 and KNO_3 in our field experiments.

During storage of pome fruits, physiological processes can affect the quality of the fruit and its nutrient composition in many aspects (*Thompson* et al., 2018; *Brizzolara* et al., 2020). Therefore, it is important to understand if there are storage-related changes in I and Se contents. Additionally, the distribution and translocation of the trace elements within biofortified fruits (fruit peel, fruit flesh and fruit core) were studied from harvest through storage.

Overall, the aim of this study was to investigate various aspects of I biofortification of apples and pears by different foliar spray treatments during cultivation in an orchard, relevant for an implementation of this method in fruit growing practice. We tested the hypothesis that by this approach these pome fruits can be enriched with I at a level sufficient for improvement of I human nutrition.

Materials and methods

Plant material and growing conditions

Field experiments were conducted on two sites in an orchard of the horticultural trial station of the Osnabrück University of Applied Sciences (site 1: 52°18'23.5"N 8°02'23.7"E; site 2: 52°18'39.1"N 8°01'42.3"E). Both neighboring locations (distance approx. 1.2 km as the crow flies) were characterized by a plaggen soil of loamy-sand texture. Soil analyses in representative samples were conducted in 2012 (for site 1) and 2017 (for site 2). The results are shown in **Table 1**. The first field experiment was carried out in 2013 on site 1 and included apple trees (*Malus domestica*) of the variety 'Jonagold' and pear trees (*Pyrus communis*) of the variety 'Alexander Lucas'. The second field experiment took place on site 2 in 2018 with apple trees of the variety 'Fuji' and pear trees of the variety 'Williams Christ'. Here, the soil was fertilized with 90 kg K_2O ha^{-1} in spring. The planting distances of the trees were 3.25 m x 1.0 m for the apple trees and 3.25 m x 1.5 m for the pear trees. This corresponds to a total number of 3,076 apple trees and 2,051 pear trees per hectare. The trees had an average height of 2.5–3.0 m and were grown in spindle form with a dominant trunk (**Figures 1A, B**). The average air temperature, precipitation quantity and number of rainy days were 14.2 °C, 392.5 mm and 88 days, respectively, between April and October 2013. For the corresponding period in 2018, the values were 16.1 °C, 268.6 mm and 70 days, respectively.

Table 1: Results of the soil analyses from the experimental sites. Capital letters indicate nutrient supply class (A: low, B: slightly low, C: optimal, D: slightly high, E: high) according to the Association of German Agricultural Analytic and Research Institutes – VDLUFA (*Kießling* and *Hoffmann*, 2016).

Soil parameter	First field trial (site 1)		Second field trial (site 2)	
	Topsoil (0–30 cm)	Subsoil (30–60 cm)	Topsoil (0–30 cm)	Subsoil (30–60 cm)
Phosphorus (CAL)*	D	D	C	C
Potassium (CAL)*	D	C	C	C
Magnesium ($CaCl_2$)	E	D	C	C
pH ($CaCl_2$)	5.6	5.9	5.0	5.1
Humus content (%)	2.3	1.7	1.8	1.1
$CaCl_2$-extractable iodine (mg kg^{-1})	<0.025	<0.025	<0.025	<0.025
Aqua regia-extractable selenium (mg kg^{-1})	-	-	0.21	0.19

*CAL extraction solution contains 0.05 *M* calcium lactate, 0.05 *M* calcium acetate, and 0.3 *M* acetic acid per liter and is buffered at pH 4.1.

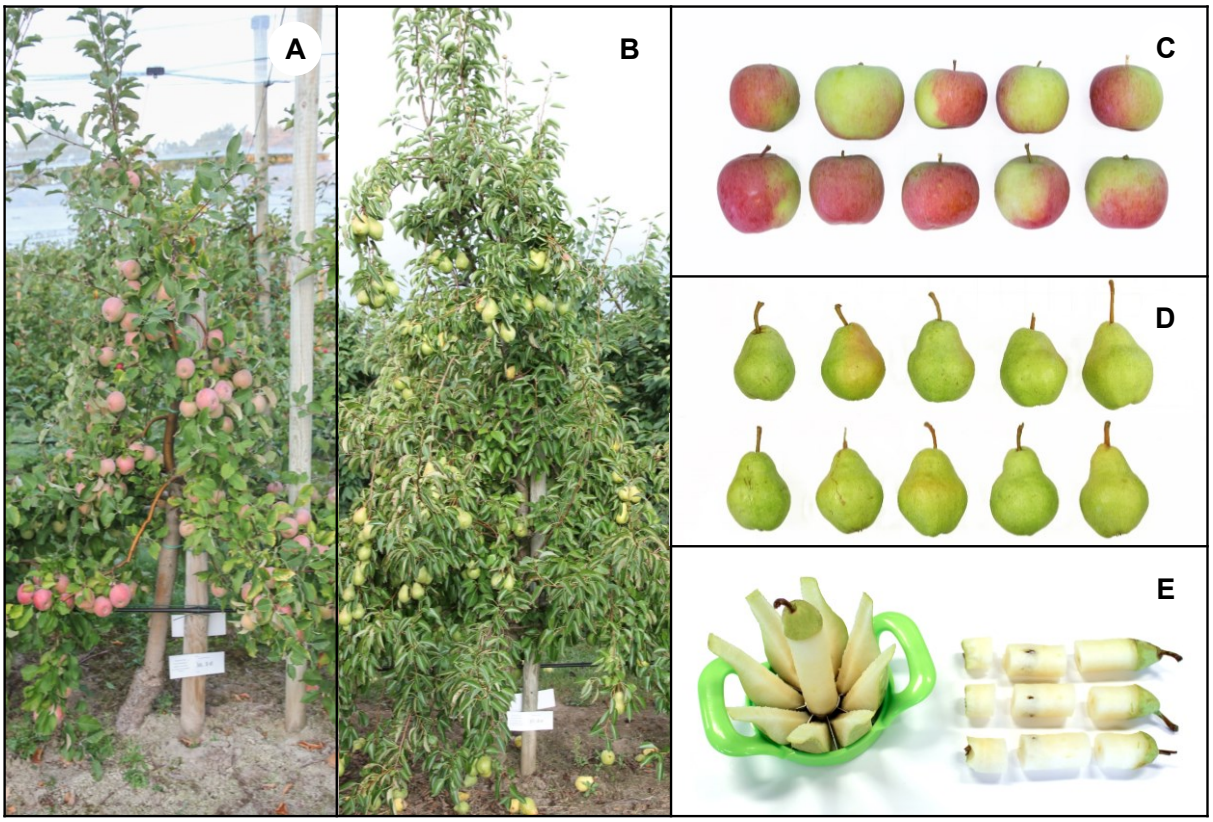

Figure 1: Examples of fruit trees included in the second field experiment and fruit appearance shortly after the harvest: Apple tree cv. 'Fuji' **(A)**, pear tree cv. 'Williams Christ' **(B)**. Selection of 10 harvested apple **(C)** and pear fruits **(D)** from treatment no. 5 consisting of a combined foliar spray with KNO_3, KIO_3 and Na_2SeO_4 which did not negatively affect external fruit characteristics. Partitioning of fruits for further preparation and analysis steps **(E)**.

Foliar spray treatments

The first field experiment was aimed at determining the influence of the I fertilizer dose and form in foliar sprays on the I accumulation in apples and pears. Here, potassium iodide (KI) and potassium iodate (KIO_3) were applied as pure salts (VWR International GmbH, Bruchsal, Germany) in three different application rates each (**Table 2**). In the second field experiment the effect of I fertilization in combination with further foliar spray treatments was investigated. The following fertilizers were used: KIO_3 as Speedfol® Iodine SP and KNO_3 as Ultrasol® K Plus, both as powder (SQM EUROPE N.V., Antwerp, Belgium) as well as sodium selenate (Na_2SeO_4), analytical-grade quality (Thermo Fisher Scientific, Kandel, Germany). Detailed information on the spray solutions are provided in **Table 2**. For all foliar sprays the surfactant Break-Thru® S 240 (AlzChem AG, Trostberg, Germany) was used in a concentration of 0.02% v/v.

Table 2: Composition of the spray solutions used in the field experiments. Applied doses are indicated per hectare and meter canopy height (CH) with the adjuvant Break-Thru® S 240 (0.02% v/v) at a water amount of 1,000 L (ha · m CH)$^{-1}$.

First field trial			Second field trial		
Treatment	**Total foliar application dose [kg (ha · m CH)$^{-1}$] and chemical form**		**Treatment**	**Total foliar application dose [kg (ha · m CH)$^{-1}$] and chemical form**	
1	0	Control	**1**	0	Control
			2	20	KNO_3
2	0.25	KI	**3**	1.5	KIO_3
3	1.0		**4**	1.5	KIO_3
4	2.5			20	KNO_3
5	0.25	KIO_3	**5**	1.5	KIO_3
6	1.0			0.05	Na_2SeO_4
7	2.5			20	KNO_3

All foliar treatments were supplied to the entire canopy of fruit trees, i.e. leaves and fruits. In the first field experiment the spray solutions were applied once two weeks before harvest of the apples or pears using a handheld sprayer (Easy Sprayer Plus, Lehnartz GmbH, Remscheid, Germany). In the second field experiment the treatments took place with a backpack sprayer (REB 15 AZ2, Birchmeier Sprühtechnik AG, Stetten, Switzerland) and were split into several dates. For apples, two applications were carried out and for pears three (**Table 3**). The water application rate was 1,000 L (ha · m CH)$^{-1}$ (CH = canopy height) in each case. The water application rates chosen ensured that the spray solutions did not run off the plant surface. The treatments were always carried out in the morning hours with no wind and in dry weather conditions.

Table 3: Splitting of the total foliar application dose, application dates and harvest dates in the conducted field experiments.

First field trial			Second field trial		
	Fruit species			**Fruit species**	
	Apple	**Pear**		**Apple**	**Pear**
Number of applications	1	1	**Number of applications**	2	3
Treatment	Sep. 15	Sep. 13	**1st treatment**	Jul. 26	Jun. 19
			2nd treatment	Aug. 31	Jul. 23
			3rd treatment	-	Aug. 6
Harvest date	Sep. 30	Sep. 24	**Harvest date**	Oct. 8	Aug. 20
			End of fruit storage	Jan. 10	Nov. 21

Data collection, sampling and sample preparation

The trees were checked for leaf and fruit damage four times during the test period and were rated accordingly (1 = no damage, 9 = very severe damage). Only fruits that were positioned in the outer part of the tree were included in the sampling for analytical investigations to ensure that they were directly wetted by the spray solution. 20 fruits per tree were harvested and the individual fruit weight was determined. In 2018 half of the fruits were stored for three months at 2 °C (**Table 3**). After harvest and storage, the external appearance of the fruits was visually evaluated and photographically documented (**Figures 1C, D**). In the second field experiment leaf samples were also taken for analytical purposes from the apple trees at harvest time. For this purpose about 20–30 leaves per tree were collected near the sampled fruits.

During fruit processing the fruits were initially divided vertically into eight equal segments and the core cylinder (**Figure 1E**). Two opposite fruit segments were then processed unwashed, washed or peeled. The washing was carried out under running deionised water. A fine peeler was used for peeling the fruit segments. The middle part of the core cylinder was used for the analytical examination and the upper and lower parts were discarded. The fruit samples were dried at 60 °C in a forced air oven (TUH 75/100, Heraeus Holding GmbH, Hanau, Germany) until the weight was constant. Using an ultracentrifugal mill (ZM 200, RETSCH GmbH, Haan, Germany), the samples were then ground at 14,000 rpm to a particle size of ≤ 0.5 mm. Until analysis the sample material obtained in this way was stored in sealed plastic beakers and dried again shortly before chemical digestion.

Analyses of iodine and selenium in plant samples

The I determination was performed according to the method *DIN EN 15111* (2007). Briefly, 1 g of dried plant substance was used and chemically digested with 25% tetramethylammonium hydroxide solution (TMAH). To ensure the quality of the analysis, certified reference material (ERM-BB422 fish muscle and NIST-1849a infant/adult nutritional milk powder) was used. Another reference material was apple powder from our own experiments, which had been previously analyzed in an external laboratory accredited for I analysis in food (LUFA Nord-West, Hameln, Germany). The I determination was performed by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700, Agilent Technologies Inc., Santa Clara, CA, United States). Selenium was determined according to the method *DIN EN 13805* (2014). For this purpose, 0.5 g of the ground plant material was digested by microwave pressure digestion in quartz glass vessels with 65% nitric acid at a temperature of 190 °C and under pressure. The digestion solution was analyzed by graphite furnace atomic absorption spectrometry (GF-AAS, Thermo Scientific - SOLAAR M Series AA Spectrometer, Thermo Fisher Scientific Inc., Waltham, MA, United States). For quality control purposes, the same certified reference materials were used as for I analysis. Again, comparative tests were performed in an external laboratory accredited for Se determination (LUFA Nord-West, Hameln, Germany). Samples with Se concentrations below 2.5 µg L^{-1} were analyzed by using the hydride technique in accordance with the method DIN 38405-23 (1994).

The I and Se content of the fruit peel was calculated from the difference between washed and peeled fruit segments. In the second field experiment the I and Se contents were also determined in unwashed and washed apple leaves, once in the control (treatment 1) and once in the variants fertilized with KIO_3 (treatment 3) and KIO_3 + Na_2SeO_4 + KNO_3 (treatment 5).

Measurement of total soluble solids content

Two segments per fruit were used to determine the total soluble solids content. The sample material was pureed and then filtered. The resulting juice was analyzed with a digital refractometer (PAL-1, ATAGO CO., Ltd., Tokyo, Japan).

Trial set-up and statistical procedures

The field experiments were designed as randomized block experiments with usually four replications. The experiment with apple trees in 2018 included six replications. Each treatment was represented by one tree per block. The selection of the trees was based on a homogeneous structure and fruit number. To avoid edge effects, the treated trees were separated from each other by at least one untreated tree. In addition, plastic foil barriers were used to isolate each tree during the spraying process to prevent contamination by drift.

The results obtained in the fruit analyses were subjected to one-way or two-way ANOVA and, if needed, logarithmized to meet assumptions of normality and homogeneity of variances. Multiple mean value comparisons were made using the Tukey-HSD test and the LSD test. The program IBM SPSS® Statistics, version 26 (IBM Deutschland GmbH, Ehningen, Germany), was used for statistical data evaluation.

Results

Iodine content of fruits and leaves

The native I content of apples and pears was 1.5 µg (100 g FM)$^{-1}$ and 1.0 µg (100 g FM)$^{-1}$, respectively. Foliar sprays with I-containing solutions significantly increased the I content of the fruits. In the first field experiment, a single treatment with 0.25–2.50 kg I (ha · m CH)$^{-1}$, carried out two weeks before harvest, led to an increase in the I content in washed fruit segments from 15.7 µg (100 g FM)-1 up to more than 200 µg (100 g FM)$^{-1}$ (**Figure 2**). The mean dry matter (DM) content was 16% for apple and pear. The aforementioned values thus correspond 0.9 to >12.5 mg I (kg DM)$^{-1}$. There was a close linear relationship between the I fertilization level and the I enrichment of the fruits. Further statistical analysis shows that the mean I content determined for the different I doses and forms were predominantly significantly different (**Supplementary Tables 1, 2**). The application of I^- resulted, averaged over both fruit species, in an I content that was 2.5 times higher than a corresponding supply of IO_3^-. However, the I enrichment of I^--treated fruits, especially at the highest supply rate, varied much more than when using the oxidized I form. **Figure 2** also shows that apples tended to accumulate more I per unit of weight

than pears. These differences in I content were related to the different individual fruit weights. Pears harvested in this trial were 19% heavier than a single apple fruit. The total amount of I per fruit contained in apples and pears was similar with the same I form and dose and reached a maximum of 508 µg/fruit for apple and 467 µg/fruit for pear at the highest I^- supply rate (**Table 4**). In I-sprayed apples, 51%, 47% and 2% of the I was localized in the fruit peel, the fruit flesh and the fruit core, respectively. For pears the corresponding values were 78%, 20% and 2%, respectively (**Figure 3**). Compared to KIO_3, the supply of KI favored the translocation of the I into the fruit flesh. Washing the fruits in running deionised water reduced the I content in the first field experiment by 14% for apples and 16% for pears.

