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Plant Growth Is Stimulated by Tea-seed Extract: A New Natural Growth Regulator?

Marianne Andresen
University of Copenhagen, Department of Agriculture and Ecology, Højbakkegård Allé 13, 2630 Tåstrup, Denmark

Nina Cedergreen[1]
University of Copenhagen, Department of Basic Science and Environment, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark

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Abstract. **Various plant extracts are being marketed claiming to enhance both crop yield and quality and being environmentally friendly. However, these claims are rarely documented by scientific data. In this study, we investigate the growth regulatory effect of Tea Seed Powder (TSP), a saponin-rich waste product from tea seed (*Camellia* sp.) oil production. The product was tested in various concentrations on *Lemna* growth and as a soil and spray application on growth of pot grown beet, mustard, oat, and barley. Finally, two treatments, 0.2 g TSP/L dry soil and weekly sprays with TSP solutions corresponding to 1.5 g TSP/m^2, were tested for effects on strawberry yield. The results showed significant growth-enhancing effects on the sterile *Lemna* of ≈20% above control, demonstrating that the growth increase was a plant physiological response to TSP rather than an indirect effect of TSP affecting pests and diseases or improving nutrient uptake. Soil-treated, pot-grown beet, oat, and barley plants showed significant biomass increases in the range of 27% to 41% above control at concentrations of ≈0.3 g TSP/L dry soil, whereas increases of 14% to 26% were observed in plants sprayed with 0.15 to 1.5 g TSP/m^2. Sprayed strawberries had a 38% higher berry yield compared with control plants in 2008, whereas no difference in leaf number and area, number of runners, and inflorescences were detected. In 2009, there were no significant observable differences between sprayed plants and controls. Soil-treated strawberry plants, however, showed a decrease in leaf number in 2008 and in strawberry yield in 2009. The study concludes that TSP has pronounced and direct physiological effects on plants, which can both increase and decrease growth and yield depending on the applied dose. The growth-enhancing effect could be used commercially to improve crop yield; however, because TSP is also known to be very harmful to earthworms, possible environmental effects of the use of TSP in agriculture and horticulture must be considered before use.**

Within the agricultural and horticultural industry, high yield and high-quality crops are of major commercial and economic importance. The demands for increased yield and improved quality can be met through improvements in crop genotypes by selection, breeding, and genetic engineering and by improvement of the crop growth environment through irrigation, fertilization, and the use of plant protection products. Chemical growth regulators are also one of the tools used to improve both crop growth and quality. Commercially registered growth regulators are all phytohormones and fall into the following categories: auxins, gibberellins, cytokinins, abscisic acid, and ethylene. Recently, however, several other hormonal compounds such as oligosaccharins, brassinosteroids, jasmonates, salicylates, and polyamines have also been shown to have growth-regulating properties (Basra, 2000). Natural products are also distributed for their growth-regulating effects, mainly as growth stimulators (see for examples: http://www.nornatur.net/uk/; http://www.agrigro.com/).

Growth-stimulating natural products are typically produced by small companies without means to verify their products' specificity and efficacy in farm trials or by scientific research. The products are mainly sold for use in home gardens and small-scale production systems, and the lack of knowledge about product specificity and efficiency hampers their credibility. As a result of the mentioned reasons, scientifically documented effects are scarce for natural growth enhancers as is information about the active components in the products, their physiological effects on plants, possible environmental effects, product stability under different environments, etc. Combining the scarce scientific literature with less validated producer information on natural growth enhancers shows that the growth-enhancing effect of different products can be roughly divided into three categories; first, compounds that claim to enhance nutrient availability, by adding it with the product, by facilitating nutrient availability through the addition of microbes or substrates for microbes, or by adding a compound claimed to facilitate nutrient uptake [U.S. Patent 6254654; gamma aminobutyric acid; Plant Growth Enhancer P-307; ODC (organically derived colloidals)]. Second are compounds that in some unspecified way decrease damage by pests (Plant Growth Enhancer P-307; ODC); and third are compounds that interfere with the plant hormone system either directly or indirectly through microbes (Fine Agrochemicals Ltc) (Ali et al., 2009). Because natural growth enhancers are often mixtures of a variety of compounds, many are likely to have multiple functions in terms of simultaneously improving nutrient availability, providing fungicidal and insecticidal effect, and possibly also hormonal effects. However, to be able to optimize the use of such products and their effect as well as to ensure manufacturing and marketing a credible and environmentally sustainable product, it is important to know what effect is generated in the plant and in its growth environment.

The company Nor-Natur is marketing products based on extracts from saponin-containing plant parts. The products have been shown to enhance the yield of tomatoes, cucumbers, and strawberries and to possess fungicidal effects (Nor-Natur APS, 2003, 2004; Wagentrisl, 2003). The producer, Nor-Natur, states that their products lead to an improved uptake of nutrients from soil and air; are effective against fungal infections, nematodes, and pathogens; and that they stimulate plant growth (http://www.nornatur.net/uk/, Nor-Natur APS, 2003, 2004; Wagentrisl, 2003). Whether the claimed growth-stimulating effect of the products is a result of their ability to combat pests or of a direct action on the physiology of the treated plant, or both, is unknown.

