Table 5 Effect of disinfectant treatments (20 min soak) on *Botrytis allii* incidence

	Percentage *Botrytis allii* infestation	
Disinfectant soak	**Batch B**	**Batch C**
Untreated control	33.5	7.3
2% Jet 5	0.8	0.3
5% Jet 5	0.0	0.0
10% Jet 5	0.0	0.0
2% sodium hypochlorite	0.0	0.8
5% sodium hypochlorite	0.5	0.0
10% sodium hypochlorite	0.0	0.0
2% Vitafect (QAC + salts)	5.3	0.8
5% Vitafect (QAC + salts)	1.0	0.5
10% Vitafect (QAC + salts)	1.0	0.5

Batch B = high botrytis; batch C = moderate botrytis.

Disinfectant treatments

There was a significant interaction effect (seed batch/disinfectant) on percentage normal seed germination. Peroxyacetic acid (20 min soak) did not affect seed germination in any of the seed batches at the concentrations tested. Sodium hypochlorite reduced germination in batches B and C, while the QAC + salts product reduced germination in batch A. Peroxyacetic acid and sodium hypochlorite reduced the incidence of *B. allii* to 0.8% or less, with treatment at 5% of product (for peroxyacetic acid) and 10% (both products) for 20 min, resulting in nil detection of the pathogen (Table 5). The efficacy of QAC + salts against *B. allii* was less consistent, although at product concentrations of 5 or 10% for 20 min, incidence was reduced to 1% or less. All of the treatments reduced but did not eliminate other microbial contaminants.

Conclusions

The work highlighted various seed treatments that, under laboratory conditions, provided effective control of *B. allii*, cause of onion neck rot. Further work is needed to determine the incidence and severity of neck rot during storage, following use of treated seed for production under a range of environmental conditions. In addition, the economic, practical and legislative implications of developing these methods commercially require consideration. Studies demonstrated the variability in onion seed batch sensitivity to chemical and physical treatments, depending on factors such as seed health and maturity.

The most effective fungicide was an experimental seed treatment formulation of boscalid and pyraclostrobin, which resulted in nil detection of *B. allii* when used at the highest rate. Work in

the USA has also shown that a combination of these fungicide active ingredients can reduce *B. allii* either when applied to seed (Du Toit *et al.*, 2004) or during the growing season as early- and mid-season applications (Seebold & Langston, 2005).

With the seed batches used (including one with high botrytis incidence), a pre-soak at 20°C for 18 h prior to hot water treatment (45°C) for 30 or 45 min reduced *B. allii* infestation to 0.5% or less with no effect on percentage germination, irrespective of seed batch.

Of the disinfectants tested, peroxyacetic acid gave the most consistent and effective control of *B. allii* in onion seed, without affecting germination.

Acknowledgements

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Quality management in seed treatment from harvesting to planting

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Introduction

The seed treatment market is the fastest-growing market segment in crop protection. The key driving factors are modern active ingredients replacing granules and foliar applications, the need to protect high-value seeds containing valuable native or built-in traits, the yield and quality benefits of the harvested crops, and the convenience for the grower in getting the required protection on the bagged seed. As a consequence, classical seed treatment technology is transformed into seed care technology, comprising chemical seed protection products and diverse add-ons such as colorants, polymers, biologicals and micronutrients. Hence complex application recipes and safety requirements need a sophisticated approach to ensure high quality standards of treated or coated seeds.

Seed quality

The quality of treated seeds is the result of a multi-step process starting with the inherent quality of the seed, which is a product of seed crop management, harvesting, conditioning, storage and processing, and, finally, the precise application of various products onto the seed.

Seed production

Seeds are produced by seed multipliers or farmers who contract this responsibility from seed companies. At this stage of seed production, seed quality can be influenced by different agronomic factors such as in-season disease and weed control management, nutrient supply to the growing crop, and weather conditions throughout the season, especially during harvest time, which will determine the initial moisture content of the collected seeds. The harvest itself is critical in terms of correct calibration of the combine harvester to avoid or minimise the amount of broken seed and to provide clean seeds as far as possible.

Seed processing

Once harvested, seeds need to be conditioned as a major step prior to marketing, and before seed treatments can be applied. This process includes drying, cleaning and sorting, which improves seed purity. High-value crops are often pelleted, which further enhances the seed by shaping it more uniformly. The final seed moisture at the end of the process is important, since any added liquids applied (e.g. seed treatments) might raise the moisture content above an acceptable level and limit the storage potential.