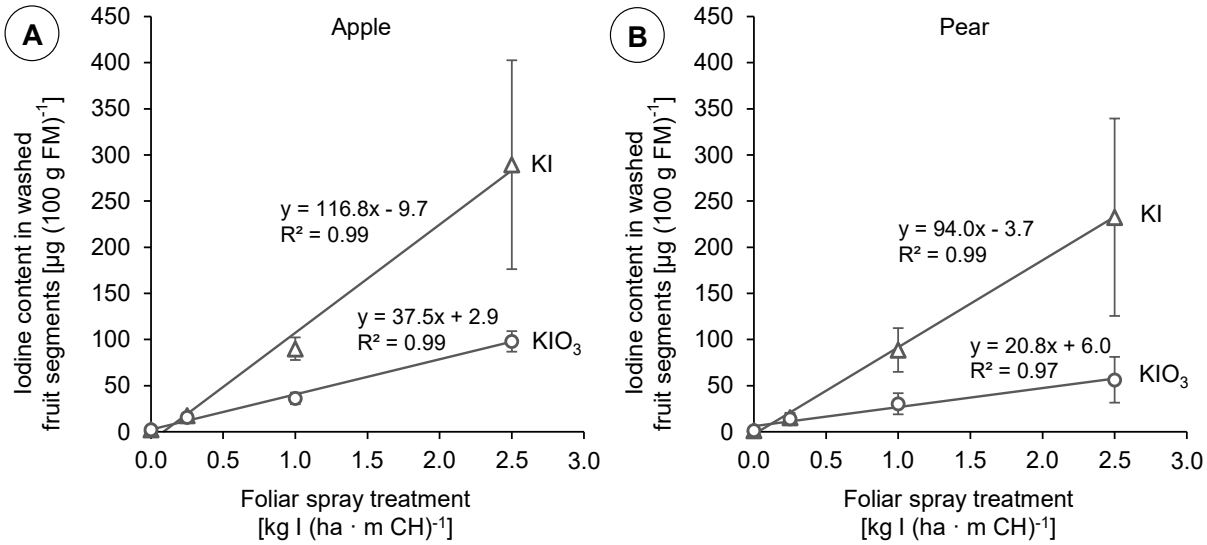

Figure 2: Iodine content in washed fruit segments of apples cv. 'Jonagold' **(A)** and pears cv. 'Alexander Lucas' **(B)** at harvest time as affected by the dose and form of iodine foliar sprays in the first field experiment. Means ± standard deviation (*n* = 4).

Table 4: Iodine amount in a whole washed fruit including core and individual fruit weight of apples and pears from the first and the second field experiment as affected by the spray solution. Means ± standard deviation (n = 4, except 'Fuji' apples second field trial n = 6). Means with same letters in one column for one field trial do not differ according to Tukey-HSD test at α = 0.05.

		Treatment[1)]		Apple		Pear	
				Iodine amount per fruit [µg]	Individual fruit weight [g]	Iodine amount per fruit [µg]	Individual fruit weight [g]
First field trial	1	Control	0	4.7 ± 0.2 a	206.8 ± 13.6 a	2.7 ± 1.5 a	230.9 ± 26.8 a
	2	KI	0.25	31.3 ± 4.7 b	195.2 ± 22.1 a	30.9 ± 10.2 b	238.8 ± 5.0 a
	3	KI	1.0	170.9 ± 23.5 d	210.9 ± 5.8 a	172.4 ± 45.9 d	232.0 ± 37.2 a
		KI	*1.5*[2)]	*294.1*		*274.6*	
	4	KI	2.5	508.0 ± 198.7e	194.0 ± 13.2 a	467.4 ± 215.8e	239.7 ± 33.7 a
	5	KIO_3	0.25	28.8 ± 8.2 b	199.4 ± 11.2 a	29.4 ± 12.1 b	246.2 ± 63.8 a
	6	KIO_3	1.0	65.2 ± 10.7 c	196.2 ± 8.6 a	59.3 ± 22.5 bc	227.5 ± 33.3 a
		KIO_3	*1.5*[2)]	*105.5*		*71.7*	
	7	KIO_3	2.5	173.9 ± 22.1 d	191.2 ± 28.8 a	107.3 ± 47.9 cd	219.6 ± 28.4 a
Second field trial	1	Control	0	1.6 ± 0.4 a	211.2 ± 39.7 a	1.8 ± 1.0 a	166.8 ± 18.0 a
	2	KNO_3	0	1.6 ± 0.7 a	214.2 ± 31.1 a	1.9 ± 0.7 a	154.5 ± 30.4 a
	3	KIO_3	1.5	102.7 ± 10.8 b	209.0 ± 21.9 a	79.9 ± 6.2 b	157.1 ± 11.6 a
	4	KIO_3 + KNO_3	1.5	89.7 ± 14.4 b	204.5 ± 37.8 a	87.8 ± 11.2 b	150.7 ± 7.1 a
	5	KIO_3 + Na_2SeO_4 + KNO_3	1.5	84.0 ± 13.5 b	194.2 ± 31.2 a	85.7 ± 13.8 b	143.0 ± 18.1 a

[1)] Iodine application rate expressed in kg (ha · m CH)$^{-1}$

[2)] Values calculated for comparison purposes are based on the regression equations indicated in Figure 2

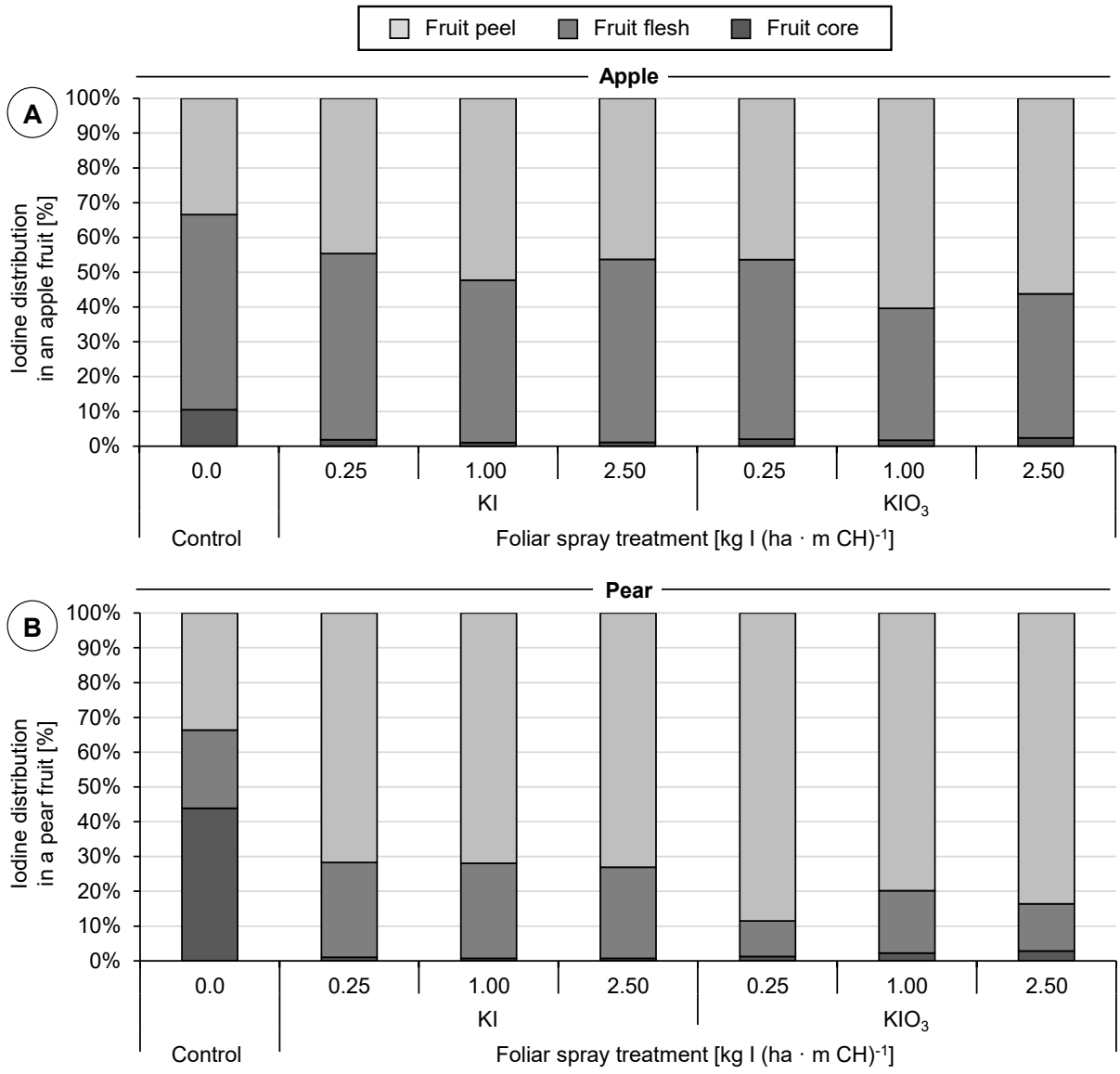

Figure 3: Iodine distribution in washed apples cv. 'Jonagold' **(A)** and pears cv. 'Alexander Lucas' **(B)** at harvest time as affected by the dose and form of iodine foliar sprays in the first field experiment.

In the second field experiment the effect of I spraying in combination with further foliar fertilization treatments was investigated. In contrast to the previous experiment, only KIO_3 with a uniform application rate of 1.5 kg I (ha · m CH)$^{-1}$ was used. Furthermore, the applications were split into two dates for apple and three dates for pear. The addition of KNO_3 and Na_2SeO_4 to the I spray solution had no clear influence on the I accumulation in washed fruit segments. At harvest time, the I content in the I-sprayed treatments varied between 47–54 µg I (100 g FM)$^{-1}$ for apples and 58–69 µg I (100 g FM)$^{-1}$ for pears, irrespective of the addition of the aforementioned salts (**Figures 4A, C**). The fruit-specific differences in I enrichment levelled out again when taking into account the individual fruit weights, which in this case were higher for apples (**Table 4**). Without I supply - in the controls and in the stand-alone KNO_3 foliar fertilization treatments - the I content of the fruits was about 1.0 µg (100 g FM)$^{-1}$. During the three-monthly cold storage the I content in I-sprayed apples decreased by 20%. In the case of pears, however, fruit storage had no significant effect on the I content.

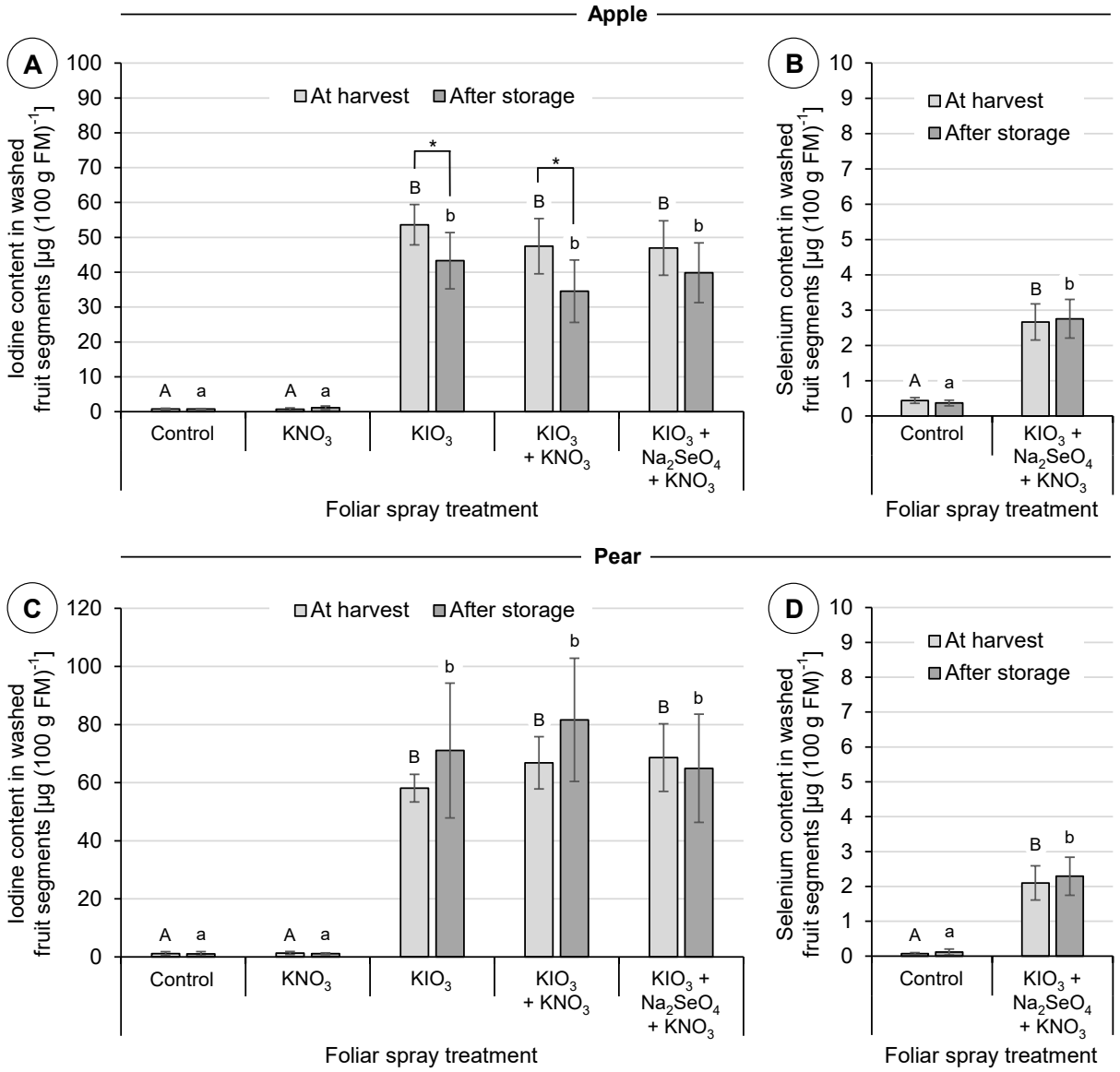

Figure 4: Iodine and selenium content in washed fruit segments of apples cv. 'Fuji' **(A, B)** and pears cv. 'Williams Christ' **(C, D)** in the second field experiment as affected by different foliar spray treatments and fruit storage at 2 °C for a period of three months. Means ± standard deviation (apple *n* = 6, pear *n* = 4). Means not sharing a letter in one chart or indicated by an asterisk are significantly different according to Tukey-HSD test at α = 0.05.

In the fruit peel of I-sprayed, washed apples and pears, the I content at harvest was 6.6 and 17.1 times higher, respectively, than in the fruit flesh. In the case of apples, this difference decreased after cold storage, as the I content in the fruit peel decreased by 45% and simultaneously increased by 14% in the fruit flesh (**Table 5**). For the pear, however, no significant change in this respect was observed.

Table 5: Iodine and selenium content in fruit peel and flesh of washed apples cv. 'Fuji' and pears cv. 'Williams Christ' in the second field experiment as affected by different foliar spray treatments and a fruit storage at 2 °C for a period of three months. Means ± standard deviation (apple n = 6, pear n = 4). Means not sharing a lower case letter in one column or an upper case letter for same type of fruit sample in one row are significantly different according to Tukey-HSD test at α = 0.05.

Apple		Iodine content [µg (100 g FM)$^{-1}$]							
		At harvest				After storage			
	Treatment	Fruit peel		Fruit flesh		Fruit peel		Fruit flesh	
1	Control	2.1 ± 0.2	a A	0.5 ± 0.2	a A	2.3 ± 0.1	a A	0.5 ± 0.1	a A
2	KNO_3	2.3 ± 0.4	a A	0.5 ± 0.4	a A	2.0 ± 0.5	a A	1.0 ± 0.5	a A
3	KIO_3	219.2 ± 53.2	b A	26.7 ± 8.3	b A	125.3 ± 75.4	b B	29.9 ± 6.5	b A
4	KIO_3 + KNO_3	172.8 ± 62.7	b A	27.1 ± 3.9	b A	84.0 ± 60.0	b B	28.8 ± 10.5	b A
5	KIO_3 + Na_2SeO_4 + KNO_3	157.2 ± 79.6	b A	29.0 ± 6.9	b A	92.3 ± 49.9	b A	36.1 ± 8.9	b A
		Selenium content [µg (100 g FM)$^{-1}$]							
1	Control	0.5 ± 0.2	a A	0.4 ± 0.1	a A	0.9 ± 0.4	a A	0.4 ± 0.2	a A
5	KIO_3 + Na_2SeO_4 + KNO_3	10.9 ± 3.2	b A	1.4 ± 0.4	b A	7.2 ± 5.3	b A	2.0 ± 0.7	b A
Pear		**Iodine content [µg (100 g FM)$^{-1}$]**							
		At harvest				After storage			
	Treatment	Fruit peel		Fruit flesh		Fruit peel		Fruit flesh	
1	Control	3.4 ± 0.7	a A	0.7 ± 0.7	a A	1.3 ± 0.8	a B	1.0 ± 0.8	a A
2	KNO_3	4.8 ± 0.5	a A	0.8 ± 0.5	a A	4.1 ± 0.3	a A	0.4 ± 0.3	a B
3	KIO_3	304.9 ± 17.4	b A	17.9 ± 3.3	b A	406.1 ± 145.9	b A	16.5 ± 5.3	b A
4	KIO_3 + KNO_3	356.3 ± 78.7	b A	19.7 ± 4.1	b A	366.7 ± 136.0	b A	35.2 ± 6.9	b A
5	KIO_3 + Na_2SeO_4 + KNO_3	355.0 ± 52.0	b A	22.0 ± 7.8	b A	331.8 ± 97.2	b A	21.5 ± 8.7	b A
		Selenium content [µg (100 g FM)$^{-1}$]							
1	Control	0.4 ± 0.0	a A	0.1 ± 0.1	a A	0.5 ± 0.4	a A	0.1 ± 0.0	a A
5	KIO_3 + Na_2SeO_4 + KNO_3	4.3 ± 2.1	b A	2.0 ± 0.5	b A	6.5 ± 5.2	b A	1.6 ± 0.7	b A

Washing the fruit segments of I-treated apples and pears under running deionised water reduced their I content at harvest time by 13% and 11%, respectively, which is in a similar order of magnitude to that observed in the first field experiment. In peeled fruit segments the I content was reduced by 51% and 73%, respectively (**Figure 5**). In stored apples the I losses due to peeling were lower, as expected, due to the previously reported decrease of I content in the apple peel.