Therefore, the objective of this study was to investigate the growth-regulating potential of water extracts of TSP, one of the ingredients used in the products from Nor-Natur. TSP is a granulated waste product from Asian tea oil production. The product contains dried seeds of the species *Camellia* sp., whose oil had been extracted. TSP contains high amounts of saponins, which are believed to be the biologically active fraction of the extract (Mølgaard et al., 2000; Pelah et al., 2002; Sparg et al., 2004). Saponins are one important class of plant secondary metabolites, which are connected to the plant's natural defense system (Wu et al., 2005). Tea seed saponins are triterpenoid saponins, and so far 20 different saponins have been identified from the seeds of the Japanese tea plant (*Camellia*

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[1]To whom reprint requests should be addressed; e-mail ncf@life.ku.dk.

sinensis) (Chaicharoenpong and Petsom, 2009; Morikawa et al., 2007). Although there are studies investigating the toxicity of saponins, few systematic investigations have been presented on the growth-stimulating activity of tea seed saponins on plants.

In this study, we systematically tested the growth-regulatory effects of TSP applying a three-step approach. At first, laboratory tests investigated growth effect on sterile *Lemna* plants. In this test system, indirect effects of pests and diseases can be excluded as well as an effect of improved nutrient uptake because the plants are grown under non-limiting nutrient conditions. Second, the product was tested in greenhouse studies on four different terrestrial plant species and by two different application methods. This allowed investigating possible species differences in sensitivity toward the product and the efficiency of soil versus leaf application. Finally, strawberry plants were used in a biennial experiment to investigate the effect on fruit development and plant growth in the season of tea seed application and during the next year of cultivation. This allowed investigating any positive or negative carryover effects of a previous year's treatment.

Materials and Methods

Tea seed extracts. The plant material TSP (Quit Slug, Batch nr.Quit Slug, Batch no. #2007-1), provided by Nor-Natur APS, Hvidovre, Denmark, was used in all tests. The water-soluble fraction of active compound extracted over 4 h was used in the *Lemna* test and the spray experiments. Seed residues were removed from the extract by sieving followed by filtering (Whatman, Schleicher and Schuell, size 10 cm filter paper).

For the soil tests, extracts were mixed with the soil without removing seed residues. Extracting tea seed did not alter the pH of the aqueous solvent.

Lemna *tests.* The tests were conducted in 10-mL six-well TC-test plates (CM. Laboratory Aps, Vordingborg, Denmark) with the duckweed *Lemna minor* L. as test species (culture strain ucc490, collected in 1977 from Wainfleet, Stinking Barn, Niagara Peninsula, Ontario, Canada, and kept at the University of Toronto Culture Collection), and K-media (pH 5) as growing media (Maeng and Khudairi, 1973). Sterile *Lemna* fronds were pre-cultured under test conditions (24 °C and a continuous photon flux density of 85 to 95 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation). The whole test was repeated twice at 24 °C and once at 20 °C.

Each test was initiated by transferring one sterile *Lemna* frond to each well of the TC-test plates. Each well was prepared with 10 mL K-media with increasing concentrations of TSP extract. Three replicates, defined as individual wells, per concentration and six replications of control were used. The TSP concentrations ranged from 100 g TSP/L to 0.000061 g TSP/L using a factor of two between doses. To determine leaf surface area, the plants were photographed with a digital camera alongside a reference area and placed in the growth cabinet. After 7 d, the plants were photographed again and leaf surface area at Days 0 and 7 were determined digitally using pixel counts of the leaf area compared with the known reference area using the computer program Corel photo paint 12. Surface area increase was calculated as fold increase [frond size day seven (cm^2)/frond size day 0 (cm^2)] because this gives the most sensitive end point in terms of measuring possible growth increases (Belz et al., 2008). Relative growth rates of controls were calculated according to: $(\ln A_T - \ln A_0)/T$, where A_0 is the initial surface area and A_T is the surface area at time T (T = 7).

Species sensitivity tests. The species sensitivity soil and spray tests were carried out from the middle of April to the middle of May 2008 in a 20 °C climate-controlled greenhouse facility at Højbakkegård, Tåstrup, Denmark. In this period, the mean day:night temperatures in the climate-regulated greenhouse were 20 ± 2:20 ± 2 °C. The light:dark ratio was 16 ± 1:8 ± 1 h, the relative humidity was 30 ± 10:40 ± 10%, and the irradiance had a range of 200 to 800 $W \cdot m^{-2}$. Two monocotyledons (oat and barley) and two dicotyledons (beet and mustard) known to respond differently to selective pesticides were chosen as test species. The cultivars used were: oat [*Avena sativa* L., cultivar Ragtar (2008)], barley [*Hordeum vulgare* L., cultivar Publican (2008)], beet [*Beta vulgaris* L., cultivar Nestor (2008)], and mustard [*Sinapsis alba* L., cultivar nr. F6313 (1993)]. Seeds were sown in a 50:50 v/v ratio mixture of sphagnum peatmoss (Pindstrup substrate; Pindstrup Mosebrug AS, Denmark) and Tåstrup A-horizon soil (dark brown, loamy to clay top-layer soil).