Seed treatment

Products are applied to seeds for different purposes. Traditionally, chemicals were and are used to disinfect and to protect the seed against seed- and soil-borne diseases. Certain technologies also allowed young seedlings to be shielded against various airborne pathogens. Systemic

insecticides such as the neonicotinoids revolutionised the seed treatment market, allowing the control not only of soil-borne, but also of early leaf-feeding and -sucking insect pests. Micronutrients and polymers can also be applied to enhance the performance of seeds and young seedlings.

Seed treatment formulation

Pure active ingredients cannot be applied to seed. Using current technologies, compounds need first to be formulated together with surfactants, stickers, anti-foam agents, dyestuff/pigments and inert carriers. Among many other aspects, the correct formulation allows the proper dosing and the avoidance of compatibility issues with other products. During the development process of a formulation, factors such as distribution, adherence and coloration on a seed batch are continuously analysed. Hence the correct design of a formulation adapted to the physical and chemical characteristics of an active ingredient is critical for excellent treatment quality. In addition, seed safety is evaluated at all steps of development with standard protocols and under different storage conditions.

Seed treatment equipment

Different technologies are used to apply seed treatment products, or combinations thereof, onto the seed. Continuous flow treaters are still widespread, especially for the treatment of cereal seeds, but increasingly are being replaced by batch treaters of different capacities. A key challenge is to distribute evenly different amounts of active ingredients (low: fungicides; high: insecticides) onto different seed types that vary in size, shape and surface structure. An exact calibration of the application equipment, and especially of the seed and chemical flow-metering systems, is critical. This task should be done before the start and routinely throughout the treatment campaign. Balancing and monitoring the volume of seeds treated with the product(s) consumed along a defined timescale and protocol will provide valuable information to deliver treated seeds of high quality ready to meet the expected biological performance.

Seed treatment quality management

Seed production and seed processing are critical steps feeding high-quality seeds into a seed treatment process. The availability of tailored formulations and sophisticated machineries are mandatory to achieve high-quality seed treatment output. Key quality requirements are:

- seed loading precision
- seed-to-seed loading uniformity
- seed flowability at bagging
- seed plantability/drillability
- seed dustiness/dust-off behaviour of formulations
- seed visual appearance.

Seed loading precision

The correct dosing of the target rate is important in order to fulfil the promise of field activity to the grower, offering peace of mind to the customers regarding seed safety and to comply with the registered label. Seed loading analysis can be done on either a bulk sample or individual seeds. Seed treatment manufacturers offer different technologies to measure seed loading.

- High-performance liquid chromatography (HPLC) is used by many laboratories within the seed treatment industry and by third-party service laboratories. HPLC allows the direct quantification of active ingredients, and is the state-of-the-art technology used for research in seed treatment. For rapid, routine and on-site quality monitoring, HPLC is still too expensive and too slow to provide feedback in time.
- Colorimetric methods rely on a built-in colour or pigment as marker to determine the loading rate and accuracy. This method is linked to the simultaneous, even distribution of the active ingredient and the colour/pigment. Syngenta has developed a portable on-site kit called SLAK™ (Seed Loading Analysis Kit), which allows monitoring of the loading of pigment coloured formulation. The test is fast, easy to handle, low-cost and offers good accuracy.
- Near infra-red technology can measure the loading of specific recipes. An advantage is that this method does not require specialised laboratories or skills, and is non-destructive, cost-efficient and fast.

Seed loading uniformity

To achieve good stand establishment, each seed needs to be protected efficiently to ensure that each one grows into a plant. The uniformity of seed loading is therefore crucial – each seed should be loaded with the targeted rate per single seed. Single seed loading accuracy is also becoming more and more important – with the increasing value of seeds (e.g. hybridisation, built-in genetic traits) and of seed treatments (e.g. seed-applied insecticide), seed companies are selling seed by number of seeds per bag or unit to optimise their cost basis and the input management of farmers.

To measure loading uniformity, either HPLC or digital imaging is the method of choice. Syngenta has developed and offers QUEST™ (Quality Evaluation of Seed Treatment) based on digital imaging technology. QUEST™ can measure different seed characteristics, such as size, shape and colour. This information can be used to support formulation development, to optimise equipment settings and to measure the seed-to-seed distribution or the uniformity on each seed of applied formulations. A prerequisite for uniform single seed loading is the sizing accuracy of the untreated seed lot.