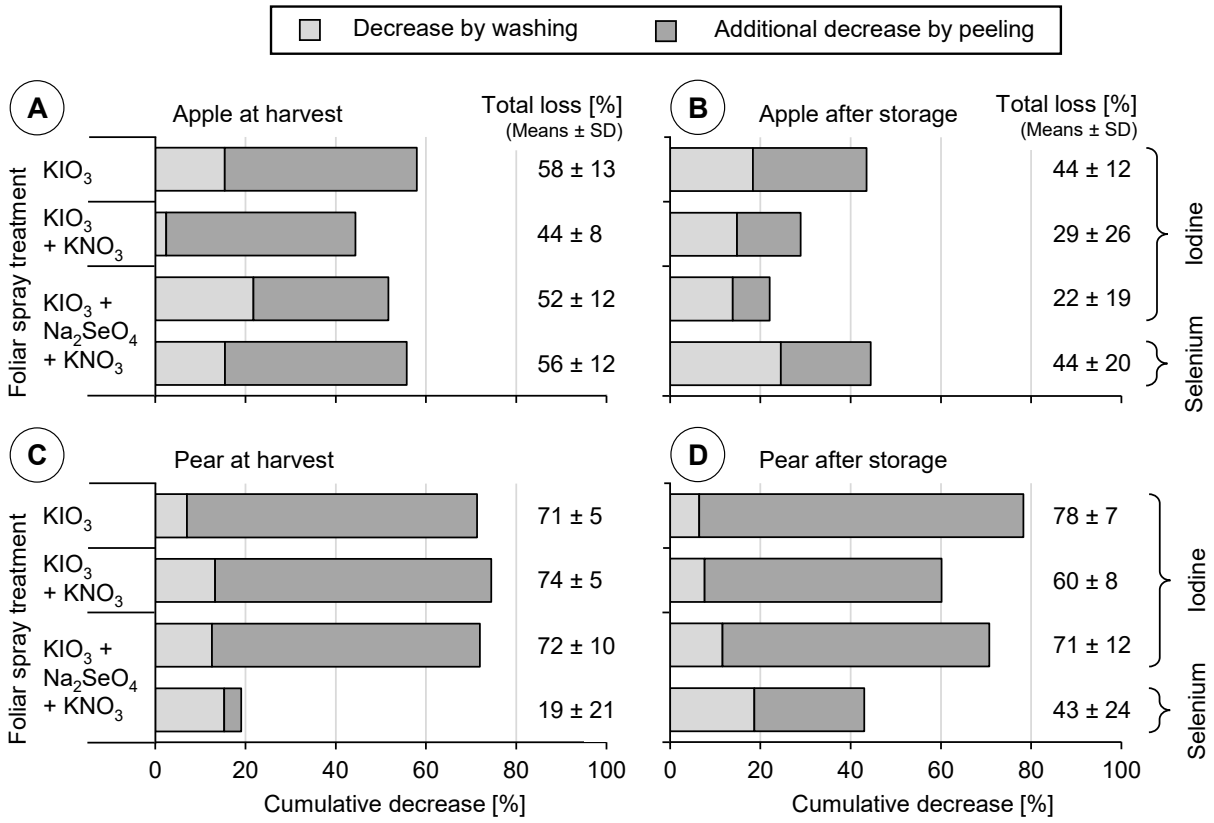

Figure 5: Cumulative decrease of the iodine and selenium content in fruit segments by washing and peeling of apples cv. 'Fuji' **(A, B)** and pears cv. 'Williams Christ' **(C, D)** in the second field experiment at harvest time and after fruit storage at 2 °C for a period of three months. Means ± standard deviation (apple $n = 6$, pear $n = 4$).

Leaves accumulated considerably more I than fruit, as exemplary analyses on apple trees revealed. Unwashed apple leaves not sprayed with I contained 166 ± 67 µg I (100 g FM)$^{-1}$. As a result of a KIO_3 foliar application, the I content increased to 10,924 ± 1,712 µg I (100 g FM)$^{-1}$. In washed leaves, this was at a similar level with 11,082 ± 1,778 µg I (100 g FM)$^{-1}$. The mean dry matter content of apple leaves was 37%. The aforementioned I content on fresh matter basis thus corresponds to 300 mg I (kg DM)$^{-1}$.

Selenium content of fruits and leaves

The native Se content of apples and pears was 0.4 µg (100 g FM)$^{-1}$ and 0.1 µg (100 g FM)$^{-1}$, respectively. Repeated foliar sprays of Na_2SeO_4 with a total of 50 g Se (ha · m CH)$^{-1}$ increased the Se content in washed fruit segments to 2.7 µg (100 g FM)$^{-1}$ and 2.1 µg (100 g FM)$^{-1}$, respectively (**Figures 4B, D**). Cold storage of the fruits had no effect on the Se content.

The foliar-applied Se was enriched in the fruit peel of apples and pears by a factor of 7.8 and 2.2, respectively, more than in the fruit flesh (**Table 5**). Washing and peeling reduced the Se content in these pome fruits 15% and 38%, respectively (**Figure 5**). At harvest time these losses were lower for pears than for apples. After storage, no differences were observed in this respect.

Apple leaves of the control treatments contained 1.9 ± 0.4 µg Se (100 g FM)$^{-1}$ in the unwashed and 1.2 ± 0.6 µg Se (100 g FM)$^{-1}$ in the washed state. Selenium fertilization increased the Se content to 303.6 ± 65.4 µg (100 g FM)$^{-1}$ in unwashed and 309.3 ± 57.2 µg (100 g FM)$^{-1}$ in washed leaves.

Phytotoxicity symptoms on leaves

The spraying of I-containing solutions on apple and pear trees resulted in leaf necrosis, starting at the leaf margins and at the leaf tip. The intensity of these symptoms increased as the number of applications increased and the growing season progressed (**Figure 6**). In the first field trial, the leaves of pear trees showed more severe damage, while in the second field trial the leaves of apple trees were more affected (**Table 6**). The degree of damage increased with increasing concentration of I in the spray solution. Iodine fertilizer form had no consistent influence on the leaf damage. Likewise, the co-application of KNO_3 and Na_2SeO_4 with I had no effect on damage pattern. When only KNO_3 was sprayed, the leaves remained undamaged as in the controls. After harvesting, accelerated leaf senescence and premature leaf fall was observed in the I-sprayed treatments. These effects also increased with increasing I supply (**Figure 7**).

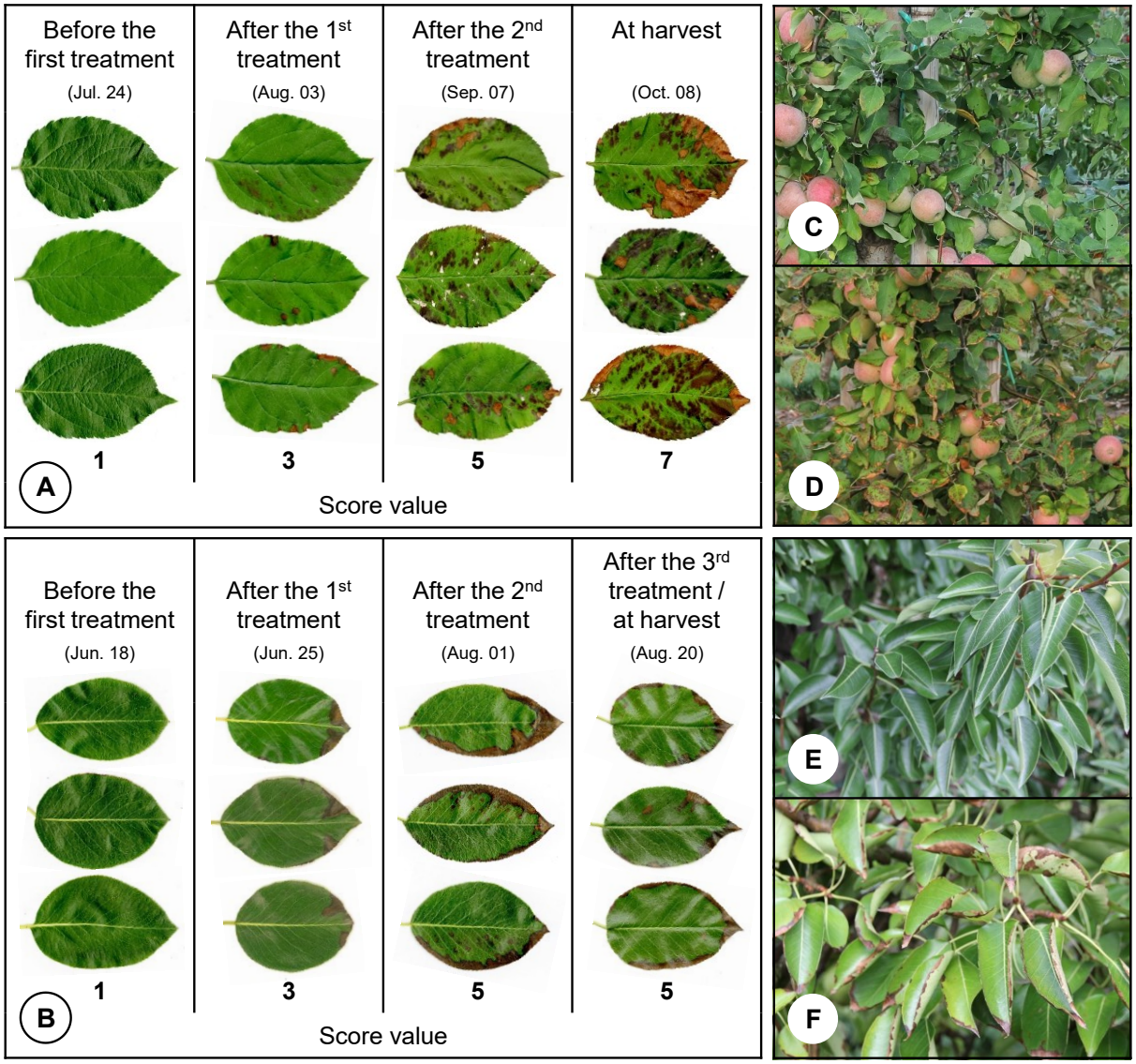

Figure 6: Development of leaf damage during the growing season until fruit harvest in the second field experiment. Images of scanned leaves of apple trees cv. 'Fuji' **(A)** and pear trees cv. 'Williams Christ' **(B)** from treatment no. 5 consisting of a combined foliar spray with KNO_3, KIO_3 and Na_2SeO_4. Score values indicate the degree of the damage (Score value 1 = no damage, 3 = slight damage 5 = moderate damage, 7 = severe damage, 9 = very severe damage). Detail view of 'Fuji' apple trees **(C, D)** and 'Williams Christ' pear trees **(E, F)** in the second field experiment at harvest time. Picture **C** and **E**: treatment no. 1 (control). Picture **D** and **F**: treatment no. 5 (spray solution composition as described above).

Table 6: Score values of leaf damage on trees of apples cv. 'Jonagold' and 'Fuji' and pears cv. 'Alexander Lucas' and 'Williams Christ' from the first and the second field experiment as affected by the spray solution. Score value 1 = no damage, 3 = slight damage 5 = moderate damage, 7 = severe damage, 9 = very severe damage. Means ± standard deviation (*n* = 4, except 'Fuji' apples second field trial *n* = 6).

	Treatment[1)]		Score values of leaf damage [1–9]	
			Apple	Pear
First field trial	1 Control	0	1.0 ± 0.0	1.0 ± 0.0
	2	0.25	3.0 ± 0.0	5.0 ± 0.0
	3 KI	1.0	3.2 ± 0.5	7.2 ± 0.5
	4	2.5	5.7 ± 1.5	7.7 ± 1.0
	5	0.25	2.4 ± 0.6	5.0 ± 0.0
	6 KIO_3	1.0	3.7 ± 1.0	6.5 ± 1.0
	7	2.5	6.7 ± 1.5	9.0 ± 0.0
Second field trial	1 Control	0	1.0 ± 0.0	1.0 ± 0.0
	2 KNO_3	0	1.0 ± 0.0	1.0 ± 0.0
	3 KIO_3	1.5	6.0 ± 1.1	3.5 ± 1.0
	4 KIO_3 + KNO_3	1.5	5.7 ± 1.0	4.0 ± 1.2
	5 KIO_3 + Na_2SeO_4 + KNO_3	1.5	5.7 ± 1.0	4.0 ± 1.2

[1)] Iodine application rate expressed in kg (ha · m CH)$^{-1}$

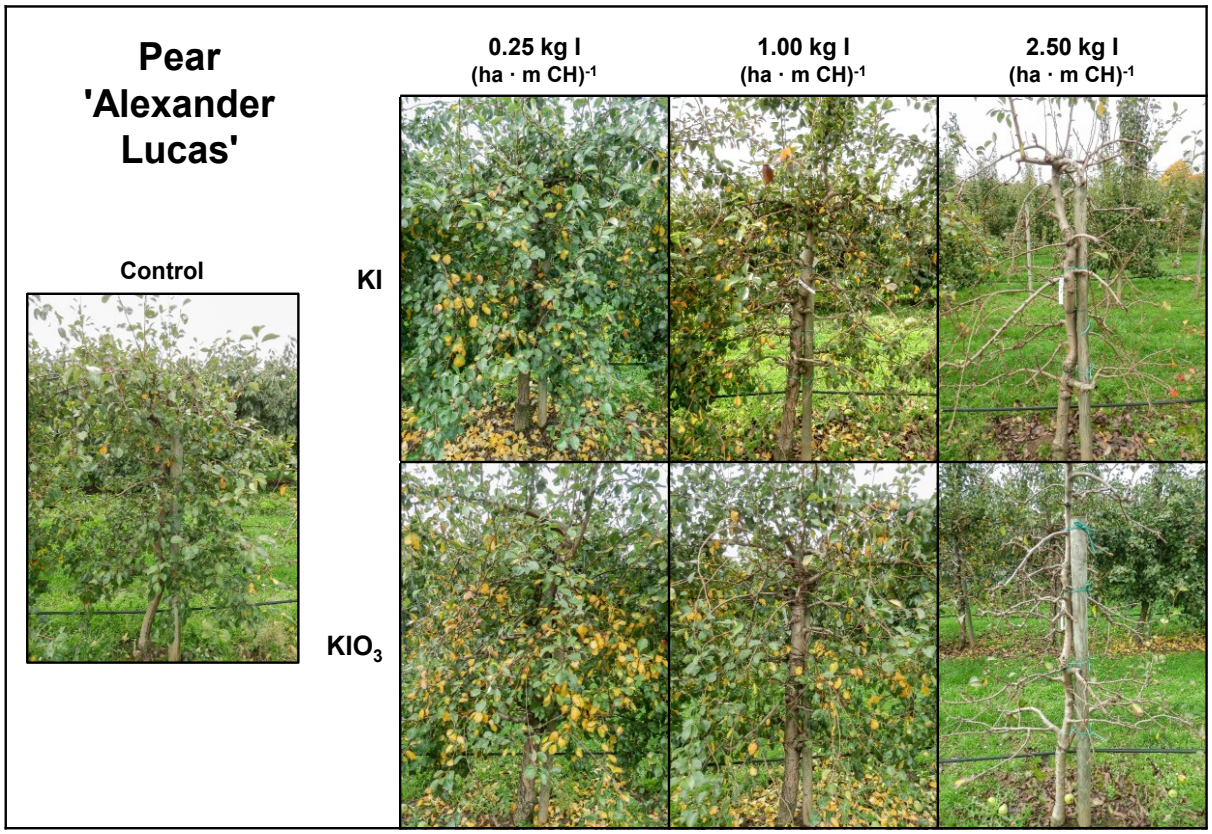

Figure 7: Appearance of pear trees cv. 'Alexander Lucas' in the first field experiment 19 days after harvest (Oct. 13) as affected by the dose and form of iodine foliar sprays applied two weeks before fruit harvest.

