Validation of a New Phytotoxicity Test (Phytotoxkit) against an Established Four-Week Growing Test with Pre-Grown Plant Plugs

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Abstract

The aim of the studies was to correlate results of a fast extract-based toxicity test with results of an established growing test with mostly pre-grown plants. A standard white peat was contaminated with four levels of TCA (trichloroacetic acid), a known toxic substance. A range was composed of five levels of TCA, including zero, 0.0013, 0.013, 0.13, and 1.3 g/L of peat. Part of the material was entered in Phytotoxkit containers and covered with a filter paper. At the other side of the filter paper two dicotyledonous species, garden cress (*Lepidium sativum*) and mustard (*Sinapis alba*) and one monocotyledon, Sorghum (*Sorghum saccharatum*) were allowed to germinate for a three day period on the extract from the substrate in a climate-controlled cabinet. The same range of amended peats was used in the standard test to grow transplanted lettuce (*Lactuca sativa*), Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) and directly sown barley (*Hordeum vulgare*). For the Phytotoxkit the above ground length and root length were recorded, and for the standard test the fresh weight and dry weight of the above ground parts were recorded. Results showed a growth reduction for all levels of TCA when using the standard method. When using the Phytotoxkit only the two highest concentrations of TCA reduced growth. For screenings of product alternatives or dissolution series the Phytotoxkit is a faster and cheaper alternative.

INTRODUCTION

Many phytotoxicity tests are in current use, and an overview of such tests has been produced by Task Group 4 of Technical Committee 223 of the European normalisation commission CEN.

Existing phytotoxicity tests may, arguably, be classified according to medium, contact of the roots to the rooting medium, duration of the contact period, technical environment, light source, possible water uptake and perhaps other criteria (Table 1). The media used in the tests range from pure extracts from growing media for bacteria, via extracts from growing media added to inert media like filter paper and rockwool to sowing on any rooting media offered for investigation. The contact of the roots to the potential toxic substance is either indirect contact via an extract from the rooting medium under investigation or direct contact with that rooting medium. The duration of the contact of a root with a potential toxin may be as short as one hour for bacterial tests and as long as 28 days for container tests in the greenhouse. The technical environment may be a climate chamber with full control of temperature or a greenhouse with natural light, varying with incidental weather conditions as well as differing with global position. The light source may be absent when just the germination is studied or fully controlled assimilation light as in a climate chamber, or natural light in a greenhouse. The water uptake may combine effects of relative humidity, temperature and light into one. It is thought to be a measure of the amount of toxin entering the plant system. As some toxins do not easily dissolve into water, the root surface in contact with the medium is of interest as well, but is usually too difficult to measure (Machrafi et al., 2006).

Many toxins affect some organisms and plant families more than other organisms. Therefore many tests use plants from different families such as Chinese cabbage (*Brassica rapa* subsp. *pekinensis*), tomato (*Lycopersicon* spp.) and garden cress

(*Lepidium sativum*). Differences in response to toxins are especially common between monocotyledons and dicotyledons (Al Mutlaq et al., 2002). Thus many tests advocate the use of at least one monocotyledon as barley (*Hordeum arvense*).

A more or less coherent overview of various tests is attempted in Table 1. A new commercially available method is the Phytotoxkit (MicroBioTests Inc.) (Blok et al., 2006). To validate the results of this new method, an experiment was designed to compare results with an established method.

MATERIALS AND METHODS

The rooting medium was a standard fertilized and neutralized milled Baltic white peat. The toxin used to prepare a range of toxicity levels was TCA, or trichloroacetic acid, a known phytotoxic substance (Cape et al., 2006; Lewis et al., 2004). 0, 0.039, 0.39, 3.9 and 39.0 g of TCA were added to five glass containers with 1.00 L demineralised water each. For each treatment 30 L of Baltic white peat were spread over a surface of about 2 m^2 with a layer thickness of less than 2 cm. Each of the solutions was sprayed evenly over a batch of peat and subsequently mixed thoroughly for 10 min. Thus a range of TCA levels was achieved including peat with zero, 0.0013, 0.013, 0.13, and 1.3 g TCA per litre of peat. From the range, 1 L sub samples were taken for the Phytotoxkit test. The peat was measured for dry bulk density and water content.