Seed flowability at bagging

The window between harvesting, processing and treatment, on the one hand, and feeding the supply chain to deliver the seed to the farmer, on the other hand, is often quite narrow. To serve their customers at the right time with the right quantity and quality of seed, companies maintain a pre-set processing and treatment capacity. Flowability of treated seeds is a key factor for a good formulation or treatment recipe. A block-building test and a funnel flow test are methods of choice to test this characteristic of treated seeds. The block-building test allows judgement of whether a formulation or recipe has the tendency for treated seeds to become sticky and block transport tubes or the bagging valves by causing bridging. The funnel test measures the speed with which treated seeds pass through a funnel, compared with untreated seeds. Both tests are simple but give a good indication of the quality of treated seeds with regard to flowability.

Seed plantability/drillability

The correct stand per hectare or acre for yield optimisation starts with the appropriate amount of seeds planted or drilled per unit area. Today most crops are sown with precision drills or planters using crop-specific metering and placement systems (cell wheels, discs or plates) in

their seed-handling units. The seed treatment quality evaluation has to ensure that seeds do not lose material as a result of mechanical stress in such units, and that cell wheels, discs or plates are always filled with a single seed delivered into the furrow. Syngenta has developed the CornCounter™ technology for plantability evaluation. The system uses photoelectric barrier technology, which allows accurate identification of the spacing of seeds, the seed rate per area unit, and the presence of skips and multiples at planting or drilling under laboratory conditions. It also provides information on the influence of additives (e.g. polymers) or mixtures on plantability/drillability factors for formulation recipe development and recommendations.

Seed dustiness/dust-off behaviour of formulations

Another key criterion for evaluating treatment quality is the adherence of applied formulations or recipes onto the seed. In addition to formulation characteristics, dust-off performance is strongly influenced by the seed surface structure (smooth or rough seed coat) and the cleanliness of the seed. To achieve low levels of dustiness of treated seeds, the purity component of seed quality is of fundamental importance. Appropriate processing and seed cleaning technologies therefore have to ensure low-dust seed quality.

Besides the quality of seeds, the quality of the seed treatment formulation is critical. In the case of product combinations, recipes of the individual components need to be tested and approved. Polymers or appropriate stickers may be necessary to guarantee low dust-off performance.

A dust management plan that continuously monitors the various seed processing steps (including cleaning) and the treatment process (including dust-off performance) may avoid or minimise any exposure risk.

The seed treatment industry mainly uses two methods or tests to measure the dust-off performance of formulations or recipes once they have been applied to the seed: the CERES test and the HEUBACH dustmeter. The former is a modification of the CIPAC test MT 194, which mainly measures the native dust-load in a seed sample after treatment. The HEUBACH dustmeter evaluates the abrasion performance of a seed treatment formulation or recipe on a specific seed type under mechanical stress. Low or minimal abrasion and dust-off is important for worker and environmental exposure, and for the bought and expected protection of the seed. To minimise the exposure of non-target arthropods to dust that may be released from pneumatic planters during planting operations, treated seeds of maize, oilseed rape and other crops have to be tested for abrasion characteristics. Currently different maximum dust levels per 100 kg seed or 100,000 seeds are mandated by different European countries (e.g. 4 g dust/100 kg or 1.3 g dust/100,000 seeds) for maize seed. These values apply for the treated seed quality after the treatment process prior to bagging. To further minimise the exposure of non-target arthropods, pneumatic seed planters have to be modified in such a way that exhausted abrasive material originating from mechanical stress on the treated seed, either from handling or the metering and placement elements in the planting equipment, is directed towards the soil surface.

Seed visual appearance

The visual appearance of treated seeds as judged by the human eye is still perceived as a first step in quality evaluation. Attractiveness of the colorants and the uniformity of the treatment film play a key role. With the increasing value of technologies applied to the seed as a delivery vehicle, laboratory- and instrument-based methods for measuring seed treatment quality become more and more important.

Seed treatment application training and auditing

Good seed treatment quality starts with skilled operators running seed processing and treatment plants. Hence it is important to train and educate operators in the correct handling and application of seed treatment products and recipes. Seed treatment manufacturers offer customised training programmes for their own staff, and also for customers. In addition, plant audits may help to identify areas for improvement and create awareness.