Fruit development and content of total soluble solids

No damage was observed on the fruits in any of the foliar fertilization treatments tested, neither at the time of harvest nor after storage. In all treatments the individual fruit weight was at the same level as in the controls (**Table 4**). KIO_3 sprays did not affect the total soluble solids content of fruits. However, repeated applications of KNO_3 promoted the accumulation of soluble solids. At harvest time the concentration of soluble solids was increased by 1.0 °Brix in apples and 0.9 °Brix in pears compared to the control (**Figure 8**). Even with simultaneous application of KIO_3 and KNO_3, apples still showed a correspondingly increased °Brix value. After cold storage of the fruits the above-mentioned differences in total soluble solids content remained.

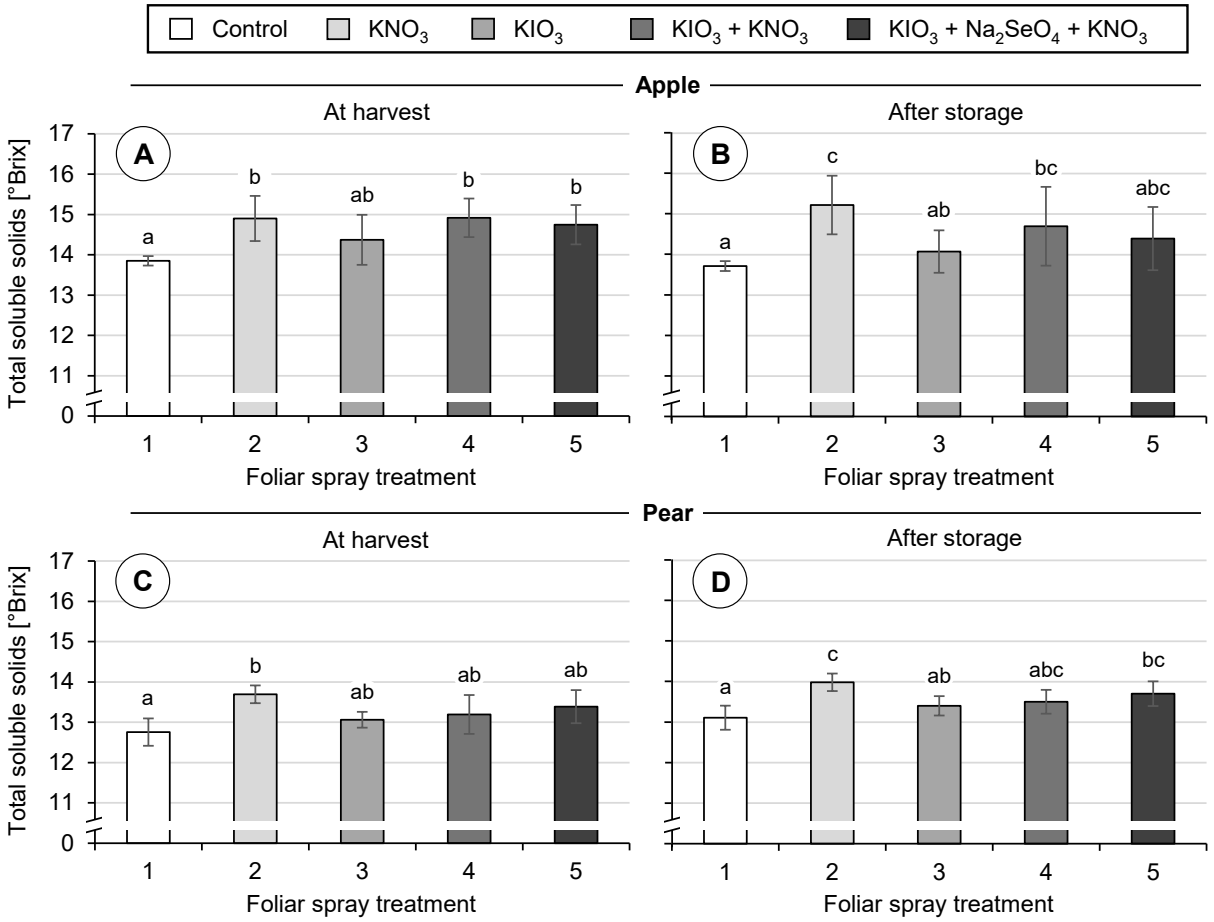

Figure 8: Total soluble solid content in fruit segments of apples cv. 'Fuji' (**A, B**) and pears cv. 'Williams Christ' (**C, D**) in the second field experiment as affected by different foliar spray treatments and fruit storage at 2 °C for a period of three months. Means ± standard deviation (apple n = 6, pear n = 4). Means with same letters for one fruit group and one time of measurement do not differ according to Tukey-HSD test at α = 0.05.

Discussion

Biofortification with straight iodine foliar sprays

By applying I-containing foliar fertilizers in an orchard, it was possible to enrich apples and pears significantly with I. While washed fruit segments of the control treatments had an I content of ≤ 1.5 µg (100 g FM)$^{-1}$, this was increased by a factor of 10 - 193 in the I-fertilized treatments and reached more than 200 µg (100 g FM)$^{-1}$ at the highest supply rate [2.5 kg I (ha · m CH)$^{-1}$] for both fruit species. A linear relationship between the I fertilizer amount and I fruit enrichment was observed (**Figure 2**). The increase was 2.5 times higher with I^--supply than with IO_3^--supply. Similar effects on the influence of the level and form of I supply were described in studies on the biofortification of strawberries (*Li* et al., 2017) and various vegetable and cereal crops (*Hong* et al., 2008; *Voogt* et al., 2010; *Cakmak* et al., 2017; *Li* et al., 2018).

The higher accumulation of exogenously applied I^- in plants is probably due to its smaller molecular weight and lower valence compared to IO_3^- (*Mackowiak* and *Grossl*,

1999). Furthermore, studies on I uptake by roots indicate that IO_3^- enters the symplast only after prior reduction to I^- (*Kato* et al., 2013). Iodide is absorbed via ion channels or chloride transporters driven by proton pumps (*White* and *Broadley*, 2009; *Medrano-Macías* et al., 2016). For foliar sprays, however, another aspect is probably of great importance, namely the difference in the point of deliquescence (POD) of the applied salts. The POD describes the relative humidity (RH) at which hygroscopic salts absorb enough water from the air to form a liquid solution. At a temperature of 20 °C, the POD for KI is 69% RH and for KIO_3 93% RH (*Greenspan*, 1977; *Apelblat* and *Korin*, 1998). This difference will influence the capacity of spray drop deposits to rehydrated under high RH conditions as affected by temperature and hence favor new cycles of I absorption by the fruits and the foliage. KI is superior to KIO_3 in this respect because it deliquesces at much lower RH. The deliquescence phenomena will be more prone to occur at night time and also in association with dew on plant organs. In our field experiments spray applications were always carried out in the morning hours when RH was usually below 94% and the thin spray liquid films formed on the surface of fruits and leaves dried relatively quickly. As a result, the dissolved salt can be converted to the solid, crystalline state. The RH at which this phase transformation begins is defined as the point of efflorescence (POE). The POE is usually below that of the POD (*Freney* et al., 2009). Recently, the importance of the POE of fertilizer salts for spray drop drying has been highlighted (*Fernández* et al., 2020). To the best of our knowledge, POE values for KI and KIO_3 have not yet been published and thus should be determined in further investigations. The translocation of I from the fruit peel to the fruit flesh was also affected by the I form as well as by the type of fruit. In the case of IO_3^- supply, 14% and 44% of the I were found in the fruit flesh of the pear and apple, respectively, while in the case of I^- supply the figures were 27% and 51%, respectively (**Figure 3**).

Despite the higher absorption and translocation of I^-, the I content in single pome fruits was subject to greater fluctuations within the treatments compared to IO_3^-, especially at high I supply. Likewise, in other published field experiments IO_3^- led to a more uniform result when applied at different locations and under varying environmental conditions (*Lawson* et al., 2016; *Cakmak* et al., 2017). This is advantageous for the practical implementation of agronomic biofortification, where the aim is to achieve the desired level of I biofortification in a way that is as reproducible as possible. For this reason, we selected KIO_3 as I salt for our second field experiment. With an application rate of 1.5 kg I (ha · CH)$^{-1}$ it was possible to increase the I content in washed fruit segments to about 50 - 60 µg (100 g FM)$^{-1}$ (**Figure 4**). An I enrichment of the same order of magnitude was achieved when apple trees cultivated under protected conditions in a plastic tunnel were sprayed with I (*Budke* et al., 2020b). This is surprising, since in an orchard it can be expected that exogenously applied I will be partially washed off the fruit trees by rain. In the second field experiment, the amount of precipitation that fell in the period from the first foliar spray to the harvest of fruits was 78.2 mm (over 75 days) for apples and 100 mm (over 63 days) for pears. However, no or very low precipitation (< 3 mm) was observed in the first two days after application. In the first field experiment, however, about 6 mm of precipitation fell one day after the treatment of the apple trees. Nevertheless, the apples investigated here were also enriched with I to an extent similar to the described plastic tunnel experiment. Obviously, rainfall in the range mentioned did not result in noteworthy wash-off losses even if the I sprayed on the fruit was probably not absorbed completely within

one day. Investigations on butterhead lettuce showed that after one day only about half of the I deposited on leaves via foliar fertilization was absorbed by the leaves (*Lawson* et al., 2016). In fruits, especially those with a thicker wax layer on the surface, the uptake of I is likely to proceed much more slowly, although this has not yet been investigated. Studies on calcium uptake in apples of the 'Cox Orange' variety showed that within 2 to 7 days a maximum of 7% and 25%, respectively, of the radioisotope $^{45}Ca^{2+}$ applied to the fruit surface penetrated to a depth of 1 mm into the fruit (*van Goor*, 1973).

When evaluating the I biofortification of apples and pears, the fruit size must be taken into account. As the fruit weight increases, the I absorbed into the fruit becomes increasingly diluted (*Budke* et al., 2020b). Accordingly, a higher I fertilizer application was required for bigger pears of the 'Alexander Lucas' variety in order to achieve an I content comparable to that of the smaller 'Williams Christ' pears. With regard to the total amount of I contained in the pears, only minor differences between both pear varieties were found. At a KIO_3 application rate of 1.5 kg I (ha · CH)$^{-1}$, pears of the 'Williams Christ' variety still contained about 10% more I than determined for 'Alexander Lucas' by calculation (**Table 4**).

The apple varieties 'Fuji' and 'Jonagold' hardly differed in fruit size and showed a similar I accumulation patterns in the fruits at the same KIO_3 application rate. The thickness of the epicuticular wax layer of the two apple varieties is also comparable and is in the middle to higher range for apples at harvest time with approx. 1.5 µm. In general, the wax deposition on the apple peel increases as the fruit develops (*Guan* et al., 2015). Therefore, a late foliar application date, as set in the first field experiment with the variety 'Jonagold' (treatment two weeks before harvest), would rather result in a lower uptake rate of I sprayed on the fruit. On the other hand, the surface area of growing fruits increases during the season. Thus, more I is retained by the fruit if the application date is late. Taken together it can be assumed that these two opposing effects compensated each other and therefore the different treatment dates in the field experiments performed had no influence on fruit I accumulation.

Preferential uptake routes for dissolved ionic solutes into the fruit are fine cracks in the cuticle and lenticels (*Harker* and *Ferguson*, 1988). The occurrence of these epidermal structures can vary considerably depending on the variety. 'Williams Christ' pears, for example, have more than three times as many lenticels as 'Alexander Lucas' pears (*Durić* et al., 2015). In our experiments, this may have additionally favored the I enrichment in the smaller fruiting 'Williams Christ' variety. 'Fuji' apples are known to have significantly more lenticels on the fruit surface than 'Jonagold' apples (*Guan* et al., 2015). However, in contrast to pears, these differences in variety did not affect the I uptake of apples. Thus, from our data we cannot conclude that lenticels play an important role for I fruit absorption.

Even though foliar sprays with I-containing fertilizers have proven to be suitable for production of biofortified pome fruits with increased I content, the efficiency of this measure is relatively low. In a normal orchard with a tree height of 2.5–3.0 m and a fruit yield of 40 t ha^{-1}, no more than about 0.5% of the applied I enters the fruits if their I content averages 50 µg (100 g FM)$^{-1}$. This calculation is based on a fertilization of 1.5 kg I (ha · m CH)$^{-1}$ in

the form of KIO_3. When using KI, the proportion of I transferred into the fruits can increase up to 1.1%, since a lower amount of I fertilizer is required for the same I enrichment.

It may be possible to increase the efficiency of I foliar fertilization by using an air-blast orchard sprayer, which is commonly used in commercial fruit growing. This application technique is likely to be superior to the handheld sprayers used in the experiment, especially with regard to sufficient wetting of the fruits covered by leaves inside the tree. This is important because they must be hit directly by the spray solution in order to be significantly biofortified. The translocation of I from leaves to fruits in apple trees was found to be negligible, which is attributed to a low phloem mobility of I in apple trees (*Budke* et al., 2020b). Most of the I applied by foliar fertilization is probably found in the foliage, which has a surface area more than 10 times larger than the fruits growing on the tree (*Knoche* and *Petracek*, 2014). The I content measured in apple leaves was more than 200 times higher than the fruits. The main reason for this is certainly the larger surface area-to-volume ratio of the leaves, which means that the increase in concentration is higher for the same amount of solutes per unit of area. In addition, the epidermis of the leaves is covered by a thinner wax layer than that of the fruits (*Fernandes* et al., 1964) and stomata are available as additional uptake routes for ionic solutes (*Eichert* and *Fernández*, 2012).

Biofortification with leaf fertilizer mixtures

The addition of KNO_3 to a spray solution containing KIO_3 had no effect on the I content of the fruits, neither for apple nor for pear (**Figure 4**). In contrast, *Cakmak* et al. (2017) found in a study on wheat plants that the uptake of foliar-applied IO_3^- is significantly increased by KNO_3. It is not yet clear what this positive effect was due to. An effect as humectant is not considered here, since KNO_3 has a relatively high deliquescence point with 95% RH (*Fernández* et al., 2013). Stronger hygroscopic salts such as $CaCl_2$ (deliquescence point of 33% RH), on the other hand, can fulfil this purpose and thus promote I uptake into the plant tissue (*Lawson* et al., 2016). Further investigations must reveal whether such tank mixtures are also useful for the I fertilization of fruit crops.

The addition of Na_2SeO_4 to a spray solution containing IO_3^- did not affect the I content of the treated pears and apples. This confirms results from previous studies on apple trees (*Budke* et al., 2020b). Likewise, in studies on the biofortification of lettuce and rice, no interactions between IO_3^- and SeO_4^{2-} were found with regard to the uptake of both trace elements (*Smoleń* et al., 2014, 2016d; *Prom-u-thai* et al., 2020). In contrast, in field experiments with carrots and wheat, a slight reduction of I accumulation in the edible plant parts was observed when Se was simultaneously applied to the soil or Se and other micronutrients to the leaf (*Smoleń* et al., 2016c; *Zou* et al., 2019). However, the effects were not consistent, but varied depending on year and location.

The combined foliar fertilization of KIO_3, KNO_3 and Na_2SeO_4 increased the Se content in the fruits 6 times compared to the control in apples and 21 times in pears. However, the maximum accumulation remained below 3.0 µg Se $(100\ g\ FM)^{-1}$ and was thus of a similar order of magnitude as previously determined for apples with a combined KI and Na_2SeO_3 foliar spray (*Budke* et al., 2020b). In both studies, the total Se fertilization

rate applied was 50 g (ha · m CH)$^{-1}$. From a human nutritional point of view, the optimal molar I/Se ratio in foods is about 6:1 (*Lyons*, 2018). For example, at a content of 50 µg I (100 g FM)$^{-1}$, the target value for Se would be 5.2 µg (100 g FM)$^{-1}$. In a study by *Groth* et al. (2020), Se content of this level was achieved in apples by a foliar spray of 150 g Se (ha · m CH)$^{-1}$, regardless of whether SeO_3^{2-} or SeO_4^{2-} was applied. Further field experiments are needed to examine the effects of appropriately increased Se fertilization rate in combination with I. In the leaves of the apple trees we examined, the Se content was several times higher than in the fruits, as already observed with I. Translocation of I and Se from leaves to seeds in wheat is mainly through phloem transport (*Cakmak* et al., 2017; *Prom-u-thai* et al., 2020), while our findings indicate that this route does not seem important for biofortification of pome fruits.