In the soil test, a total of 300 seeds per species were sown in plastic pots (diameter 10 cm, (0.15 m^2, 2.6 dL), with five seeds per pot, five pots per treatment, 10 pots for controls, and 10 different TSP treatments ranging from 9.3 g TSP/L soil to 0.018 g TSP/L soil decreasing with a factor of two between treatments. The soil was moistured up to 80% field capacity and pots were placed randomly on two greenhouse tables. The plants were watered by an automatic subirrigation system with 10 min flooding everyday with nutrient-enriched water (Pioner NPK Makro 14-3-23 + Mg combined with Pioner Mikro with iron, Brøste, Denmark) adjusted to a pH of 5.5 and an electrical conductivity of 2.0. All above-ground biomass was harvested after 14 d, dried at 80 °C, and weighed.

A similar setup was used for the spray treatment but excluding the TSP from the soil. To obtain plants at the two-leaf stage at spraying, beats were sown 14 d before spraying and the oat, mustard, and barley were sown 9 d before spraying. Ten different concentrations of TSP were used and the concentrations ranged from 100 g TSP/L water to 0.195 g TSP/L water decreasing with a factor of two between concentrations. Controls were sprayed with demineralized water. The spraying was done in a spray cabin using Hardi 02-110 nozzles, a pressure of 4 bar, and a spraying volume of 150 $L \cdot ha^{-1}$. All above-ground biomass was harvested 8 d after spraying, dried at 80 °C, and weighed.

Strawberry tests. The strawberry cultivar, Honeoye (*Fragaria ×ananassa* Duchesne), was obtained as plantlets from Svanemosegård in Roskilde, Denmark. The plantlets were planted in June 2008 in sphagnum peatmoss (Pindstrup substrate; Pindstrup Mosebrug AS) and were watered as described previously. From June to the end of Aug. 2008, the mean day:night temperatures in the greenhouse were 25 ± 3:20 ± 3 °C. The light:dark ratio was 16 ± 2:8 ± 2 h, the relative humidity was 30 ± 10%:50 ± 10%, and the irradiance ranged from 200 to 800 $W \cdot m^{-2}$.

The TSP concentrations used in the strawberry soil and spray test were selected according to previous test results as 0.2 g TSP/L soil in the soil test and 100 g TSP/L water in the spray test corresponding to 1.5 $g \cdot m^{-2}$ using the spray setup described previously. These concentrations either initiated a growth increase or at least did not have adverse effects on the growth of the four previously tested species. For the soil test, 50 strawberry plantlets were planted in TSP-spiked soil in plastic pots (diameter 20 cm) with one plantlet in each pot. The TSP was added to the soil as described for the species sensitivity test. After 4 weeks, the plants were treated again by watering each pot with a solution of 1 g TSP in 500 mL water. For the spray test, another 50 plantlets were planted in clean soil. The plants were sprayed as described previously 1 week after planting and once a week for the next 8 weeks. Seventy-four plantlets served as untreated controls for both soil and spray tests.

All plants were placed randomly on the greenhouse tables and were moved every week at the time of spraying. Number of leaves, runners, flowers, berries, and the berry weight were recorded every week. After 9 weeks of cultivation, total number of ripe berries, inflorescences, leaves, dead leaves, runners, total length of runners, and total number of leaves on runners were recorded for all plants. Finally, the above-ground biomass of half of the plants (24 soil-treated plants, 25 spray-treated plants, and 37 control plants) were harvested and the total leaf area of each plant was measured with an area meter (LI-3100; LI-COR Inc., Lincoln, NE). The plants were dried at 80 °C and weighed.

The remaining plants overwintered outside at the Pometum (http://www.pometet.life.ku.dk/) and stayed outside for the berry season of 2009 being watered with nutrient-enriched water. During the period of berry harvest in 2009, temperature ranged from 6 to 28 °C with an average of 15 °C and the average irradiance between 0800 HR and 2000 HR was 590 ± 306 $W \cdot m^{-2}$. The number and weight of ripe berries per plant was monitored weakly from 19 June to 9 July. At 9 July, the entire plants were harvested and the number and weight of ripe berries and green berries were determined. Also, the number of

inflorescences, leaves, runners, and rooted plants per pot was counted and total above-ground fresh weight biomass was determined. Unfortunately, plants were discarded before dry weight determination. Hence, to be able to compare plant weights across years, leaf dry weight data were calculated from the fresh weight data using a dry weight (DW) content of 41% as found in Darnell and Stutte (2001).

Plant protection. Plant protection was only carried out in the strawberry test because there was no problem with either pests or diseases in the other experiments. Two yellow sticky traps (Borregaard Bioplant ApS) were set up in the beginning of the test to catch and monitor flying pests (mainly thrips and aphids) in the greenhouse. Because thrips and aphids were identified in Week 2 of 2008, four new yellow sticky traps and two roller traps (Borregaard Bioplant ApS) were placed at each table in Week 3 of 2008. Furthermore, Duxon insecticidal soap (distributed by Borregaard Bioplant ApS) was applied in Week 3. Approximately 30 min after spraying, plants were washed carefully with water to remove the soap. The plants in the spray test were not sprayed with TSP in the week of soap application. One d after the treatment, Swirskii predatory mites (*Amblyseius swirskii*; Borregaard Bioplant ApS) were set out in the plants to keep the amount of thrips down to a minimum. The thrips were later controlled by setting out one new population of Swirskii predatory mites in Week 5 of 2008. There were no further problems with pests in the following and there were no diseases observed.

Statistics. Statistical analyses and modeling of data were made using the statistical program "language and environment R," Version 2.6.2 with the add-on package "drc" and the computer program Microsoft Office Excel 2003 (Microsoft Inc., Redmond, WA). In the *Lemna* test, the data were fitted with two different dose–response models, a monotonic log-logistic dose–response model, and a modified biphasic log-logistic model including a parameter for hormesis (Brain and Cousens, 1989).

The log-logistic model is described in Eq. 1:

$$Y = \frac{d}{1 + (x/e)^b} \quad (1)$$

where y is the measured response, d is the upper limit of the curve corresponding to the untreated control values (lower limit corresponds to zero), e is the dose that reduces the response by 50% (EC_{50}), and b is proportional to the slope of the dose–response curve around EC_{50} (Cedergreen et al., 2005).

The modified biphasic logistic model used was that of Brain and Cousens (Brain and Cousens, 1989) described in Eq. 2:

$$Y = \frac{d + fx}{1 + (x/e)^b} \quad (2)$$

where y is the measured response, d is the upper limit of the curve corresponding to the untreated control values, e determines the inflection point of the decreasing part of the curve, and the size of b determines the steepness of the curve after the maximal hormetic effect. In this equation, f is the parameter describing the degree of growth stimulation at low doses in combination with the slope parameter. The hormetic effect increases with increasing values of f as long as f is positive. To determine which of the models best described the data, the monotonic and biphasic dose–response models were compared with a lack-of-fit F-test (Cedergreen et al., 2005).

In the *Lemna* tests and the species sensitivity soil and spray tests, comparisons between single doses and controls were done with a two-tailed t test assuming equal variance. For the strawberry tests, the analysis of variance followed by the Tukey honestly significant difference test was used to investigate if there were a significant difference in parameters between treatments. The probability level defining significance was set to 0.05 in all tests.

Results

Lemna *tests.* In all three tests, the data were better described by the biphasic dose–response model than the monotonic model, indicating that the TSP has a direct physiological effect on the leaf area-specific growth rate of the *Lemna* plants (Fig. 1; Table 1). The growth increase occurred at concentrations of 0.01 to 0.1 g·L^{-1} and was within the size of 13% to 38% stimulation relative to the untreated controls. The mean relative growth rates for the controls ranged from 0.30 to 0.34 d^{-1}, which exceeds the 0.27 d^{-1} minimum required by the Organisation for Economic Cooperation and Development (Sims et al., 1999).

Species sensitivity tests. For all four species in the soil test, the above-ground plant biomass increased just below or above TSP concentrations of 0.3 g TSP/L soil (0.58 g, 0.29 g, 0.29 g. and 0.15 g TSP/L water for beet, mustard, oat, and barley, respectively) (Fig. 2). This increase was significant for all species except mustard (t test: $P = 0.011$, $P = 0.005$, and $P < 0.001$ for beet, oat, and barley, respectively), and the size of the biomass increase was 27%, 39%, and 41% in beet, oat, and barley, respectively. The growth increase was visible not only in DW, but also by the eye. A small growth-inhibiting effect was observed for beet and mustard at the two highest concentrations (t test; $P < 0.05$). Germination percentage was between 98% and 100% for all species, all controls, and for all concentrations of TSP.

The results for plants sprayed with a TSP extract differed from the soil test results (Fig. 3). For these plants, there was a tendency of increasing above-ground biomass with increasing doses of TSP extract. However, although the majority of the treated plants were bigger than the control plants, only a few treatments had significantly higher DWs with treated plants being up to 26%, 15%, and 14% heavier than control plants for beet, oat, and barley, respectively (Fig. 3; t test: $P < 0.05$).

Strawberry tests. The setting of leaves, runners, and mature berries of plants exposed to the two TSP treatments over time in 2008

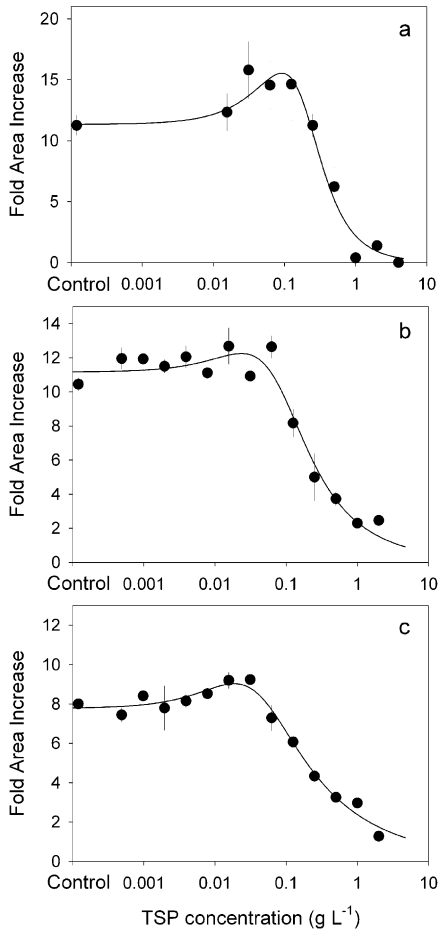

Fig. 1. The fold area increase of *Lemna* after 7 d as a function of increasing concentrations of Tea Seed Powder (TSP) extract. The test was repeated three times either at 24 °C (**A**–**B**) or at 20 °C (**C**). Data presented as mean ± SE (n = 3) and are modeled by Eq. 2 (fitted parameters according to Table 1).

are shown in Figure 4 together with the harvest data of mature berries in 2009. The results showed that adding TSP to the soil had a negative impact on the development of leaves and runners, whereas the berry yield in 2008 was unaffected by the soil treatment (Fig. 4A–C). The year after, however, also strawberry yield was lower for the soil-treated plants (Fig. 4D).

The TSP-sprayed plants, however, increased the yield of berries with 39% in 2008 (Fig. 4C) but were no different from control plants in 2009 (Fig. 4D). There was no difference in strawberry weight per berry between treatments, but berry weight varied with time with the largest berries maturing in the beginning of the season both years (two-way analysis of variance: $P = 0.37$ and 0.73 for treatment effect in 2008 and 2009 and $P << 0.001$ for effect of time on mean berry weight).

Looking at the destructive harvest data for both years, the negative impact on vegetative growth of soil-treated plants could also be observed as a decrease in number of leaves on runners, total leaf area, number of dead leaves, and plant weight in 2008, although

Table 1. Parameter estimates (mean± SE) and the *P* value for the lack-of-fit *F*-test of the *Lemna* data described by the monotonic log-logistic model (Eq. 1) and the biphasic model (Eq. 2).[z]

Expt.	Model	Model parameters				*F*-test
		b	*d*	*e*	*f*	*P*
TSP a	1	3.60 ± 0.36	13.0 ± 0.6	0.48 ± 0.07		0.0165
	2	2.20 ± 0.36	11.3 ± 0.9	0.18 ± 0.07	85 ± 53	
TSP b	1	1.33 ± 0.21	11.7 ± 0.29	0.28 ± 0.04		<0.0001
	2	1.58 ± 0.09	11.2 ± 0.3	0.08 ± 0.02	126 ± 63	
TSP c	1	1.09 ± 0.13	8.34 ± 0.18	0.37 ± 0.05		<0.0001
	2	1.43 ± 0.04	7.78 ± 0.20	0.04 ± 0.01	224 ± 77	

[z]The model parameters and their significance are described in the text. At a propability of $P < 0.05$, the biphasic model (Eq. 2) describes the data significantly better than the monotonic model in all three experiments (a–c).
TSP = Tea Seed Powder.

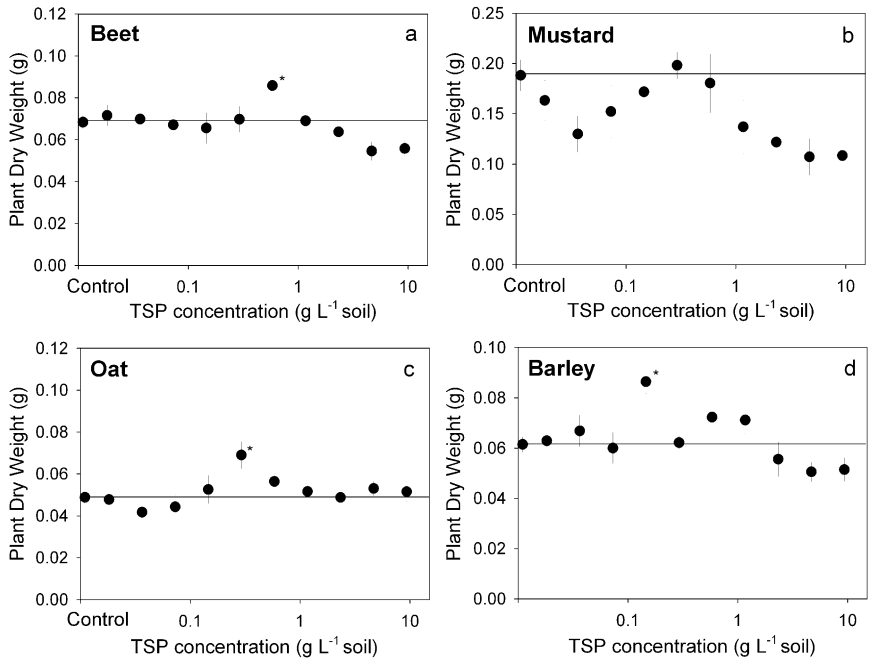

Fig. 2. The harvest dry weight 2 weeks after sowing of the four species beet, mustard, oat, and barley exposed to an increased Tea Seed Powder (TSP) concentration in the soil. Data are given as mean weight per plant in each pot ± SE (n = 10 pots for controls and 5 per treatment). The horizontal line marks the control mean. Treatments significantly different from control values (*t* test, $P < 0.05$) are marked with an asterisk.

only significantly for dead leaves (Fig. 5C, E–G). In 2009, the number of berries and leaves, the number of inflorescences, and the number of plants per pot decreased significantly in soil-treated plants, whereas the number of runners increased (Fig. 5A–B, D–E, H–I). Apart from the increased number of strawberries of the sprayed plants in 2008, no other measured end point of the sprayed plants deviated significantly from the untreated control plants (Fig. 5).

Discussion

Growth-stimulating effects. The repeatable growth-stimulating effect on sterile cultures of *Lemna* indicate that the stimulating effect of TSP on plant growth and yield is not exclusively caused by its adverse effect on pathogens (Heijne et al., 2007) nor by an increase in nutrient uptake, because *Lemna* is grown under non-nutrient-limiting conditions (Sims et al., 1999). The results of this study therefore indicate that TSP contains substances with hormone-like properties or substances that interfere with hormones that can stimulate or affect biomass allocation in plants.

The species sensitivity tests and strawberry test show that a growth-stimulating effect can be achieved in different species when TSP is applied either in soil or as a spray. Other studies have also shown that treating soil or spraying plants with saponin-containing plant products based on the bark from the soap bark tree (*Quillaja saponaria*) can have a positive effect on the yield and quality of strawberries, tomato, and grapes; yield increases of 18% and 12% were achieved in strawberries and tomato, and a quality improvement was achieved in grapes (Nor-Natur APS, 2003, 2004; Wagentrisl, 2003). The fact that the growth-stimulating effects stem from addition of plant extracts containing saponins does not exclude that other active compounds in the products such as polyphenols could be the compounds causing the effect on growth. More studies on specific fractions of the TSP and *Quillaja* extract are needed to get indications as to which compounds or combinations of compounds induce the growth-enhancing effect.

Adverse effects. Apart from the growth-stimulating effects, TSP also displayed growth-reducing effects at increased doses in *Lemna*, mustard, barley, and the soil-treated strawberries. Growth-reducing effects have also been found for early watergrass (*Panicum crus-galli* L.), green foxtail (*Setaria viridis* Beauv. L.), and white clover (*Trifolium repens* L.) at saponin concentrations of 0.1, 0.05 and 0.01 g saponins/L water for the three different species, respectively (Kohata et al., 2004). Assuming 0.13 to 21 g tea seed saponins per gram TSP, as is the range measured in various samples (Chaicharoenpong and Petsom, 2009), this study indicates an inhibition of *Lemna* growth at concentrations above ≈0.01 to 0.10 g saponins/L water. These values correspond well with the values found by Kohata et al. (2004).

The effect on strawberry yield. According to Hancock (1999), the amount of berries on a strawberry plant are proportional to the leaf area, meaning that the more and bigger leaves the plant has, the more berries it should be able to produce. TSP in some way uncouples this relation. This uncoupling is particularly evident for the sprayed plants, in which the 39% increase in berry yield in 2008 was not a result of an increased leaf area or any other measured plant parameter. It could be hypothesized that the TSP could have induced a change in resource allocation from vegetative growth to berry biomass as a response to the low stress imposed by the TSP. This hypothesis is often proposed in different forms to explain biphasic dose–response relationships (Cedergreen et al., 2007) and allocating resources from vegetative to fruit biomass forms the theoretical basis of most pruning practices in horticulture (Tromp et al., 2009). However, the increased berry biomass did not appear to occur at the expense of any other trait either in 2008 or in 2009, when control and sprayed plants performed equally well (Figs. 4 and 5). The physiological processes behind the berry increase in 2008 therefore remain unresolved.

The adverse effects of the soil TSP treatment were probably the result of too high TSP doses and it remains unknown whether a berry increase could be achieved by addition of lower TSP doses to the soil. The results from beet, mustard, oat, and barley in this study and from soil treatments with soap bark tree extracts (Braverman, 2003), which all enhanced biomass or yield, confirm that a yield increase with soil application should be possible. The adverse effects of the soil TSP addition were more severe in 2009, when berry yield decreased by 24% and leaf number, the number of inflorescences, and

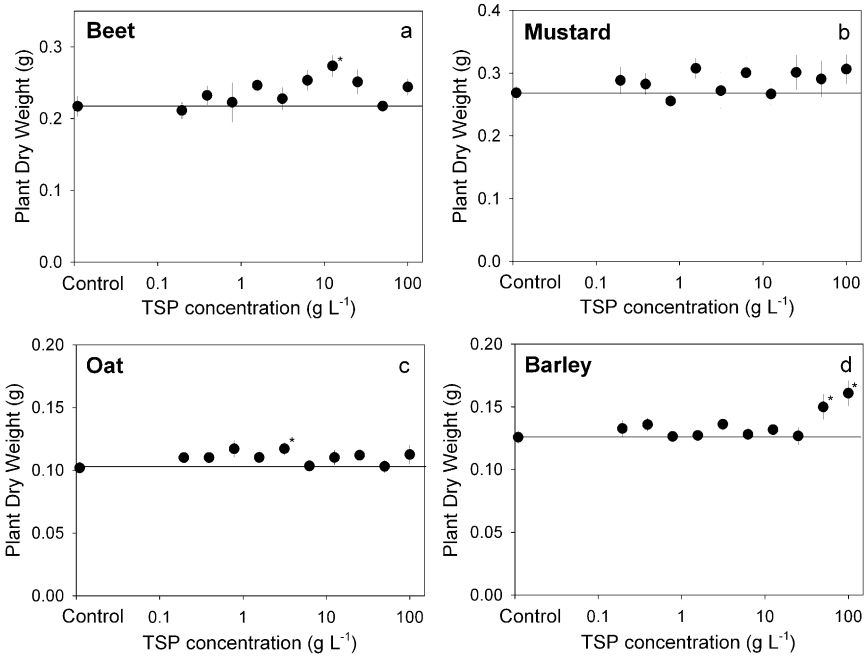

Fig. 3. The harvest dry weight 17 to 22 d after sowing of the four species beet, mustard, oat, and barley sprayed with increasing doses of Tea Seed Powder (TSP). Data are given as mean weight per plant in each pot ± SE (n = 10 pots for controls and 5 per treatment). The horizontal line marks the control mean. Treatments significantly different from control values (*t* test, $P < 0.05$) are marked with an asterisk.

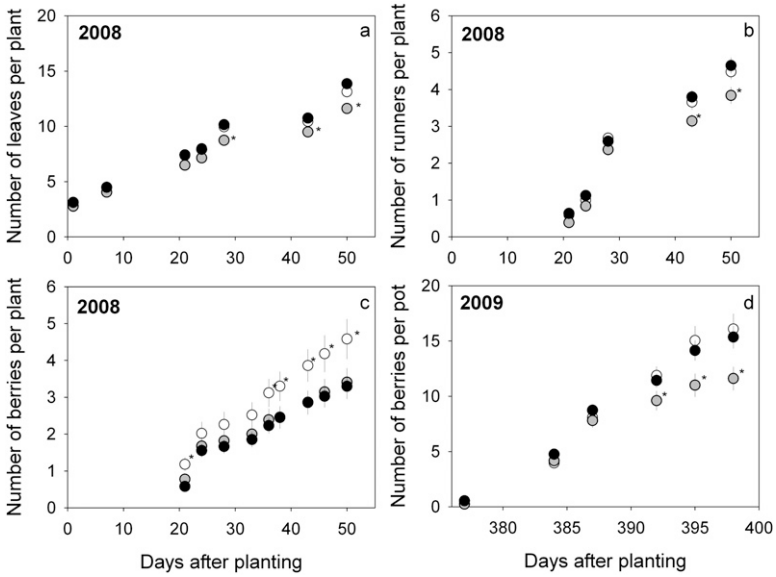

Fig. 4. End points measured over time from planting strawberry plantlets until the end of the harvest season in 2008 and throughout the harvest season in 2009 for untreated control plants (black symbols), soil-treated plants (gray symbols), and sprayed plants (open symbols). The end points are (**A**) the number of leaves per plant, (**B**) the number of runners per plant, and (**C**–**D**) the cumulated number of mature berries harvested per pot in 2008 and 2009, respectively. Data are given as mean± SE, n = 74 for control and 50 for treated plants in 2008 and n = 37 for control and 25 for treated plants in 2009. Treatments deviating significantly from untreated control are marked with an asterisk (*t* test, $P < 0.05$).

plants per pot decreased 20%, 24%, and 27%, respectively, compared with 2008, when only the number of leaves and runners were adversely affected by 16% and 17%, respectively. These results emphasize the importance of monitoring carryover of an adverse effect from one year to the other to obtain the full effect of a treatment. Another option for the more severe adverse effects observed in 2009 could be the formation of more toxic metabolites by degradation of TSP. The only trait that increased in 2009 in response to the soil treatment was the number of runners, which was 13% higher in soil-treated plants compared with control plants. Increasing the number and length of runners is a typical plant response to unfavorable growth conditions whereby they can escape and search for better conditions (de Kroon and Hutchings, 1995).

Conclusion

This study shows that extracts from TSP have a pronounced and direct physiological effect on plants, which can both increase growth and decrease growth depending on the applied dose. The stimulatory plant physiological effects can be used in agriculture and horticulture together with the documented fungicidal effect (Heijne et al., 2007) to enhance crop yield and quality. Small companies already market TSP and other saponin-containing products for their growth-enhancing and fungicidal effects, whereas Kohata et al. (2004) proposed to use tea seed saponins as a herbicide, taking advantage of its adverse effects. However, no matter what the purpose of using TSP and other bioactive plant products in horticulture and agriculture, the probability of adverse effects on other species than the target, including the crops, should be taken into account in the same way as it is done when synthetic pesticides are authorized. According to the Internet advertisements of various TSP products, TSP has proven to be efficient against slugs, golf course insect pests, and scrimps in fish farms, but unfortunately also harmful to beneficial organisms such as earthworms at the doses recommended for use on golf courses (Potter et al., 2010). A comprehensive study of the ability of using TSP to remove earthworms from golf course putting greens showed that doses of 0.29 $g \cdot m^{-2}$ drove out and killed a large number of worms thereby reducing earthworm casts with more than 80% compared with the controls during the one month after treatment that was monitored (Potter et al., 2010). Although earthworms might be undesired in putting greens, this is not the case for agricultural and horticultural land, where earthworms are considered highly beneficial. The doses found to be growth-stimulating in the species sensitivity soil test were between 5.8 and 23.2 g TSP/m^2 soil and the sprayed concentrations corresponded to 1.5 g TSP/m^2 soil, all exceeding the concentrations shown to be detrimental to earthworms. Until more is known about possible adverse effects of TSP in the environment, the use of the product could be confined to greenhouse crops and growth systems where the spreading of the product to the environment is limited. The study by Potter et al. (2010) did also investigate the effect of TSP on other beneficial groups of organisms in the golf course and found that earthworms, and possibly other worm species, were far the most vulnerable species with insect and mite populations not being acutely (72 h) affected by the mentioned treatments. Finally, yield is not the only important parameter in food production. Crop quality is as important. Hence, it must be studied how plants respond to the product as well as whether the use influences the appearance, taste, and shelf life of the exposed fruits. Many saponins have a bitter

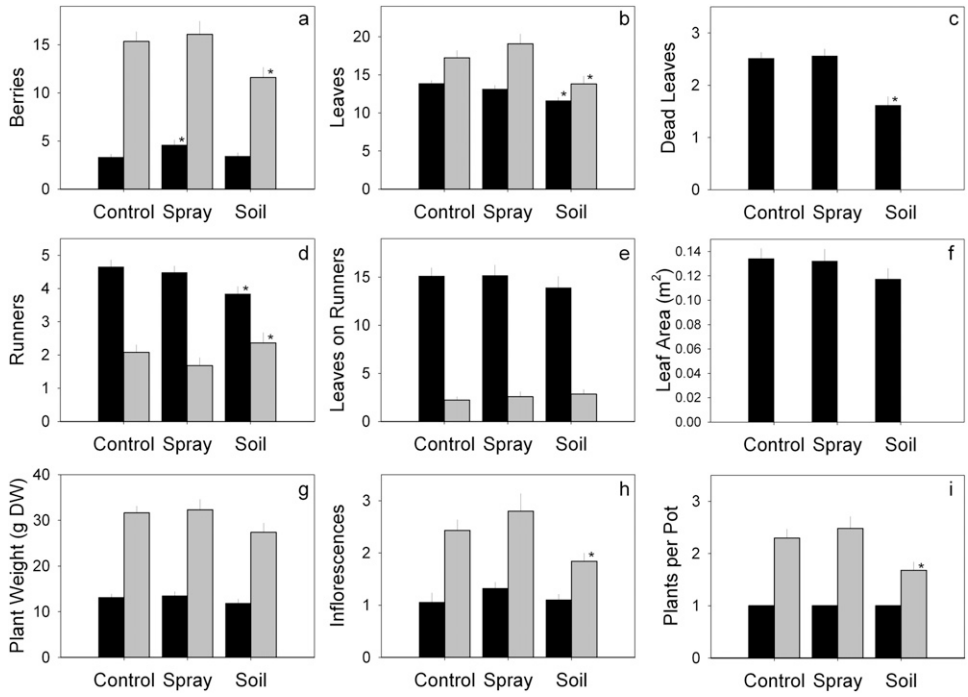

Fig. 5. Destructive and non-destructive end points measured by the end of the harvest in 2008 (black bars) and 2009 (gray bars). The end points are: (**A**) cumulated number of berries, (**B**) number of leaves, (**C**) number of dead leaves, (**D**) number of runners, (**E**) cumulated number of leaves on runners, (**F**) leaf area of all leaves, (**G**) dry weight of the above-ground biomass per pot, (**H**) number of inflorescences, and (**I**) the number of plants per pot. Unless otherwise stated, the end point unit is number per pot. Data are given as mean± SE with n = 74 for controls and 50 for treated plants for 2008 data of **A, B, C, D, E, H,** and **I**, whereas n = 36–37 for controls and 25 for treated plants for 2008 data for **F** and **G** and for all 2009 data. Treatments deviating significantly from untreated control are marked with an asterisk (*t* test, $P < 0.05$).

taste (Hostettmann and Marston, 1995), and this bitterness should preferably not be transferred to the fruit.

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