Summary and conclusion

Quality management in seed treatment is uppermost in the minds of all parties involved in this crop protection technology. Driving factors are the increasing value of seeds, the more expensive and innovative protection and enhancement technologies compared with previous methods, the increasing regulatory requirements, and last but not least, the need for input/output optimisation of all steps of the value chain in food, feed and biomaterial production. Seed treatment has an advantage compared with field application, as the process is operated under controlled conditions in seed plants. Parameters can be set and monitored. Both the seed industry and the seed treatment manufacturers have to combine their expertise to deliver top seed treatment quality to their customers – the farmers. A broad range of tools is available today with which to evaluate and monitor quality factors such as seed loading, treatment uniformity, plantability, abrasion and many other measurable characteristics. The progress of technology will allow new tools to become available, which will either replace or improve current methods.

Uptake of model compounds by soybean, switchgrass and castor seeds applied as seed treatments

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Summary

The efficacy of systemic seed treatments depends on the ability of applied chemical compounds to be taken up and then transported into the seedling. The objective of the present work was to study if organic compounds can diffuse through the seed coat and are able to penetrate the embryo of different crop seeds. Attention was focused on the differences in diffusion processes caused by the unique seed coat permeability properties. The selection of crop seeds provided a range of seed coat morphologies and compositions, including the presence of semi-permeable layers. A number of fluorescent and coloured tracers were employed to mimic transport of systemic seed treatments and for visualisation of chemical movement. These tracers provided a range of log K_{ow} values (hydrophilic/hydrophobic nature) and charge (non-ionic, cationic or anionic). Tracers were applied to seeds by a dry coating process, placed in moistened sand for 18–24 h at 20°C and removed prior to visible germination. Imbibed seeds were hand-dissected, and the location and intensity of fluorescence observed under long-wavelength UV light. Large-seeded legumes such as soybeans have seed coats permeable to fluorescent tracers. Castor seed coats were impermeable to the tracers, indicating the presence of the semi-permeable barrier surrounding the embryo. Switchgrass has a semi-permeable seed coat composed of suberin or cutin, and uptake was dependent on the chemical nature of the tracer. In conclusion, the ability of a particular compound to diffuse the seed coat was determined by the chemical nature of the seed covering tissue and physicochemical properties of the compound applied (log K_{ow} and charge).

Introduction

Systemic seed treatments are used commercially for efficient pest management, especially for foliar pest control, and have potential for eradicating seed-borne pathogens. Systemic compounds should penetrate the seed coat or seed-covering tissues to be effective. In particular, active ingredients that affect the embryo prior to visible germination must reach their target. Pesticide absorption varied with different species, and was related to chemical properties of the pesticide, seed composition (proteins, lipids) and seed coat characteristics (Phillips *et al.*, 1972; Garcinuno *et al.*, 2003). Lipophilicity, charged state, molecular weight and H-bonding capacity are the major characteristics that determine the effectiveness of chemicals to be taken up by plants. Lipophilicity assesses the affinity of the compounds for the lipid phase of plant tissues (plasma membrane, waxes, cutin, suberin, etc.). Lipophilicity is determined by the octan-1-ol/water partition coefficient and expressed as log K_{ow} or log P. Figure 1 illustrates the effect of log K_{ow} on systemic activity (curve adapted from Briggs *et al.*, 1982).

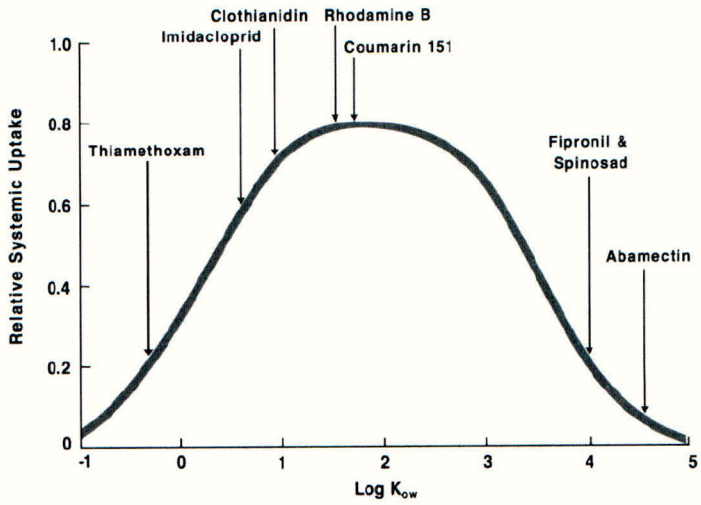

Figure 1 Relative systemic uptake of selected seed treatments and fluorescent tracers