Effects of fruit storage

Cold storage of I-sprayed apples at 2 °C for three months reduced the I content of the fruit by about one fifth. In contrast, no statistically significant changes were observed in pears (**Figure 4**). In the apples, the storage-related reduction of the I content was limited to the fruit peel, while the content in the fruit flesh remained relatively stable (**Table 5**). At harvest time, the I content in the fruit peel was 6.6 times higher than in the fruit flesh. However, after storage this difference was reduced to about half.

In I-biofortified nectarines, which were stored at 5 °C for two weeks, the I content also remained unchanged (*Caffagni* et al., 2012). Gaseous emissions associated with the activity of methyltransferases have been detected in numerous plant species. These enzymes catalyze the formation of methyl iodide (CH_3I), a volatile compound, which can escape into the atmosphere (*Itoh* et al., 2009). Besides a role in plant defense, this mechanism may serve to prevent toxic levels of I accumulation in higher plants (*Gonzali* et al., 2017). Additionally, I volatilization can be catalyzed by vanadium-dependent haloperoxidase, leading to synthesis of volatile hydrogen halides. Recently, activity of these enzymes in relation to I uptake has been demonstrated for lettuce (*Smoleń* et al., 2020). In brown alga *Laminaria digitata* volatilization of cellular I by vanadium-dependent haloperoxidases is thought to be a potential tool in defense against pathogens and I volatilization is important to maintain osmotic balance (*Verhaeghe* et al., 2008). However, to the best of our knowledge the activity of I-specific halide methyltransferases or haloperoxidase in pome fruits has not been studied.

Fruit storage did not affect the Se content of apples or pears (**Figure 4**). Nevertheless, it is known that plants are able to form volatile Se compounds such as dimethyl selenide [$(CH_3)_2Se$] and dimethyl diselenide [$(CH_3)_2Se_2$] from Se-containing amino acids (*Malagoli* et al., 2015). However, these processes obviously do not play a significant role in stored pome fruit at the Se enrichment level achieved in our study. To what extent a longer storage time – apples can be stored under controlled atmosphere conditions until the following year's harvest – affects fruit Se and I losses should be examined in further trials.

Effects of fruit washing, peeling and core removal

When segments of I-biofortified apples and pears were washed under running deionised water shortly after harvesting, this reduced their I content by 14%. Losses of a similar magnitude were observed for Se in the Se-fertilized treatment (**Figure 5**). This shows that most of the I and Se detected in the fruits was completely absorbed or adhered so firmly to the fruit peel that it could not be removed by normal washing procedures. A strong sorption of foliar-applied I on to cuticular waxes was observed on leaves of field beans (*Shaw* et al., 2007). Losses of I of up to 30% were observed when washing strawberries that received a final I spray six days before harvest. Longer pre-harvest intervals reduced I losses to below 20% (*Budke* et al., 2020a). In our experiments the pre-harvest interval was at least two weeks and in the second field trial with apples the last foliar fertilization was carried out almost six weeks before harvest.

After I foliar sprays, the fruit peel contains much higher concentrations of I compared to the flesh. Therefore, peeling lowers the I content of the fruit. In freshly harvested apples of the I-fertilized treatments, it decreased by 51% and in pears by as much as 78%. Similarly, high peeling-related I losses were previously reported for apples (*Budke* et al., 2020b). For nectarines, however, the peeling of I-biofortified fruits did not lead to a significant change in the I content (*Caffagni* et al., 2012). This may be due to the differences in fruit peel properties between pome and stone fruits affecting the penetration of I into the fruit. Furthermore, it should be noted that the I enrichment in the nectarines was lower by more than a factor of 10 compared to pome fruits that we investigated. For Se the peeling effects were subject to stronger fluctuations, which is probably due to the relatively low Se content of Se-sprayed fruits. In general, peeling of pome fruit is not recommended, since not only are larger amounts of biofortified I and Se lost but also health-promoting secondary plant compounds from the group of flavonoids, which are mainly localized in the fruit peel (*Drogoudi* et al., 2008).

The fruit core of I-fertilized apples and pears always had the lowest I content within the fruit [5.6 µg (100 g FM)$^{-1}$]. In total, not more than 1–3% of the I contained in a fruit was found in the fruit core. This indicates that only a small part of the I absorbed via the fruit surface penetrated to the center of the fruit.

As the core of apples and pears is usually not consumed, the limited translocation of the I in the fruit is advantageous with regard to its utilization for human nutrition. The I content in fruits without the core was about 9% higher in apples and about 14% higher in pears than in the whole fruit. This difference must be taken into account when in future the I content needs to be determined for quality control procedures and the marketing of I-biofortified pome fruit. In this case, it is useful to analyse the I content in washed, cored fruits in order to indicate adequately the contribution of the products to the dietary I intake.

Content of total soluble solids

The total soluble solids content is often used as an indicator for the sugar content and sweetness of fruits (*Charles* et al., 2017). These fruit characteristics have a significant influence on the taste and consumer acceptance of apples and pears (*Hoehn* et al., 2003; *Predieri* et al., 2014). Spraying KIO_3 alone did not lead to a significant change in total soluble solids content in either of the two types of pome fruits analyzed. In apples, a combined application of KIO_3 and KNO_3 increased the total soluble solids content by about 1.0 °Brix. An increase of the same order occurred in apples as well as in pears when a pure KNO_3 leaf fertilizer was applied. These positive effects remained even after three months of cold storage of the fruits (**Figure 8**). In accordance with this, *Shen* et al. (2016) report that foliar sprays with KNO_3 in 'Kousui' Japanese pears led to an increase of fructose and sucrose content in the fruits and thereby significantly increased their sweetness. Other potassium-containing fertilizers also had a beneficial effect in this respect. Potassium plays an important role in the photosynthesis of the leaves and the translocation of the assimilates into the fruits (*Zörb* et al., 2014). Nevertheless, the positive influence of potassium foliar fertilization on the sugar content of the fruits in our field experiment is surprising, since the plant-available potassium content of the soil at the experimental site was in the optimal range [class C according to VDLUFA (*Kießling* and *Hoffmann*, 2016)]. The effect of I on sugar accumulation in fruits can vary considerably depending on the amount of I applied, as shown by studies on strawberries. In hydroponically cultivated strawberries, a moderate increase of the I concentration in the nutrient solution promoted the accumulation of soluble sugars in fruits. In contrast, high I concentrations in the nutrient solution reduced the fruit sugar content (*Li* et al., 2017). Likewise, after repeated KI sprays on strawberries grown in the field, a significant reduction of total soluble solids content was observed when a total of 0.8 kg I ha^{-1} was applied. In contrast, I fertilizer applications of ≤ 0.4 kg I ha^{-1} had no such adverse effects (*Budke* et al., 2020a).

The addition of Na_2SeO_4 in fertilizer mixtures with KIO_3 and KNO_3 did not affect the total soluble solids content of apples and pears. *Pezzarossa* et al. (2012), however, reported that pure Se spraying of pear trees led to a significant increase in the total soluble solids content of fruits. In this field experiment Na_2SeO_4 was also used, but with a significantly lower concentration in the spray solution (1.0 mg Se L^{-1}) than in our study (50 mg Se L^{-1}). In peaches, which were also included in the aforementioned study, no corresponding effects were found depending on Se fertilization (*Pezzarossa* et al., 2012). In hydroponically cultivated strawberries, it was possible to increase the total soluble solids content by about 2.0 °Brix if about 8 mg Se L^{-1} was added to the nutrient solution as Na_2SeO_4 (*Mimmo* et al., 2017). In grapes, the content of glucose, fructose and sucrose correlated closely with the Se content of the fruits. Here, Se was added by application of a leaf fertilizer containing 120 mg L^{-1} organically bound Se in the spray solution (*Zhu* et al., 2017).

Taken together, it appears that, in addition to potassium, I and Se can also promote the accumulation of sugar in fruits. However, there are differences in this respect depending on the type of fruit, the fertilization level, the form of fertilization and probably also the application technique, which need to be further investigated.

Phytotoxic effects

Spraying with I-containing fertilizers caused leaf necroses on apple and pear trees, which increased with increasing I doses (**Figures 6 and 7**). Similar damage was previously observed in different plant species (*Caffagni* et al., 2011; *Kato* et al., 2013; *Kiferle* et al., 2013; *Cakmak* et al., 2017; *Incrocci* et al., 2019; *Budke* et al., 2020a, 2020b). At equal concentrations I^- usually causes stronger phytotoxic effects than IO_3^-. One reason for this might be that I^- inhibits the activity of superoxide dismutase, while IO_3^- can promote its activity. This enzyme plays a key role in the defense against reactive oxygen species and thus in the prevention of cell damage (*Blasco* et al., 2011). In our study, however, no consistent differences between the two I species were observed with respect to the intensity of leaf damage.

The fruits of the apple and pear trees did not experience any sort of damage (**Figure 1**), even after three months of cold storage. The individual fruit weight also remained unaffected (**Table 4**). Furthermore, as discussed before, the total soluble solids content of the fruits was not reduced by I applications, and in combination with KNO_3 even increased significantly in some cases. Thus, we assume that the observed leaf damage had no negative influence on the fruit development. In the year after application, no abnormalities, e.g. with regard to fruit set or fruit development, were observed on the I- and Se-fertilized trees in the experiments conducted as well as in other investigations not yet published. However, we cannot exclude the possibility that long-term I and Se-supply have an adverse effect on fruit trees. To clarify this, fertilization trials in orchards over several years are necessary.

Implementation of iodine biofortification in pome fruit production

The biofortification of pome fruit with I can be integrated into fruit growing practice by means of foliar fertilization with relatively little effort and at acceptable costs. The application can be done with a standard orchard sprayer. With a raw material price of 60 US-$ per kg KIO_3 in food grade, an exchange rate of 1.18 US-$ per € and a fertilization quantity of 1.5 kg I (ha · m CH)$^{-1}$, the pure I fertilizer costs in an orchard with 2.5–3.0 m high trees amount to about 320–385 € ha^{-1}. In addition, there are the application costs, which are estimated to be about 50 € ha^{-1} per treatment (*Weitgruber*, 2016). Overall, with an average yield of 40 t, the I biofortification would result in additional costs of around 1.0–1.3 euro cents per kg of fruit. In the case of apple cultivation, for example, this would correspond to about 2.5–3.5% of total production costs (*Lang* and *Thomann*, 2008). The application costs are omitted or arise only proportionately if the I treatment can be combined with other sprays. KNO_3 and Na_2SeO_3 have proved to be suitable mixture components in the concentrations tested in our experiments.

Repeated calcium sprays are common in pome fruit cultivation, among other things to prevent physiological disorders such as bitter pit in apples or flesh browning in pears (*Blanco* et al., 2010; *Wójcik*, 2012). Therefore, further investigations should be carried out to determine whether I can also be applied together with this plant nutrient. However, when using IO_3^- as I species, miscibility is limited here by the low water solubility of $Ca(IO_3)_2$, which is 2.43 g L^{-1} at 20 °C (*John*, 2019). In 600 liters of water, which are usually applied

with an orchard sprayer per hectare, up to 0.95 kg I could be dissolved as $Ca(IO_3)_2$. Thus, for the application of 3.75–4.50 kg IO_3^--I ha^{-1}, four to five treatments with such a spray solution are necessary. If I^- is used, the required I supply can be achieved with fewer treatments, since CaI_2 is much more soluble in water [676 g L^{-1} at 20 °C (*John*, 2019)]. In field experiments with lettuce, the addition of calcium nitrate [Ca(NO3)2] to an IO_3^--containing spray solution had no effect on I uptake into the foliage, while $CaCl_2$ was beneficial (*Lawson* et al., 2016). Tank mixtures of KIO_3 with selected pesticides were also successfully tested in the aforementioned study.

It was also possible to achieve the I enrichment targeted for apples and pears by a single foliar fertilization with KIO_3 or KI (**Figure 2**). In our first field experiment, this treatment was applied two weeks before harvest. At the highest fertilization level, with 2.5 kg I (ha · m CH)$^{-1}$, the trees were largely defoliated three weeks after harvest (**Figure 7**). This conspicuous side effect of I sprays could possibly be used in pome fruit cultivation to promote the coloration of the fruits, especially of varieties with red peel color, by improving exposure to light. In further investigations it will be necessary to check which treatment date and which I application quantities are particularly suitable for this purpose. Currently, a technique is being tested for pre-harvest defoliation of apple trees in which the outer leaves are removed by means of compressed air two to four weeks before harvesting (*Andergassen* and *Pichler*, 2019). This requires first of all the purchase of a special defoliation machine. Furthermore, it should be noted that the pneumatic defoliation can lead to increased fruit drop and pressure marks on the fruit. Last but not least, the associated treatment costs of around 1,600 € ha^{-1} (*Andergassen* and *Pichler*, 2019) are significantly higher than for I sprays.

A premature leaf fall in apple trees could also be interesting from a phytosanitary point of view. The ascospores of the apple scab (*Venturia inaequalis*), from which the primary infection starts in spring, overwinter on the fallen leaves. In order to ensure a rapid conversion of the leaf material, urea sprayings are carried out after harvesting and the fallen leaves are then mulched (*Holb*, 2006; *Singh*, 2019). The earlier this is done in autumn, the more complete the decomposition process can progress. To what extent a late I application is useful in this respect and whether such a treatment can contribute to the reduction of scab infestation in an apple orchard should be investigated in further field trials.

Conclusion

Pome fruits can be biofortified with I to an extent appropriate for human nutrition when cultivated under orchard conditions by means of foliar fertilizer sprays. The supply of KIO_3 at a total application rate of 1.5 kg I (ha · m CH)$^{-1}$ increased the I content in washed apples and pears to about 50–60 µg (100 g FM)$^{-1}$ without affecting the development and marketability of the fruits. The consumption of such an I-enriched fruit of average size (about 175 g) would cover about two thirds of the recommended daily I intake of 150 µg for an adult (*EFSA*, 2006). Foods declared and marketed in the European Union with nutritional claims must have a certain I content in accordance with Regulation (EC) No 1924/2006 (*European Commission*, 2011). With an I content of ≥ 22.5 µg (100 g FM)$^{-1}$, corresponding to 15% of the recommended daily allowance for I, foods may be labeled as

a "source of iodine". If the I content is twice as high, the products can be labeled as "rich in iodine". Such foods may also be advertised with health claims such as "iodine contributes to normal thyroid function" according to Regulation (EU) No. 432/2012 (*European Commission*, 2012). The approach thus offers fruit producers an interesting option for increasing the nutritional value of their products, and to take advantage of this in marketing.

In the field experiments performed, only fruits hanging on the outside of the tree and thus those directly wetted by the spray solution were examined. It can be assumed that fruits from inside the tree, which were partially or entirely covered by leaves, had lower I contents. Therefore, in further investigations variations in the range of the I enrichment of the fruits depending on their position on the tree should be investigated. In this context, the application technique used might also play an important role. With air-blast orchard sprayers, as used in commercial tree fruit cultivation, a significantly better penetration can probably be achieved than with the hand sprayers and backpack sprayers used in our experiments. With regard to the practical use of I biofortification in pome fruit cultivation, it also remains to be clarified what influence foliar fertilizer additives such as adhesive agents as well as tank mixtures with calcium-containing fertilizers and pesticides have on the effectiveness of the process. Furthermore, it is important to discover how fast the sprayed I penetrates the fruit and to what extent weather conditions affect this.

Supplementary material

Supplementary Table 1: Log-transformed iodine content in washed fruit segments of apples cv. 'Jonagold' at harvest time as affected by the dose and form of iodine foliar sprays in the first field experiment (n = 4).

Foliar spray treatment [kg I (ha · m CH)$^{-1}$]	KI	KIO_3	Mean Doses
0.00	0.354	0.343	0.349
0.25	1.243	1.184	1.213
1.00	1.951	1.554	1.753
2.50	2.436	1.989	2.213
Mean Forms	1.496	1.268	
Analysis of Variance			
Forms (F)	***		
Doses (D)	***		
F x D	***		
LSD 5% Forms	0.059		
LSD 5% Doses	0.084		
LSD 5% F x D	0.118		

Supplementary Table 2: Log-transformed iodine content in washed fruit segments of pears cv. 'Alexander Lucas' at harvest time as affected by the dose and form of iodine foliar sprays in the first field experiment (n = 4).