Reference Test

The reference test prescribes the use of lettuce (*Lactuca sativa*), Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) and barley (*Hordeum vulgare*). Lettuce and Chinese cabbage were pre-sown and transplanted, while barley was directly sown. For lettuce sowing, trays were filled with 4 cm of untreated Baltic white peat and then gently levelled by pressing with a flat panel. 10 seeds of Lettuce were sown and kept for 14 days before transplanting. The trays were stored for 24 h at 12°C and then brought in the greenhouse at 22/20°C (day/night) and RH >80%. Chinese cabbage was sown a week after the Lettuce and transplanted at the same time. On the day of transplanting thirty 800 ml containers of 12 cm diameter per medium treatment were filled. There were one hundred and fifty containers in total with five substrates × three seeds × ten repetitions. The lettuce and Chinese cabbage were transplanted with one plant per container and the barley was sown directly on the containers, with 10 seeds per container. Water was added regularly. After 2 weeks the plants were harvested and the fresh and dry weight of the above ground parts were measured (Fig. 3).

Phytotoxkit Microbiotest

The Phytotoxkit tests were used according to the Standard Operational Procedure of this assay and with the materials included in the commercial Phytotoxkit. The seeds of three different plant species, garden cress (*Lepidium sativum*), mustard (*Sinapis alba*) and sorghum (*Sorghum saccharatum*) were used as prescribed in the method.

Ninety ml of the peat, based on the previously measured dry bulk density and water content measurements, was transferred to the bottom compartment of a 21×16 cm test plate. A volume of distilled water calculated as necessary to reach saturation of the substrate was added. The wet peat in the test plates was then flattened with a spatula and covered with a 1.5 mm thick filter paper. Ten seeds of one species were subsequently placed on the filter paper in a single row, close to the dividing ridge. All assays were performed in four replicates for each of the three plant species used. The test plates were closed with the transparent lid. The test plates were placed vertically in a cardboard holder and incubated for three days at 25°C, in darkness. At the end of the incubation period a picture of each test plate was taken with a digital camera (Fig. 3). Root and shoot length measurements were made with the aid of an image analysis programme.

RESULTS AND DISCUSSION

Table 2 shows a growth reduction for all levels of TCA when using the standard method. Table 3 shows that when using the Phytotoxkit, only the two highest concentrations of TCA reduce growth. The effects of both methods are displayed in Figures 1 and 2. The figures show a clear, but not linear, relationship between the greenhouse cultivation in gram fresh weight along the X-axis and the Phytotoxkit test in mm shoot length along the Y-axis. Each set of three data points as seen from the X-axis represents one toxicity level treatment: T0, T1, T2, T3 and T4. T0 (without TCA) displayed the highest weight and length. T4 with the highest level of TCA displayed the lowest growth.

Growth of barley in the reference test is reduced at lower doses and to a larger extent than the length reduction in any of the plants grown in the Phytotoxkit. TCA seems to affect barley more than the two dicotyledonous species.

The growth in the Phytotoxkit in Figure 1 shows a possible optimum for the lowest level of TCA, T1, except for cress. The same is visible in Figure 2. Many toxic substances are known to stimulate growth at low doses (Duke et al., 2006).

The reference method is, as expected because of the higher water uptake associated with a longer uptake period and larger plants, much more sensitive than the Phytotoxkit test. It is shown that small growth reductions at T1 and T2 may remain unnoticed with the Phytotoxkit test. The reference test is a more stringent test than the Phytotoxkit and may be therefore necessary where critical evaluations are required. For screenings of product alternatives, the Phytotoxkit is a faster and cheaper alternative. It may also be used for dissolution series if these contain enough samples with higher levels of toxin compared to the reference test.

The validation of toxicity in this experiment can only be extrapolated to other substances with caution as many toxins are transported in specific ways and many toxins affect plant growth in a variety dependent way.

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Tables

Table 1. Various classification criteria for phytotoxicity tests.