Most systemic seed treatments have a log K_{ow} between approximately 0 and 4. Systemic seed treatments Cruiser (thiamethoxam, Syngenta), Gaucho (imidacloprid, Bayer CropScience), Poncho (clothianidin, Bayer CropScience) and Mundial or Regent (fipronil, BASF) have a log K_{ow} of –0.13, 0.57, 1.05 and 3.7, respectively, while the non-systemic Avicta (abamectin, Syngenta) has a log K_{ow} of 4.4. The partition coefficient varies with pH for Entrust (spinosad, Dow AgroSciences) with log K_{ow} of 2.8, 4.0 and 5.2 at pH 5, 7 and 9, respectively. Compounds with high log K_{ow} (>4) are not systemic as they are strongly retained in the plant lipid constituents, and have limited water solubility. The electrical charge of molecules influences migration, and positively charged molecules are bound by negatively charged cell walls. The optimal number of H-donors and acceptors in pesticide molecule was suggested to be fewer than five and 10, respectively, based on Lipinski parameters. According to Briggs (1997), the 'limit' number of H-donors for agrochemicals should not exceed three. Increasing molecular weight was reported to impair molecule penetration across the plasma membrane. Mobile agrochemicals were found to have a molecular weight around 300 or less (Briggs, 1997).

Uptake of systemic compounds applied as seed treatments was reported for soybean, wheat and lupin. However, an analysis was not performed in terms of relationship between physicochemical properties of applied chemicals and seed coat permeability. The great majority of earlier studies used radioactive tracer techniques (^{14}C-radiolabelling) to localize compounds in seed tissues. In the current study, fluorescent or coloured tracers were chosen to mimic seed treatments and to visualise diffusion of compounds through the seed coat. Such tracers avoid disadvantages of radio-labelling techniques, including cost, availability, worker safety and disposal issues. Fluorescent tracers were used as model compounds and represented diversity of chemical structure, with special attention to the value of log K_{ow}, charge, H-bonding capacity and molecular weight. Collectively, the objective of the present work was to study the seed coat permeability of selected species with different morphology and anatomy using fluorescent tracers as model compounds.

Materials and methods

Plant materials

Three crops were studied: soybean AG 1901 (*Glycine max*), castor Hale (*Ricinus communis*) and switchgrass Shawnee (*Panicum virgatum*). Castor is commonly, but incorrectly, known as castor bean as it is not a legume.

Model compounds

Seven fluorescent dyes and two colour-forming compounds were used with different values of K_{ow}, charge, pKa, molecular weight and H-bonding capacity. Two fluorescent coumarin derivatives, coumarin 1 (log K_{ow} 3.0) and coumarin 151 (log K_{ow} 1.8) were selected to mimic non-charged moderately lipophilic pesticides. Three fluorescent tracers, fluorescein (log K_{ow} 1.8), carboxyfluorescein (log K_{ow} –1.9) and uranine (log K_{ow} –1.3) were chosen to simulate weak acid pesticides. Tetrazolium red (log K_{ow} –2.4) and tetrazolium violet (log K_{ow} –1.2) represented hydrophilic cationic pesticides. Rhodamine B (log K_{ow} 1.5) and sulforhodamine B (log K_{ow} –2.0) were used, as they differ in lipophilicity and are zwitterionic compounds.

Dye application and microscopy

Tracers were applied as dry powders to seeds to avoid exposure to water during treatment. Treated seeds were placed in moistened builders' sand at 20°C and removed prior to, or after, visible germination, as needed. The location and intensity of fluorescence in hand-dissected imbibed or germinated seeds were observed under long-UV (365 nm) light with an Olympus SZX12 stereomicroscope, equipped with a SPOT Insight camera and software (ver. 4.5).

Coumarin uptake by soybean seeds under different water regimes

Soybean seeds were used to study tracer uptake under water stress conditions and at 100% relative humidity (RH). For the water stress treatments, different concentrations of polyethylene glycol 8000 (PEG) were prepared to obtain solutions of different water potential. Seeds were treated with coumarin 1 powder, placed in sand moistened with solutions of 0 MPa (water), –1 MPa or –2 MPa water potential, and incubated in closed containers at 25°C. For the 100% RH treatment, coumarin 1-treated seeds were placed on screens above water in closed containers producing a water vapour-saturated atmosphere at 25°C. Seeds were examined for dye penetration after 12, 48, 96 and 144 h.