Foliar spray treatment [kg I (ha · m CH)$^{-1}$]	KI	KIO_3	Mean Doses
0.00	0.084	0.084	0.084
0.25	1.165	1.126	1.145
1.00	1.936	1.463	1.699
2.50	2.335	1.716	2.026
Mean Forms	1.380	1.097	
Analysis of Variance			
Forms (F)	***		
Doses (D)	***		
F x D	**		
LSD 5% Forms	0.112		
LSD 5% Doses	0.158		
LSD 5% F x D	0.224		

Chapter 3
General Discussion

To investigate the suitability of fruit crops for biofortification with iodine, strawberry, apple and pear were selected as plant species. These species have a great importance in terms of cultivation and consumption in Germany and corresponding iodine-rich fruits could thus contribute to the improvement of the iodine supply of the population. Soil and foliar fertilization were investigated as possible approaches in several years of trials within the framework of this work. An examination of other factors, such as the translocation of iodine in the plant or the influence of fruit storage, was also carried out. In the following chapter the results of the various experiments are discussed collectively in an overarching perspective and the hypotheses generated in section 1.7 (pp. 14ff) are evaluated. First, a description and discussion of a modification in the extraction of iodine from plant matrices is given.

3.1 Methodological improvement of iodine extraction from plant matrices

In this dissertation the methodical procedure was described in the corresponding subchapters in section 2 (pp. 17–92) Since the necessary laboratory work was very extensive, it was examined whether an optimization of the work steps is possible here. The extraction of iodine from plant matrices was carried out with TMAH over a period of 3 hours at 90 °C according to the method *DIN EN 15111* (2007). For this purpose, the sample substance is weighed into vessels that can be well sealed. Screw-top laboratory bottles with appropriate caps are usually used for this purpose. After the extract has cooled down, it is transferred to a volumetric flask and filled up to a defined mark with ultrapure water. The screw-top glass vessel must be filled several times with the water in small steps, closed and shaken to collect as much of the extract as possible and transfer it to the volumetric flask. The entire contents of the volumetric flask are then transferred to centrifuge tubes and centrifuged for 15 min. Reuse of the materials requires cleaning with a 0.5 percent TMAH rinse solution and the use of the laboratory dishwasher. Therefore, as part of the comprehensive laboratory work, it was investigated whether these steps could be modified.

Significantly higher working efficiency was achieved when weighing and extraction were performed directly in the centrifuge tubes. Steps such as the transfer of the solutions into the volumetric flasks and the cleaning of the tubes and caps can be omitted. In addition, there is no need for appropriate laboratory glassware with this procedure. The consumption of the TMAH solution is lower, since no rinsing solution is required, and the disposal costs of the solution are also eliminated. Further cost and time savings result from the fact that there is no need to use laboratory dishwashers. Furthermore, this circumvents the problem of residues contaminating a new sample if cleaning is inadequate. For this procedure, the centrifuge tubes must have an accurate scale so that filling to a defined quantity is possible. The accuracy is somewhat lower than with a volumetric flask (content of centrifuge tubes 45 mL ± 0.3 mL compared to content of volumetric flasks 50 mL ± 0.08 mL). However, it is advantageous that all extraction steps take place in one vessel and possible losses due to incorrect transfer of the extract are eliminated. During heating to 90 °C, the plastic tubes become softened and the caps may deform under certain

circumstances, leading to leaks. Therefore, the tubes should first cool down well after this step and damaged caps should be replaced. This was the case for about 5% of the centrifuge tubes used in this work (article number 525-1101, VWR International GmbH, Bruchsal, Germany).

Comparative measurements between this method and the conventional procedure showed no significant deviations ($R^2 = 0.97$, annex **Figure 1**, p. 128). In addition to certified reference material [ERM-BB422 fish muscle and NIST-1849a infant/adult nutritional powder (milk)], apple and lettuce powder from own trials which had previously been analyzed by an accredited laboratory (LUFA Nord-West, Hameln, Germany) was used here. The measurement range investigated was between 0.6 and 15.6 µg I per g dry matter. This corresponds to the measurement range in which the iodine content in this work was normally found. Therefore, it can be confirmed that this modified method is suitable for iodine extraction from plant matrices. The maximum sample quantity per working day was 48 samples for the conventional method. With the modified procedure, up to 80 samples per day can be processed.

3.2 Identification of an appropriate application method

With increasing iodine application, the iodine content in the fruits also increased in the soil and foliar fertilization trials conducted. A dose-dependent effect of iodine application was also confirmed in many other trials (*Medrano-Macías* et al., 2016; *Gonzali* et al., 2017). This effect is irrespective of the application method or the iodine form. A very close linear relationship ($R^2 = 0.97–0.99$) was found for iodide and iodate in apples and pears after foliar application (Section 2.3 First field experiment, pp. 62ff).

In all trials, it was also shown that foliar applications, in which the fruits are directly wetted, is clearly superior to soil fertilization. Thus it was also possible to confirm this hypothesis. After foliar fertilization with a lower iodine application rate, a higher iodine content was always achieved in the leaves and fruits of the experimental plants than after a treatment of the growing medium. The main problem with iodine soil fertilization seems to be the low plant availability of iodine. This explains the low iodine content in the strawberry fruits after relatively high iodine application rates of up to 7.5 kg ha^{-1}. On the basis of the model experiment performed with peat substrate (Section 2.2, **Figure 9**, p. 55), it was possible to capture the dynamics of the iodine. Already a few hours after application, a large part of the iodine was no longer available to plants. *Lawson* et al. (2015) were also able to show that a sustainable effect of soil fertilization was also not present. Lettuce and radish cultivated on the same area where iodine fertilization had been applied in the previous growing season showed a significantly lower iodine content. Measurements on a random basis of fruit and leaves of apple trees, one year after soil fertilization, confirmed this observation (Section 2.2, p. 59). The extent to which the iodine-rich algae already mentioned (*Weng* et al., 2008a, 2008b, 2013) could be usefully applied as fertilizer at this point would have to be clarified in further experiments.

After soil fertilization with iodine and uptake via the roots, translocation occurred mainly to the leaves of apple trees. In appropriately fertilized experimental variants, over 300 times more iodine per 100 g FM was determined in leaves compared to fruits. A similar relationship was shown in other plant species (*Landini* et al., 2011; *Caffagni* et al., 2012; *Li* et al., 2017). Thus, it is evident that after uptake by the root, iodine is primarily translocated to the more transpiring leaves via the xylem transport pathway. In agreement with this, *Li* et al. (2017) measured between 7 and 12 times more iodine in leaves after iodine fertilization of strawberries in a hydroponic system.

Foliar fertilization with iodine requires that the fruits must be hit directly by the spray solution. It was possible to confirm this by corresponding test variants with strawberries (dense natural foliage canopy) and apple trees in the plastic tunnel (fruits deliberately shielded). A high iodine content in the leaves of the investigated crops is irrelevant, since a transfer to the fruits occurred only to a very small extent. The same is the case for younger leaves formed after foliar application. The phloem mobility or the retranslocability of iodine can therefore be classified as low in the plant species studied. Comparable results were obtained by *Humphrey* et al. (2019) in spinach plants. Here, a phloem mobility of less than 2% was detected. However, studies on tomato and cereal plants showed that appreciable amounts were present in fruits and seeds after foliar application (*Landini* et al., 2011; *Cakmak* et al., 2017; *Zou* et al., 2019). These different translocation patterns are probably due to plant genotypic differences and should therefore be tested individually in follow-up experiments.

In most cases, foliar application of iodide resulted in a higher iodine content in the plant mass compared to application of iodate. In apple and pear trees in the open field (Section 2.3, First field experiment, p. 72), up to 2.5 times more iodine was thus detected in the fruit at the same application rate. The advantageous physicochemical properties of potassium iodide, such as its lower molecular weight and valence (*Umaly* and *Poel*, 1971, *Mackowiak* and *Grossl*, 1999), thus obviously favor its uptake into plant tissue. However, this greater uptake was found to be accompanied by large fluctuations in iodine content. Therefore, iodate is preferable for practical use, as it can more reliably achieve the desired target ranges in plant mass (*Lawson* et al., 2016; *Cakmak* et al., 2017).

3.3 Influence of fruit type on iodine biofortification

In principle, it was possible to increase the iodine content in the fruits of strawberries, apples and pears to the desired target range between 50 and 100 µg I per 100 FM. By consuming 100 to 200 g of such an enriched fruit, the iodine deficit in the german population could thus be counteracted. Depending on the iodine form, an optimal application rate can be determined for each type of fruit to achieve this target range. However, there are specific limitations related to the different growth and fruit characteristics that must be considered.

For example, it was assumed that the strawberry is particularly well suited for iodine biofortification due to its relatively thin fruit skin. An application of 0.2 kg I^--I per ha was

therefore already almost sufficient to reach the target range. For apple and pear trees, 1.5 kg IO_3^--I per ha and m canopy height (i.e. approx. 3.75 kg for trees with a height of 2.5 m) was necessary for this purpose. However, field-grown strawberries have crucial disadvantages that make practical use of this berry fruit in the context of iodine biofortification difficult. For example, the iodine application must take place a few days before harvest for a successful biofortification. Since strawberries are harvested over several weeks and a dilution effect occurs due to fruit growth, one-time iodine sprays at the beginning of the harvest time are not effective. Furthermore, fruits that form after the application are not covered by the spray solution at all. Many applications during the harvest period, each with low doses of iodine, would therefore be necessary. Another major disadvantage is that the plants form a much denser canopy after the first year of cultivation. This canopy shielded the fruits to such an extent that they were not sufficiently hit by the iodine solution. As mentioned earlier, iodine translocation from leaves to fruit is low. Therefore, this fruit type is not suitable for successful biofortification in the cultivation method investigated or is associated with increased additional effort. The extent to which other cultivation methods, such as systems with fertigation in protected cultivation, are suitable here would have to be clarified in further trials. *Li* et al. (2017), for example, were able to cultivate strawberries with a relatively high iodine content in the fruit mass in a hydroponic system.

The application of iodine to apple and pear trees was sometimes carried out several weeks before harvest. Therefore, the time interval here is significantly longer compared to the treatment dates in the trials with field-grown strawberries. Nevertheless, iodine enrichment in the target range was achieved in these pome fruit species. This is due to the fact that the fruits were already formed at the time of application. In contrast to strawberries, the ripening and size increase of the fruits thus takes place more uniformly. The application rate necessary to enable reaching the target range can therefore be calculated relatively well. Since apple trees in commercial orchards are usually cultivated as slender spindles, this method of cultivation allows good wetting of the fruits. However, it is unclear to what extent strongly covered fruits are affected by the iodine solution and how the content in the fruit mass may vary as a result. In the trials conducted, there were either no hidden fruits (Section 2.2, e.g. **Figure 1a**, p. 45) or they were intentionally not included in the harvest (Section 2.3, pp. 62ff). However, hand-held application systems were used here (electric spray gun or backpack sprayer), which have a significantly lower penetrating power compared to professional orchard sprayers. Appropriate equipment and differentiated sampling must therefore be considered in further trials. In trials on selenium biofortification on 'Elstar' apple trees, it was shown that covered fruits contained only about half as much selenium as well-exposed fruits. Here, an over-row sprayer was used (results not published). A comparable order of magnitude is therefore conceivable for iodine.

In terms of fruit surface, apple and pear varieties differ in certain details such as the number of stomata and lenticels per cm² or the thickness of the cuticular wax layer (*Belding* et al., 1998, *Durić* et al., 2015, *Guan* et al., 2015). However, fruit size at harvest time was found to have a much greater influence on iodine content in the fruit mass. Therefore, smaller 'Williams Christ' pears, for example, had more iodine in the fruit mass than the larger 'Alexander Lukas' pears with the same calculated application rate. The higher fruit weight also means a higher volume and thus a potential dilution effect for absorbed iodine.

In addition to adjusting the amount of iodine to the appropriate size of the trees (m canopy height), it would be conceivable to apply a correction factor for particularly large fruit at this point. In relation to the amount of iodine in the whole fruit, however, this difference is set into relation again, so that the values were relatively close to each other (72 µg I per fruit for 'Alexander Lukas' and 80 µg I per fruit for 'Williams Christ').

3.4 Plant compatibility of iodine fertilization

The extent to which a fertilization measure is compatible with plants can be evaluated by means of various parameters. For example, the yield or the visual appearance of plants and fruits are possible criteria. The amount of iodine applied, the form of iodine, the application method and the number of applications are decisive influencing factors. Various authors state that iodide can cause more severe damage to plants compared to iodate (*Mackowiak* and *Grossl*, 1999; *Zhu* et al., 2003; *Caffagni* et al., 2011; *Cakmak* et al., 2017). Therefore, it was assumed that this was also the case for the fruit species studied.

However, contrary to expectations, there was no significantly greater leaf damage after iodide application to apple and pear trees compared to iodate (Section 2.3, First field experiment, **Table 6**, p. 80 and **Figure 7**, p. 81). The iodine dose had a more significant effect on damage symptoms than the iodine form at this point. In the strawberry plants, violet discoloration occurred on the leaves, among other symptoms. This is due to increased synthesis of anthocyanins and has already been observed in other experiments (*Blasco* et al., 2008). Furthermore, chlorosis and necrosis were observed in all fruit species after iodine application. These damage symptoms were also described in other experiments and explained with an intracellular oxidation to elemental iodine (*Mynett* and *Wain*, 1971, 1973; *Kiferle* et al., 2013). Fruit weight represents another measurable parameter. The trials with field-grown strawberries showed that there was no reduction in yield in any variant. The average individual fruit weight was also not affected. In the trials with apple trees in the plastic tunnel and the trials in the orchard, care was taken from the outset to ensure a homogeneous fruit set. However, the parameter "fruit yield" could not be reliably used to make a statement, since significantly more trees would be necessary here for valid results. The average individual fruit weight was also not influenced by the fertilization measures.

None of the test variants carried out resulted in damage to the fruits. The iodine measurements showed that the fruits accumulated significantly less iodine than the leaves. Undamaged fruits are very important for later marketing as dessert fruit. However, should this occur, it would be conceivable to divide the iodine dose over several applications before harvest. For the apple and pear trees in 2018 (Section 2.3, Second field experiment, pp. 62ff), the iodine dose was divided into 2 to 3 dates. Nevertheless, it was possible to achieve reliably the targeted iodine content in the fruit mass.

Besides chlorosis and necrosis, increased leaf fall was also observed in apple and pear trees (Section 2.3, **Figure 7**, p. 81). However, this side effect could be specifically

used in fruit growing to improve the coloration of the fruit shortly before harvest. Further trials on a relatively large scale are necessary to determine the optimum iodine quantity and the correct application time for this purpose. The average fruit coloration can then be reliably determined via sorting machines.

3.5 Iodine distribution in the fruit, effect of fruit preparation and storage

Peeling the apples and pears significantly reduced the iodine content. This means that a large proportion of the iodine is bound in the fruit peel or cuticular waxes (51–73%, Section 2.3, **Figure 5**, p. 77). Strong sorption of iodine after foliar application was also observed in the waxy layer of leaves in broad bean (*Shaw* et al., 2007). However, since the fruit peel contains many valuable constituents apart from iodine, it is not recommended to peel pome fruit anyway (*Drogoudi* et al., 2008).

Furthermore, it was suspected that the iodine continues to diffuse into the fruit flesh over the 3-month storage period. During fruit storage, transpiration losses cause the fruit to become somewhat lighter (approximately 3%, results not shown). Therefore, the iodine content should generally be somewhat higher after storage. However, it was found that in apples, significant losses of about 20% occurred in the fruit peel in some cases, while the content in the fruit flesh remained relatively constant. This reduction can only be explained by gaseous release. Plants are capable of emitting iodine as methyl iodide (CH_3I) (*Itoh* et al., 2009). This could be considered as a kind of detoxification process. The extent to which this process occurs during cold storage of apples and whether this is really the cause of the loss rates would have to be clarified in follow-up experiments. However, for the marketing of corresponding apples and the associated declaration of the iodine content, these losses would have to be taken into account. Fluctuations in the range of +45% and -35% of the declared value are, nevertheless, permissible according to Regulation (EU) No 1169/2011 (*European Commission*, 2011). Damage to the fruit or other abnormalities were also not observed after storage.

On average, iodine losses due to fruit washing were found to be about 14% for apples and pears at harvest time. After storage, the value was about 12% (Section 2.3, **Figure 5**, p. 77). A certain amount of iodine thus remains externally attached to the surface during storage and is not further absorbed into the fruit. In freshly harvested strawberries, it was possible to determine iodine losses of up to 30% by washing the fruit if iodate was applied 6 days before harvest. For iodide, this value was about 24%. *Lawson* et al. (2016) were also able to show in experiments with lettuce that leaves absorbed iodide more strongly. The losses due to washing were greater here for iodate. In summary, it can be stated that the uptake of iodide is faster than the uptake of iodate and that a certain amount of iodine, which adheres externally to the plant surface, is not taken up even after a longer period of time. Iodate was nevertheless preferred in our experiments because, as already mentioned, this form of iodine led to more uniform results and various authors attested that plants tolerated it better.