Contact	Method	Contact time	Process time (days)	Water uptake during contact* (g)
Extract	Bacteria scintillation	1 h	1	-
Extract	Lemna (Duck weed)	48 h	3	-
Extract	Petri dish/filter paper	72 h	4	2
Extract	Phytotoxkit	72 h	4	2
Extract	Seed lobes 1 extract	168 h	8	20
Direct	Seed lobes 2 direct	7 d	8	40
Direct	Cultivation sown	21 d	22	250
Direct	Cultivation transplanted	14 d	32	500

*Estimation and highly dependent upon the exact technical setting and climate.

Table 2. Greenhouse container test weight data for 4 TCA levels and three plant species.

	Shoot fresh weight in g (*n*=10)			Shoot dry weight in g (*n*=1)*		
	Barley	Chinese cabbage	Lettuce	Barley	Chinese cabbage	Lettuce
T0	4,61 d	3,19 b	0,96 c	0,326	0,195	0,064
T1	4,20 d	2,86 b	0,71 ab	0,321	0,177	0,049
T2	1,54 c	2,74 b	0,63 bc	0,134	0,185	0,046
T3	0,54 b	2,39 b	0,53 ab	0,056	0,167	0,046
T4	0,03 a	0,57 a	0,27 a	0,002	0,036	0,018

a-d: Values which do not share suffixes a, b, c or d are significantly different. LSD (least significant difference) ranges between 0.3 and 0.7.
*Samples joint before weighing.

Table 3. Phytotoxkit data for 4 TCA levels and three plant species.

	Root fresh length in mm (*n*=40)			Shoot fresh length in mm (*n*=40)		
	Garden cress	Mustard	Sorghum	Garden cress	Mustard	Sorghum
T0	41,7 a	50,8 ab	64,4 b	45,2 b	57,3 c	67,8 c
T1	51,2 a	57,6 bc	46,8 a	61,7 c	59,4 c	58,6 bc
T2	54,9 a	66,4 c	67,2 b	59,6 c	43,8 b	71,1 c
T3	46,2 a	60,8 bc	43,8 a	44,2 b	38,0 ab	40,8 ab
T4	46,0 a	43,0 a	36,4 a	21,2 a	25,6 a	24,1 a

a-d: Values which do not share suffixes a, b, c or d are significantly different. LSD (least significant difference) ranges between 10 and 13.