Results and discussion

Seed coat permeability to applied tracers

Soybean (*Glycine max*)

The results for seed coat permeability of soybean to applied tracers are summarised in Figure 2(a). Based on observations, seed coats of soybeans were permeable to most of the fluorescent tracers: coumarins, fluorescein, carboxyfluorescein, uranine and rhodamine B. After seeds were imbibed and seed coats removed, embryos revealed strong uniform fluorescence on the cotyledons and embryo axis tissues. Both tetrazolium salts penetrated soybean seed coat; however, red and purple formazan coloration on the surface of the cotyledons was not uniformly distributed. Sulforhodamine did not permeate the seed coats.

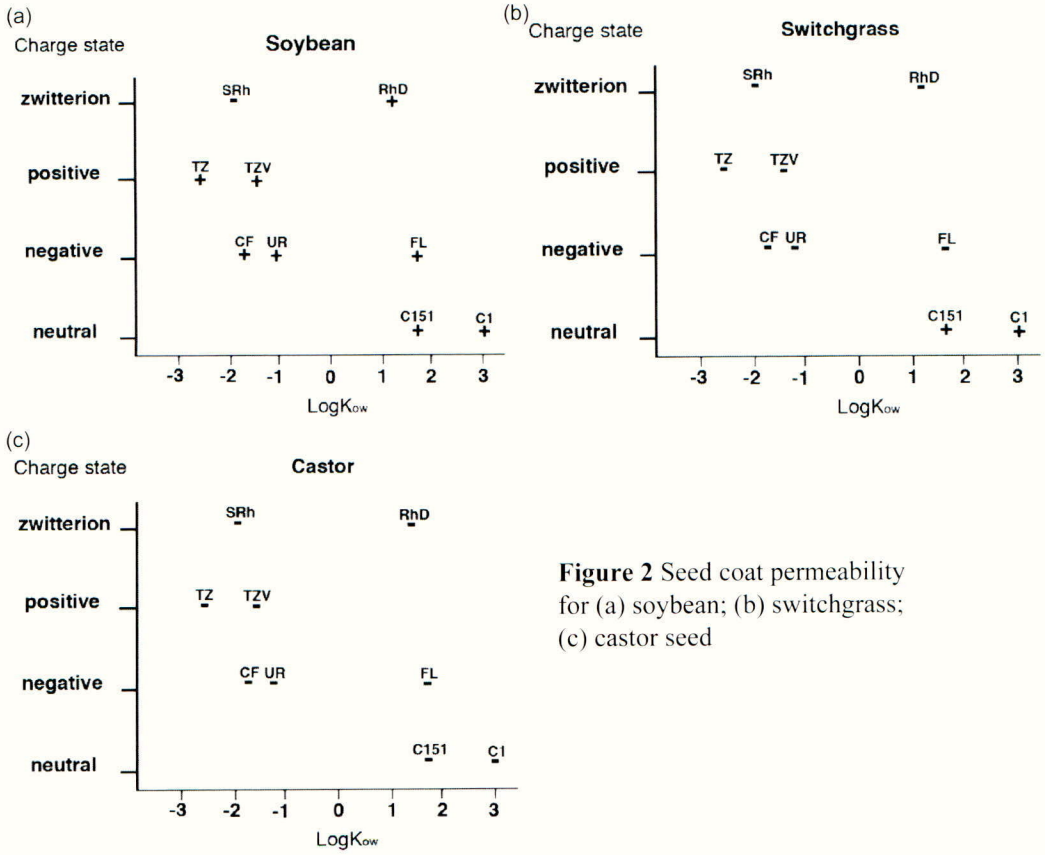

Figure 2 Seed coat permeability for (a) soybean; (b) switchgrass; (c) castor seed

Switchgrass (*Panicum virgatum*)

The results for seed coat permeability of switchgrass are presented in Figure 2(b). The seeds were found to be permeable only to non-ionisable fluorescent tracers (coumarin 1 and 151). A strong fluorescence was revealed only in embryo tissue, while no fluorescence was recorded in endosperm of the grain.

Castor (*Ricinus communis*)

No fluorescence or staining was observed in the embryo tissue after imbibition of castor seeds treated with range of tracers (Figure 2c).