3.6 Influence of iodine fertilization on the sugar content of fruits

The taste of the fruit, in addition to health-related aspects, is also an important purchase criterion (*Wortmann* et al., 2018). The sugar content is of particular importance here, and soluble solids can serve as an indicator (*Aprea* et al., 2017; *Charles* et al., 2017). In strawberries, it was confirmed that with increasing leaf damage, soluble solids content in fruits slightly decreased. *Li* et al. (2017) came to a similar conclusion. Here, a slight increase was indeed recorded at first. However, above a certain level of iodine, a decrease in sugar content occurred. In the same study, a similar relationship was observed for vitamin C. Up to a certain dose, which depends among other things on the plant species and the cultivation system, stimulation of the synthesis of plant constituents is thus possible before the harmful effect predominates. *Blasco* et al. (2008) were also able to demonstrate this in experiments with lettuce for total phenolic content, flavonoids and anthocyanins.

The apple and pear trees showed significant damage to the leaves after an application of 1.5 kg IO_3^-I (ha · m CH)$^{-1}$. Contrary to expectations, this did not result in a significant decrease in sugar content, but tended to increase it (**Figure 8**, p. 82). Taking into account the results of *Li* et al. (2017), this means either that the iodine dose was not yet high enough for a reduction, or that the pome fruit generally has a different reaction scheme at this point or a higher tolerance. Achieving the target level of between 50 and 100 µg I per 100 g FM, however, does not require a further increase in the iodine dose.

3.7 Combination of an iodine application with other foliar fertilization measures

The extent to which iodine biofortification can be combined with other foliar fertilization measures was also investigated as part of this work. This would make it conceivable to bundle several measures in one step. In the experiments with pome fruit (Section 2.2, pp. 41ff and 2.3, pp. 62ff) it was shown that a combined foliar application of iodine (KI or KIO_3) and selenium (Na_2SeO_3 or Na_2SeO_4) could increase the content of both trace elements. No effect of selenium on iodine content was found. Comparable results were observed by *Smoleń* et al. (2014, 2016d) in lettuce, where likewise no interactions between iodate and selenate were found. However, there are also indications that combined application may decrease the iodine content in plant mass (*Smoleń* et al., 2016c; *Zou* et al., 2019; *Cakmak* et al., 2020). Iodine application rates may need to be increased if a combined application with selenium results in a significant reduction. Nevertheless, this does not appear to be the case for pome fruit.

Washing of apples and pears at harvest time resulted in an average reduction of between 10 and 15% in selenium content. After uptake into the fruit, most of the selenium was localized in the fruit peel. Here, the 3-months of storage did not lead to further translocation into the fruit flesh. Thus, the uptake and translocation pattern are relatively

similar for iodine and selenium. However, a reduction of the selenium content during storage did not occur here. Plants are, in general, able to release selenium in the form of volatile selenium compounds (*Malagoli* et al., 2015). Although this was not observed in the trials performed, measurements in this respect should be carried out again in follow-up trials over a longer storage period.

Selenium application had a positive effect on the sugar content of pears, strawberries and table grapes in studies by other authors (*Pezzarossa* et al., 2012; *Mimmo* et al., 2017; *Zhu* et al., 2017). However, in the experiments conducted with apples and pears, such an influence could not be confirmed (Section 2.3, pp. 62ff). Possibly, the amount of selenium would have to be increased somewhat for this purpose. Nevertheless, *Pezzarossa* et al. (2012) were able to record positive results in pears already from a low selenium concentration of 1 mg Se L^{-1}. In the same study, however, no significant change was observed in peaches either.

Contrary to expectations, the additional application of potassium nitrate did not affect the iodine content. In contrast, *Cakmak* et al. (2017) found a significantly higher iodine uptake after foliar fertilization with iodate and potassium nitrate. Although, it is not entirely clear which cause led to this increase.

Potassium nitrate significantly increased the soluble solids of the fruits. Moreover, this increase was measurable after storage of the apples and pears. In 'Kousui' Japanese pears (*Pyrus pyrifola*), *Shen* et al. (2016) were also able to increase fructose and sucrose content with potassium nitrate after a foliar application. Potassium plays an important role in leaf photosynthesis and translocation of assimilates to fruits (*Zörb* et al., 2014). The soil contained an optimal amount of plant-available potassium at the time of the experiment [class C according to VDLUFA (*Kießling* and *Hoffmann*, 2016)]. It is therefore astonishing that further potassium fertilization had an increasing influence here.

In summary, it can be stated that the combination of iodine application with other foliar fertilization measures is, in principle, possible. On the one hand, it is important that the mixture of substances is adapted to the corresponding crop and the desired target ranges and tested in trials. On the other hand, certain substances can influence solubility, which can lead to undesirable precipitation products. For example, the solubility of calcium and iodate is very limited [2.43 g $Ca(IO_3)_2$ L^{-1} at 20 °C (*John*, 2019)]. However, calcium sprays are commonly used in commercial fruit production to counteract, among other things, physiological nutritional disorders in fruits (*Blanco* et al., 2010; *Wójcik*, 2012). The solubility of calcium iodide is significantly higher [676 g CaI_2 L^{-1} at 20 °C (*John*, 2019)]. Therefore, iodide could possibly be considered for combined application at this point.

Chapter 4
Conclusion

Due to the low translocation of iodine after uptake via the root, soil fertilization proved to be completely unsuitable for iodine biofortification. Only foliar fertilization, in which the fruits are directly wetted, can be used for a successful biofortification. The trial results showed that strawberries, apples and pears can, in principle, be biofortified with iodine and the target range of 50 to 100 µg I per 100 g FM can be achieved. In strawberries, however, some extra effort is required through regular spraying just before harvest to achieve this result and only plants in the first year of cultivation can be used. Therefore, biofortification of field-grown strawberries is relatively difficult. Using foliar applications, it was possible to raise the iodine content in apples and pears to the desired level with relatively less effort. With regard to the general efficiency of an iodine application, the application rate per area unit must be calculated in relation to the amount of iodine in the edible parts of the plant. For pome fruit, this efficiency is about 0.5% (use of KIO_3) to 1.1% (use of KI). In lettuce, where almost the entire above-ground portion of the plant is consumed, this efficiency is much higher at a maximum of 33% after foliar fertilization (*Lawson* et al., 2015). However, for a sustainable improvement of the nutritional supply for humans, it is beneficial if as many different fruits and vegetables with increased iodine content as possible are commercially available. From this point of view, the apple in particular is very well suited, since it has a wide, year-round availability.

The use of nutrition and health claims is useful in the marketing of such products. According to Regulation (EC) No 1924/2006 (*European Commission*, 2011), a food may be declared as a "Source of iodine" from a value of 22.5 µg I (100 g FM)$^{-1}$. Furthermore, according to Regulation (EU) No. 432/2012 (*European Commission*, 2012), statements such as "Iodine contributes to normal thyroid function" may be used. This could boost the sale of such products and thus create financial incentives for traders and growers.

For the implementation of iodine biofortification of pome fruit in commercial orchards, further trials on a larger scale are necessary. Common orchard sprayers should be used for this purpose. Furthermore, tests would have to be carried out as to whether a relatively constant level with regard to the iodine content in the fruits is achievable or whether high fluctuations occur at this point. The miscibility with various additives, pesticides and fertilizers also needs to be further investigated in order to keep the workload as low as possible by combining the measures.

The costs of an iodine application amount to about 320–380 € ha^{-1} (assumptions: 60 US-$ per kg KIO_3 in food grade, exchange rate of 1.18 US-$ per €, fertilization quantity of 1.5 kg I (ha · m CH)$^{-1}$, trees with a height of 2.5–3.0 m). In addition, there are application costs of about 50 € ha^{-1} (*Weitgruber*, 2016) which, however, would not apply or would have to be credited proportionally if the iodine is applied in combination with other plant protection products and fertilizers. At an average yield of 40 t ha^{-1}, this would result in costs of about 1.0 to 1.3 euro cents per kg, which corresponds to about 2.5 to 3.5% of the total costs in apple cultivation (*Lang* and *Thomann*, 2008). For the fruit grower, this additional expense is only bearable if it is offset by higher revenue.

No negative effects in terms of plant tolerance were observed from the combined application of selenium. Therefore, fruits enriched with both trace nutrients could address the deficiency in human nutrition in two cases at once. However, it is necessary to adjust

the amount of selenium to reach the optimal molar ratio of 6:1 from a nutritional point of view (*Lyons*, 2018). Furthermore, tests should be carried out as to whether the positive effect of KNO_3 co-application on total soluble solids in experimental scale can also be reproduced under practical conditions. The extent to which iodine fertilization, individually or in combination with other substances, can increase the sugar content or other bioactive substances, e.g. the increase in antioxidant compounds (*Blasco* et al., 2008), should also be investigated in this context.

Summary

Iodine is an essential nutrient for humans, which is often not ingested through food in adequate quantities. Currently, Germany is once again one of the countries in which there is an iodine deficiency in the population. Women between the ages of 20 and 40 are particularly affected, a critical situation since pregnant and lactating women have an increased iodine requirement. Iodization of table salt is a widely used prophylactic measure. However, this method is not sufficient and may become less important in the future if further dietary salt reduction occurs, as nutritionists are demanding. Alternative approaches are therefore needed to improve the supply.

One of these approaches is the agronomic biofortification of food crops. In this process, iodine is applied via fertilization measures during the cultivation of the plants. This gives the plants the ability to take up the mineral, which is only available in the soil to a very limited extent. In recent years, many studies have been published on the biofortification of vegetables and cereals. Foliar fertilization measures have proven to be significantly more efficient than soil fertilization measures. Nevertheless, up to now few results are available on the biofortification of fruit crops. However, fruit is also important for a healthy diet and the iodine supply of humans can only be improved if as many iodine-rich foods as possible are available. Therefore, the aim of this work was to investigate iodine biofortification of berry and tree fruit species in more detail.

In order to be able to achieve this objective, trials were performed over several years with strawberries, apple and pear trees. In addition to suitable application methods, the aim was to determine the iodine form (iodide and iodate) and the necessary iodine quantity. On the one hand, the measured iodine contents in the fruit and leaf tissue allowed conclusions to be drawn about the translocation of iodine in the plant. On the other hand, this made it possible to evaluate the basic suitability for iodine biofortification of the fruit crops investigated. Since iodine has a phytotoxic effect above a certain amount, the plant compatibility should also be tested. In addition, common household processing methods, such as washing or peeling the fruit, as well as fruit storage over several months, should provide information on the extent to which such measures could reduce the iodine content. Another study parameter was the soluble solids content, as there is evidence that iodine can affect the sugar content of fruit. Furthermore, a combined application of potassium nitrate and selenium was carried out and their influence on iodine and sugar content was investigated. Selenium is also an essential trace element, which is usually inadequately absorbed through the diet.

The results of the investigations showed that it was possible, in principle, to raise the iodine content of strawberries, apples and pears to a level of 50 to 100 µg iodine per 100 g fresh mass. In the case of strawberries, however, this was only feasible if the plants were in their first year of cultivation and the iodine was applied by foliar fertilization shortly before harvest. In the 2nd and 3rd year of cultivation, the plants had a very dense canopy, which prevented direct wetting of the fruit. However, direct wetting of the fruit surface with the iodine solution is imperative, as this was the only way to achieve a reliably high iodine

content in the fruit mass. Soil fertilization proved to be completely unsuitable in trials with strawberries and apple trees. The translocation of iodine after soil fertilization occurred mainly via the xylem transport into the strongly transpiring leaves and not into the fruits. In addition, compared to a foliar application, a significantly higher iodine application rate was required. Furthermore, experiments with apple trees cultivated in a plastic tunnel, protected from precipitation, showed that the iodine transfer via the phloem into the fruits was only marginal.

With regard to the phytotoxic effect of iodine application, no consistent difference was observed between potassium iodide and potassium iodate. Both forms of iodine did not affect yield or average individual fruit weight. Damage to fruit was not observed in any variant. However, with increasing iodine levels, significant damage to leaves was noticeable. Apple and pear trees also showed early leaf fall. Iodide generally led to significantly higher iodine contents in the plant mass after foliar application, but this was also associated with high fluctuations. With iodate, it was possible to reliably achieve the targeted iodine content in the fruit mass of apple and pear trees with an application rate of 1.5 kg iodine per hectare and meter canopy height.

Washing the fruit reduced the iodine content of strawberries by up to 30%. For apples and pears, this value was about 14% at harvest and about 12% after 3 months of storage. Peeled apples and pears showed a significantly reduced iodine content. 51% of the iodine in apples was bound in the fruit peel or the cuticular waxes. A reduction of 73% was determined for pears. Cold storage for 3 months resulted in a significant loss of iodine in parts of the apple peel. At this point, the release of volatile iodine compounds is probably the cause of the reduction. However, this would still have to be confirmed by further investigations.

Iodine application had a negative effect on the soluble solids content of strawberries above a certain level. It was not possible to observe significant changes for pome fruit in the trials conducted. However, the application of potassium nitrate (alone and in combination with iodine) resulted in an increase. Iodine uptake remained unaffected by the combined application of potassium nitrate and selenium. However, it was shown that selenium has a comparable uptake and translocation pattern to iodine and that a combined biofortification with both minerals is, in principle, possible.

Accordingly, apple and pear trees are well suited for biofortification with iodine by foliar fertilization. However, further trials in commercial orchards are necessary to implement this process. In the future, appropriately fortified fruit could make an important contribution to the alimentary iodine supply for humans.

Zusammenfassung

Iod ist ein essentielles Nährelement für Menschen, welches häufig nur unzureichend über die Nahrung aufgenommen wird. Aktuell zählt Deutschland wieder zu den Ländern, in denen ein Iodmangel in der Bevölkerung besteht. Frauen im Alter von 20 bis 40 Jahren sind hiervon besonders häufig betroffen, was kritisch ist, da schwangere und stillende Frauen einen erhöhten Iodbedarf aufweisen. Die Iodierung von Speisesalz ist eine weitverbreitete Prophylaxemaßnahme. Allerdings reicht diese Methode nicht aus und könnte in Zukunft noch an Bedeutung verlieren, wenn eine weitere Salzreduktion in der Nahrung stattfindet, wie es Ernährungsmediziner fordern. Daher sind alternative Ansätze zur Verbesserung der Versorgungssituation notwendig.

Einer dieser Ansätze ist die agronomische Biofortifikation von Nahrungsmittelpflanzen. Dabei wird während des Anbaus der Pflanzen Iod über Düngungsmaßnahmen appliziert. Die Pflanzen werden dadurch in die Lage versetzt, den im Boden nur sehr begrenzt verfügbaren Mineralstoff aufzunehmen. In den letzten Jahren wurden bereits viele Studien zur Biofortifikation von Gemüse und Getreide veröffentlicht. Dabei erwiesen sich Blatt- gegenüber Bodendüngungsmaßnahmen als deutlich effizienter. Allerdings liegen bislang nur wenig Ergebnisse zur Biofortifikation von Obstkulturen vor. Für eine gesunde Ernährung ist Obst jedoch ebenfalls wichtig und die Iodversorgung des Menschen kann nur dann verbessert werden, wenn möglichst viele iodreiche Lebensmittel zur Verfügung stehen. Daher war das Ziel dieser Arbeit, die Iod-Biofortifikation von Beeren- und Baumobstarten näher zu untersuchen.

Um dieses Ziel erreichen zu können, wurden mehrjährige Versuche mit Erdbeeren, Apfel- und Birnbäumen durchgeführt. Dabei sollte neben geeigneten Applikationsmethoden unter anderem die Iodform (Iodid und Iodat) und die notwendige Iodmenge bestimmt werden. Die gemessenen Iodgehalte in der Frucht- und Blattmasse ließen einerseits Rückschlüsse auf die Verlagerung des Iods in der Pflanze zu. Andererseits war so die Bewertung der grundsätzlichen Eignung für eine Iod-Biofortifikation der untersuchten Obstkulturen möglich. Da Iod ab einer bestimmten Menge eine phytotoxische Wirkung aufweist, sollte ebenfalls die Pflanzenverträglichkeit geprüft werden. Haushaltsübliche Aufbereitungsmethoden, wie das Waschen oder Schälen der Früchte sowie eine Fruchtlagerung über mehrere Monate sollten zudem Aufschluss darüber geben, inwieweit solche Maßnahmen den Iodgehalt mindern könnten. Ein weiterer Untersuchungsparameter war der Gehalt an löslicher Trockensubstanz, da es Belege dafür gibt, dass Iod den Zuckergehalt von Früchten beeinflussen kann. Des Weiteren wurde eine kombinierte Applikation von Kaliumnitrat und Selen durchgeführt sowie deren Einfluss auf den Iod- und Zuckergehalt untersucht. Selen ist ebenfalls ein essentielles Spurenelement, welches über die Nahrung in der Regel in zu geringem Umfang aufgenommen wird.