Figures

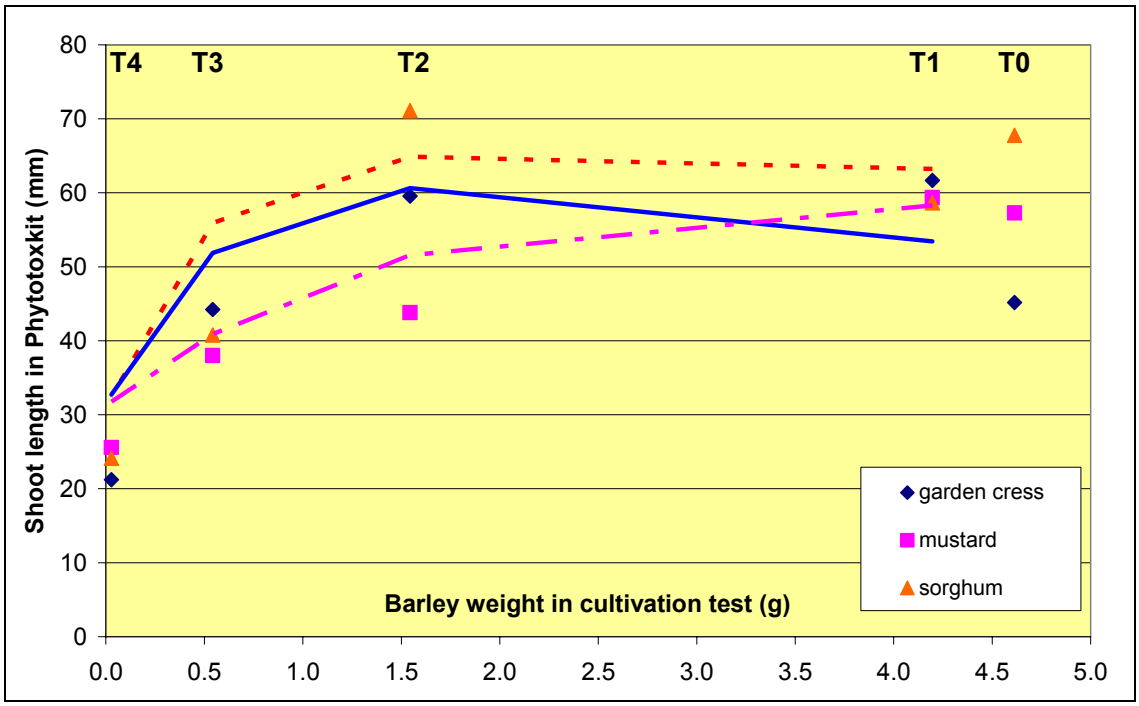

Fig. 1. Relationship between the fresh weight harvest of barley grown in peat and the shoot length of garden cress (——), mustard (----) and sorghum (….) grown in the Phytotoxkit with peat. Lines are a 2 period moving average. The levels indicated in the figure are T0=none, T1=0.0013, T2=0.013, T3=0.13, and T4=1.3 g TCA per litre of peat.

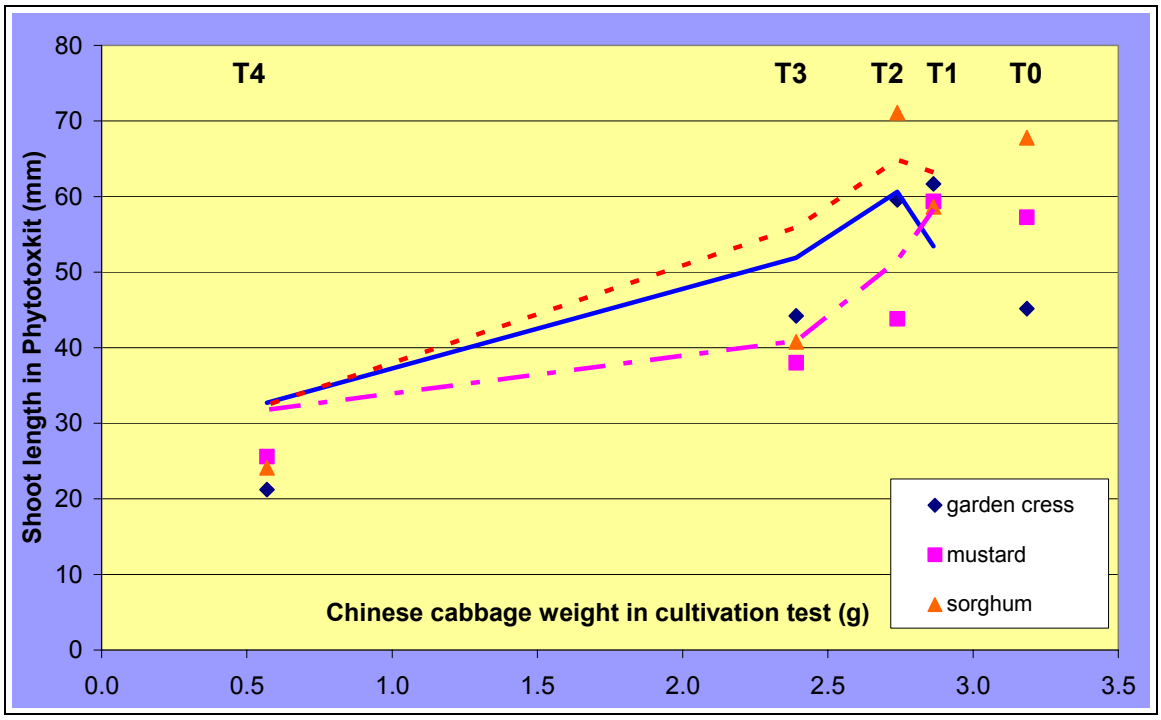

Fig. 2. Relationship between the fresh weight harvest of Chinese cabbage grown in peat and the shoot length of garden garden cress (—), mustard (----) and sorghum (….) grown in the Phytotoxkit with peat. Lines are a 2 period moving average. The levels indicated in the figure are T0=none, T1=0.0013, T2=0.013, T3=0.13, and T4=1.3 g TCA per litre of peat.

Fig. 3. Left to right: Reference test showing examples of barley, Chinese cabbage and lettuce at all five toxicity levels and the Phytotoxkit with garden cress at T2 and T5 respectively.