Coumarin uptake under different water regimes

Tracer uptake by soybean seed at different medium water potentials and 100% RH is summarised in Table 1. Uptake was rapid from seeds imbibed in sand moistened with water, and treated seeds revealed fluorescence in the embryo after 12 h. All seeds were germinated after 48 h that were hydrated with water. Tracer uptake rate was slower when seeds were sown in sand moistened with –1 or –2 MPa PEG solutions compared with the water check. However, no visible germination was recorded at either water stress by 144 h. Maintaining seeds at 100% RH resulted in limited tracer penetration after 96 h, while at 144 h strong fluorescence was observed in the embryo. Therefore liquid water is not needed for diffusion of coumarin through the seed coat.

Table 1 Tracer uptake by soybean seeds

Medium	12 h	48 h	96 h	144 h
0 MPa	+	G	G	G
–1 MPa	–	+	+	+
–2 MPa	–	+	+	+
100% RH	–	–	F	+

F, faint; G, seeds germinated.

Based on a structure–activity relationship (SAR) model (Horobin & Rashid, 1990), non-ionised moderate lipophilic tracers (coumarin 1 and coumarin 151) should be taken up by plant tissues. Indeed, both coumarins were observed in soybean and switchgrass embryos, indicating that these tracers permeated the seed coats of these crops. The charged molecules (rhodamine B, uranine, fluorescein, carboxyfluorescein, tetrazolium red and tetrazolium violet) penetrated only soybean seed coats, indicating that a semi-permeable layer is not present in this large-seeded legume. For many grasses, a semi-permeable cutinised or suberised membrane in the caryopsis integuments restricts solute transport through the seed coat (Simpson, 1990). Assuming similar histochemistry in switchgrass caryopses, the apparent source of seed coat impermeability is cutinised/suberised layers of the integuments. Therefore in grass species, a compound's ionisation status determines its ability to penetrate seed coats containing semi-permeable layers. In contrast, castor seed coats were not permeable to any compound tested. However, both coumarins were able to diffuse through thick, lignified outer seed coat, but did not permeate the inner seed coat and the tracers accumulated as crystals on its surface.

Collectively, seed coats are the primary factor regulating chemical movement from the seed surface to the embryo during imbibition. The histochemical nature of seed covering tissues differs by plant species and may attenuate transport of compounds. Physicochemical properties of compounds in combination with seed coat histochemistry can predict the penetration of substances through seed coats.

In summary, seed coat permeability to solutes can be grouped into three categories: (1) permeable, (2) semi-permeable and (3) non-permeable. Soybean had a seed coat permeable to a wide range of applied chemicals, whereas swithchgrass had semi-permeable characteristics that transmitted only non-ionic compounds with moderate lipophilicity to penetrate. Castor had a seed coat non-permeable to applied compounds. Therefore systemic uptake of compounds must occur through roots in castor.

An outcome from this research is that seed coat permeability characteristics can be determined for other species of seeds. Tracers are applied as dry powders by placing seeds with tracer in a closed container and shaking for 1 min. The excess powder is discarded, and the uniformly treated seeds sown in moistened sand. Alternatively, agarose is dissolved (0.6% w/v) in de-ionised water saturated with fluorescent tracer in a microwave oven. The solution is poured into Petri dishes to obtain a 1-cm-thick gel layer and subsequently cooled at room temperature. Seeds are immersed in solid agarose gel and incubated at 20 or 25°C. Seeds are removed from either moistened sand or agarose gel prior to visible germination or any signs of seed coat cracking. The seed-covering tissues are removed, and embryos are examined under long-UV

light (365 nm). Magnification may be needed depending on seed size. Seed coat permeability to solutes can be grouped as (1) permeable, (2) semi-permeable or (3) non-permeable by testing each species with coumarin 151 and rhodamine B. Positive embryo staining with coumarin 151 and rhodamine B indicates permeable seed coats. Positive embryo staining with coumarin 151 and negative staining with rhodamine B indicates semi-permeable seed coats. Negative embryo staining for both coumarin 151 and rhodamine B indicates non-permeable seed coats. The method is simple, and all chemicals can be purchased from scientific chemical vendors.