Die Ergebnisse der Untersuchungen zeigten, dass es grundsätzlich möglich war, den Iodgehalt von Erdbeeren, Äpfeln und Birnen auf einen Gehalt von 50 bis 100 µg Iod pro 100 g Frischmasse anzuheben. Bei Erdbeeren war dies allerdings nur möglich, wenn

es sich um Pflanzen im ersten Standjahr handelte und die Iodapplikation mittels einer Blattdüngung kurz vor der Ernte erfolgte. Im 2. und 3. Standjahr wiesen die Pflanzen ein sehr dichtes Laubdach auf, was eine direkte Benetzung der Früchte verhinderte. Die direkte Benetzung der Fruchtoberfläche mit der Iodlösung ist allerdings zwingend notwendig, da nur so ein zuverlässig hoher Iodgehalt in der Fruchtmasse erreicht werden konnte. Bodendüngungen erwiesen sich bei Versuchen mit Erdbeeren und Apfelbäumen als gänzlich ungeeignet. Die Verlagerung von Iod nach einer Bodendüngung erfolgte hauptsächlich über den Xylemtransportweg in die stark transpirierenden Blätter und nicht in die Früchte. Außerdem war verglichen zu einer Blattapplikation eine deutlich höhere Iodaufwandmenge notwendig. Durch Versuche mit Apfelbäumen, die in einem Folientunnel, geschützt vor Niederschlag kultiviert wurden, konnte zudem gezeigt werden, dass die Iodverlagerung über das Phloem in die Früchte nur marginal war.

In Hinblick auf die phytotoxische Wirkung einer Iodapplikation wurde kein einheitlicher Unterschied zwischen Kaliumiodid bzw. Kaliumiodat erfasst. Beide Iodformen beeinflussten nicht den Ertrag bzw. das durchschnittliche Einzelfruchtgewicht. Schäden an den Früchten wurden in keiner Variante beobachtet. Allerdings war mit steigender Iodmenge eine deutliche Schädigung der Blätter zu erkennen. Bei Apfel- und Birnbäumen zeigte sich zudem ein frühzeitiger Blattfall. Iodid führte in der Regel nach einer Blattapplikation zu deutlich höheren Iodgehalten in der Pflanzenmasse, damit waren jedoch ebenfalls hohe Schwankungen verbunden. Mit Iodat konnte mittels einer Aufwandmenge von 1,5 kg Iod pro Hektar und Meter Kronenhöhe zuverlässig der angestrebte Iodgehalt in der Fruchtmasse von Apfel- und Birnbäumen erreicht werden.

Das Waschen der Früchte reduzierte den Iodgehalt bei Erdbeeren um bis zu 30 %. Bei Äpfeln und Birnen lag dieser Wert bei ca. 14 % zum Erntezeitpunkt und bei ca. 12 % nach der 3-monatigen Lagerung. Geschälte Äpfel und Birnen wiesen einen deutlich geminderten Iodgehalt auf. 51 % des Iods waren bei Äpfeln in der Fruchtschale, bzw. den kutikulären Wachsen gebunden. Eine Minderung von 73 % konnte hier für Birnen ermittelt werden. Die Kühllagerung über 3 Monate führte teilweise zu einem signifikanten Iodverlust in der Apfelschale. Wahrscheinlich ist an dieser Stelle die Abgabe volatiler Iodverbindungen für die Minderung ursächlich. Dies müsste allerdings noch durch weitere Untersuchungen bestätigt werden.

Eine Iodapplikation wirkte sich ab einem gewissen Level negativ auf die lösliche Trockensubstanz von Erdbeeren aus. Signifikante Veränderungen konnten für Kernobst bei den durchgeführten Versuchen nicht beobachtet werden. Jedoch führte die Applikation von Kaliumnitrat (allein und in Kombination mit Iod) zu einer Steigerung. Die Iodaufnahme blieb durch die kombinierte Applikation von Kaliumnitrat und Selen unbeeinflusst. Es zeigte sich allerdings, dass Selen ein vergleichbares Aufnahme- und Verlagerungsmuster wie Iod aufweist und eine kombinierte Biofortifikation mit beiden Mineralstoffen grundsätzlich möglich ist.

Apfel- und Birnbäume sind demnach gut für eine Biofortifikation mit Iod mittels einer Blattdüngung geeignet. Weitere Versuche in Obstbaubetrieben sind allerdings notwendig, um dieses Verfahren zu implementieren. In Zukunft könnte entsprechend angereichertes Obst einen wichtigen Beitrag für die alimentäre Iodversorgung des Menschen leisten.

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Annex

Methodological improvement of iodine extraction from plant matrices

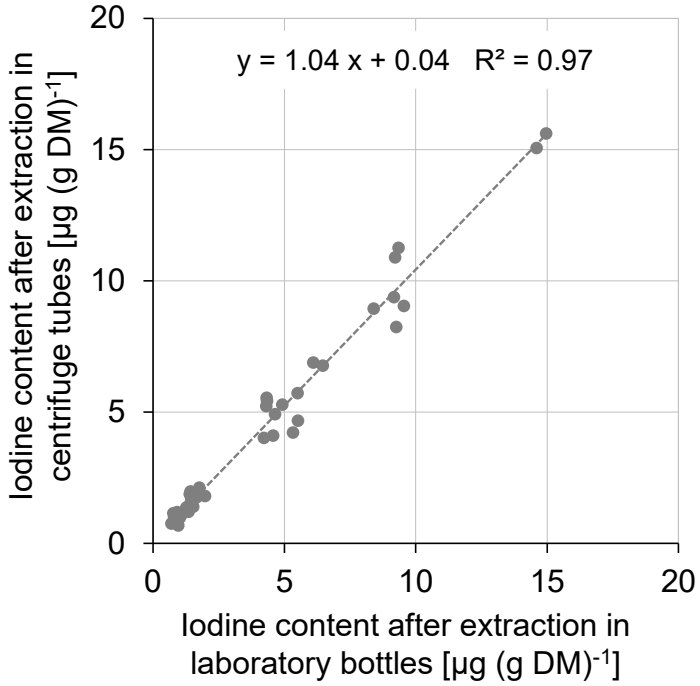

Figure 1: Comparison of the iodine content of different sample material [ERM-BB422 fish muscle and NIST-1849a infant/adult nutritional powder (milk), apple and lettuce powder] after extraction in laboratory bottles and centrifuge tubes (*n* = 48).

Abstracts of co-authored papers

Influence of a Selenium Biofortification on Antioxidant Properties and Phenolic Compounds of Apples (*Malus domestica*)

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* corresponding author | DOI: 10.3390/antiox9020187 | *Antioxidants* **2020** 9, 187

Abstract

Biofortified apples seem to be a suitable produce. In this study, different selenium forms and application levels were applied to the two apple varieties ‘Golden Delicious’ and 'Jonagold', grown in the years 2017 and 2018 in order to increase the selenium uptake within a typical Western diet. It was shown that the biofortification, which was performed as a foliar application implemented in usual calcium fertilization, led to significantly increased selenium contents in the fruits. Furthermore, biofortification affected the total phenolic content (TPC), the polyphenol oxidase activity (PPO), as well as the antioxidant activity (AOA), the latter measured with the two well-known assays Trolox Equivalent Antioxidant Capacity Assay (TEAC) and Oxygen Radical Absorbance Capacity Assays (ORAC). The varying selenium forms and application levels showed a differing influence on the parameters mentioned before. Higher fertilizer levels resulted in higher selenium accumulation. It was found that PPO activity fluctuates less in biofortified apples. With regard to TPC, selenate led to higher amounts when compared to the untreated controls and selenite resulted in lower TPC. AOA analysis showed no clear tendencies as a result of the selenium biofortification. In the case of 'Jonagold', a higher AOA was generally measured when being biofortified, whereas, in the case of 'Golden Delicious', only one form of application led to higher AOA. Additionally, differences in the amount of major phenolic compounds, measured with High Performance Liquid Chromatography Mass Spectrometry (HPLC-DAD-ESI-MS^n), were observed, depending on the conditions of the biofortification and the variety.

Keywords:
apple, selenium, agronomic biofortification, antioxidant activity, phenolic compounds, TEAC, Total Phenolic Content, phenoloxidase

Selenium biofortification of different varieties of apples (*Malus domestica*) – Influence on protein content and the allergenic proteins Mal d 1 and Mal d 3

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[*] corresponding author | DOI: 10.1016/j.foodchem.2021.130134 | *Food Chem* **2021** 362, 130134

Abstract

As allergy towards apples is widespread, the evaluation of various cultivation and postharvest influences on the allergenic potential is of great importance. Therefore, the analysis of the Mal d 1 content was the focus of this work, originally dealing with investigating the influence of a selenium biofortification on apple quality. The content of Mal d 1 of seven different apple varieties was determined with a direct ELISA. Protein patterns of the apples, especially with regard to the proteins of the Mal d family, were studied with SDS-PAGE. A LC-MS/MS analysis confirmed the presence of the allergens. The Mal d 1 content of apples was in most cases reduced when the fruits were biofortified with 3,1 – 8,7 µg selenium per 100 g fresh weight. Apple variety and climatic conditions were identified as further influencing factors for the Mal d 1 content of the fruits. Due to the significantly higher intensity of the Mal d 3 bands of the selenium-fertilized apples, a promotion of the synthesis of this protein by this treatment cannot be excluded. The separate analysis of the peel and the fruit flesh of the variety 'Elstar' showed that the content of Mal d 1 in the fruit flesh was significantly lower in the biofortified samples than in the controls. In conclusion, the results indicate that the selenium biofortification of apples and biochemical mechanism behind can reduce the allergenic potential regarding the content of Mal d 1.

Keywords:
Apple, selenium, agronomic biofortification, Mal d 1, Mal d 3, SDS-PAGE, ELISA, allergy

Relationship between Phenolic Compounds, Antioxidant Properties, and the Allergenic Protein Mal d 1 in Different Selenium-Biofortified Apple Cultivars (*Malus domestica*)

Sabrina Groth[1], Christoph Budke[2], Timo Weber[2], Susanne Neugart[3], Sven Brockmann[1], Martina Holz[1], Bao Chau Sawadski[1], Diemo Daum[2] and Sascha Rohn[1,4 *]

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[*] corresponding author | DOI: 10.3390/molecules26092647 | *Molecules* **2021** 26(9), 2647

Abstract

Notable parts of the population in Europe suffer from allergies towards apples. To address this health problem, the analysis of interactions of relevant allergens with other substances such as phenolic compounds is of certain importance. The aim of this study was evaluate correlations between the total phenolic content (TPC), polyphenol oxidase (PPO) activity, antioxidant activity (AOA), and the phenolic compound profile with the content of the allergenic protein Mal d 1 of six apple cultivars. It was found that the PPO activity and the content of individual phenolic compounds had an influence on the Mal d 1 content. With regard to the important constituents flavan-3-ols and phenolic acids, it was found that apples with a higher content of chlorogenic acid and a low content of procyanidin trimers and/or epicatechin had a lower allergenic potential. This is probably based on a reaction of phenolic compounds (when oxidized by the endogenous PPO) with proteins, being able to change the conformation of the (allergenic) proteins, which further corresponds to a loss of antibody recognition. When apples were additionally biofortified with selenium, composition of apples with regard to TPC, phenolic profile, AOA, and PPO was significantly affected. Consequently, this innovative agronomic practice seems to be promising for reducing the allergenic potential of apples.

Keywords:
Apple, biofortification, selenium, antioxidant properties; phenolic compounds, polyphenol oxi-dase, Mal d 1, allergy

Conference contributions as first author

Oral presentations

Budke, C., *Heinlein, A.-K., Wortmann, L., Enneking, U., Daum, D.* (2016): Selen - ein nützliches Spurenelement für den Apfelanbau? 45. Osnabrücker Kontaktstudientage, Hochschule Osnabrück, 11.-12.11.2016, Osnabrück, Germany.

Budke, C., *Wortmann, L., Enneking, U., Daum, D.* (2017): Äpfel - ein geeignetes Zielobjekt für die Biofortifikation mit Selen? Deutsche Gesellschaft für Qualitätsforschung (Pflanzliche Nahrungsmittel) e.V., 51. Vortragstagung, Hochschule Geisenheim, 22.-23.03.2017, Geisenheim, Germany.

Budke, C., *Daum, D.* (2018): Biofortifikation von Obst mit Selen - Verfahrenstechniken und Perspektiven als funktionelle Lebensmittel. 26. Jahrestagung der Deutschen Gesellschaft für Pflanzenernährung e. V., Hochschule Osnabrück, 13.-14.09.2018, Osnabrück, Germany.

Budke, C., *Daum, D.* (2019): Biofortifikation von Obst mit Iod und Selen. Osnabrücker Geographisches Kolloquium - Forschungskolloquium im Master Boden, Gewässer, Altlasten, Universität Osnabrück, 09.01.2019, Osnabrück, Germany.

Budke, C., *Weber, T., Frieman, A., Holthusen, H., Daum, D.* (2021): Biofortification of apples with selenium under orchard conditions. 4th International Symposium on Horticulture in Europe. Virtual Congress from March 09.-11.03.2021.

Budke, C., *Schachtschneider, M.-L., Daum, D.* (2021): Pathways of exogenously applied iodine in apple trees. 4th International Symposium on Horticulture in Europe. Virtual Congress from March 09.-11.03.2021.

Poster presentations

Budke, C., *Daum, D., thor Straten, S., Mühling, K. H.* (2016): Fortifikation von Äpfeln mit Iod durch eine Nachernte-Tauchbehandlung. Deutsche Gesellschaft für Qualitätsforschung (Pflanzliche Nahrungsmittel) e.V., 50. Vortragstagung, Julius-Kühn-Institut, 13.-15.03.2016, Berlin, Germany. Awarded as best poster contribution

Budke, C., *Bommes, C., Körner, M., Martin, J., Dingwerth, E.-M., Daum, D.* (2016): Faktoren der Iodanreicherung in Äpfeln bei einer Nachernte-Tauchbehandlung. 45. Osnabrücker Kontaktstudientage, 11.-12.11.2016, Osnabrück, Germany.

Conference contributions as co-author

Oral presentations

Daum, D., ***Budke, C.,*** *Lawson, P. G.* (2017): Verfahrenstechniken zur Erhöhung des Jodgehaltes in Gemüse und Obst. Deutsche Gartenbauwissenschaftliche Gesellschaft e.V., 51. Gartenbauwissenschaftliche Jahrestagung, Hochschule Osnabrück, 01.-04.03.2017, Osnabrück, Germany.

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Weber, T., ***Budke, C.,*** *Daum, D.* (2018): Einfluss einer Ioddüngung auf den Botrytisbefall von Kopfsalat. 47. Osnabrücker Kontaktstudientage, Hochschule Osnabrück, 09.-10.11.2018, Osnabrück, Germany.

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Groth, S., Holz, M., Brockmann, S., ***Budke, C.,*** *Daum, D., Rohn, S.* (2020): Einfluss einer Selen-Biofortifikation von Äpfeln unterschiedlicher Sorten auf die allergenen Proteine Mal d 1 und Mal d 3. Deutsche Gesellschaft für Qualitätsforschung (Pflanzliche Nahrungsmittel) e.V., 54. Vortragstagung, Universität Hohenheim, 04.-06.03.2020, Stuttgart, Germany.

Groth, S., ***Budke, C.,*** *Daum, D., Rohn, S.,* (2021): Selen-Biofortifikation von Äpfeln und deren Einfluss auf den Mal d 1-Gehalt. Informationsaustausch verschiedener Arbeitsgruppen (Deutschland, Italien) zum Thema Apfelallergenpotential. Online-Veranstaltung, organisiert durch die Hochschule Osnabrück, 26.02.2021.

Groth S., Oest M., Brockmann S., Holz M., Sawadski B. C., ***Budke C.,*** *Weber T., Neugart S., Daum D., Rohn S.,* (2021): Zusammenhang zwischen phenolischen Verbindungen, antioxidativen Eigenschaften und dem allergenen Protein Mal d 1 in verschiedenen Selen-biofortifizierten Apfelsorten. Jahrestagung des Regionalverbandes Nord der Lebensmittelchemischen Gesellschaft, Online-Tagung, 18.03.2021.

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Poster presentations

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Further contributions

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Curriculum Vitae

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Professional career

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04/2020 - current	Research assistant University of Applied Sciences Osnabrück
03/2015 - 03/2020	Research associate University of Applied Sciences Osnabrück 01.03.2016 - 31.03.2019 research project: BiofortiSe - Biofortifikation von Äpfeln mit Selen zur Verbesserung der Fruchtqualität, der Lagerfähigkeit und des gesundheitlichen Wertes
02/2012 - 01/2014	Research associate University of Applied Sciences Osnabrück Research project: Green Growing

Education

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09/2008 - 11/2011	Bachelor of Science University of Applied Sciences Osnabrück Thesis: „Verbesserung der Applikationsqualität an einem handgeführten Flächenspritzgerät für gärtnerische Kulturen“
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