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Transmission and spread of *Xanthomonas campestris* pv. *campestris* in brassica transplants: implications for seed health standards

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Summary

Xanthomonas campestris pv. *campestris* is well known as an important seed-borne pathogen of brassicas. Seed health assays should be designed to have a high probability of detecting unacceptable seed lots. Mathematical models have been developed both for transmission of the pathogen from seed to seedling and subsequent spread in module-raised brassica transplants. Using these models, with different initial parameters, the potential for development of disease epidemics can be explored for negative results obtained by seed health assays with different sensitivities (detection limits) and tolerance standards. Examples of different scenarios will be presented, and suggest that the greatest risk arises when negative test results are obtained from seed lots with a relatively high proportion of infested seeds but low number of bacteria per seed.

Introduction

Xanthomonas campestris pv. *campestris* (*Xcc*) is well known as an important seed-borne pathogen of brassicas. Seed health assays should be designed to have a high probability of detecting unacceptable seed lots. The problem is to define an 'unacceptable seed lot', and in recent years there has been much dispute over the value of the most sensitive seed health assays and the tolerance standards required to achieve satisfactory control of *Xcc* in brassicas. Schaad *et al.* (1990) suggested a tolerance standard of 0.01% for a direct-drilled brassica crop, but that this was inadequate for transplant production. Most vegetable brassicas are grown as transplanted crops, but most seed is still tested to a tolerance standard of 0.01%. This paper will present the results of work done over several years to develop models to describe the transmission and spread of *Xcc*, which have then allowed us to examine the potential development of disease epidemics for seed lots with different seed health scenarios and the likelihood of their detection in seed health assays with different sensitivities.

Models

A model for the transmission of *Xcc* from seed to seedling was devised using data from glasshouse experiments. Seed was inoculated with different concentrations of bacteria, sown in commercial module trays, and subjected to different watering regimes (Roberts *et al.*, 1999). Visible symptoms were recorded, and leaf washings were carried out to detect

the pathogen on symptomless plants. The results were consistent with a one-hit model for infection/transmission:

$$P = 1 - \exp(-w.d^x)$$

where P is the probability of transmission, w is the 'one-hit' probability, d is the dose (number of *Xcc* per seed) and x is a dose coefficient.

A model for the spread of *Xcc* in brassica transplants was developed using data from a series of glasshouse experiments designed to simulate a typical commercial module plant raising system with overhead gantry irrigation (Roberts *et al.*, 2007). Primary inoculum was introduced as inoculated seeds in one or more cells. Disease symptoms were mapped and the presence of the pathogen on samples of plants was monitored by leaf washing, dilution and plating on a selective medium. Spread of symptoms and spread of contamination followed a similar pattern, but the proportion of plants contaminated was much greater than the proportion showing symptoms, approaching 100% after 6 weeks in the gantry-watered trays within 50 plants distance from a single primary infector. Models relating the proportion of plants with symptoms, or contaminated, to the distance from primary infector and time since sowing were fitted to the data:

$$\ln[p/(1-p)] = \ln(a) + b\ln[c + (k.x^2 + y^2)^{½}] + r.t$$

where p is the proportion of plants contaminated, a is an intercept parameter, b is the gradient, c is a truncation parameter, k is a directional scaling parameter, x,y are the distance from the primary infector in the x and y directions, r is the relative contamination rate, and t is time.

These models were used to explore the potential for development of disease epidemics in commercial-scale blocks of transplants for seedlots with different proportions of seed infested and different numbers of bacteria on those infested seeds. Using model parameters from different spread experiments, the expected proportions of contaminated transplants were calculated for a block of approximately 100,000 transplants, assuming uniform distribution of infested seedlings and assuming 100% transmission.

The average percentage contamination of transplants was then calculated by multiplying the expected proportion obtained from the spread models above by the probability of transmission obtained from the transmission model for the different seed infestation scenarios.

For each seed infestation scenario, the probability of detection was also calculated for seed health assays with different sensitivities (detection limits; resulting from the inclusion/omission of a centrifugation step). The probability of at least one infested seed being contained in the sample is given by:

$$P_{cont} = 1 - (1 - \theta)^n$$

where θ is the true proportion of infested seeds in the lot and n is the total number of seeds in the sample. Then, if present, the probability of detecting an infested seed in a sub-sample is given by:

$$P_d = 1 - e^{-\lambda v}$$

where λ is the mean density of bacteria in the suspension (the number of bacteria per infested seed divided by the volume in which the sub-sample is suspended) and v is the effective volume plated. Thus the probability of a positive result for the test is given by: