A Bioassay for Asparagus (*Asparagus officinalis L.*) Resistance to *Stemphylium vesicarium*

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ABSTRACT

A Bioassay for Asparagus (Asparagus officinalis L.) Resistance to *Stemphylium vesicarium*

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Purple spot, caused by *Stemphylium vesicarium* (Wallr.) E. Simmons, is one of the most important diseases facing asparagus producers in Ontario. Four cultivars, Jersey Giant, Guelph Millennium, Guelph Eclipse, and Gijnlim were examined in the field and in detached-spear controlled environment assays for reaction to *S. vesicarium* infection. Consistent results were achieved in the controlled environment assays and cultivar, location on the spear, isolate, wounding, and lighting were each found to affect lesion formation. In the field, an interaction was identified between cultivar resistance and acute weather, especially heavy rainfall, before and during a critical infection period. In controlled environment studies there was an interaction between cultivar and location of inoculation on the spear, whether near the top or base. The bioassay was useful for assessing some mechanisms of resistance, but inoculating attached spears in the field, while accounting for weather, is recommended for determining realistic field results.

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1. Introduction

Purple spot, caused by *Stemphylium vesicarium,* is one of the most important diseases facing asparagus (*Asparagus officinalis)* producers in Ontario and globally. In the spring, symptoms of the disease develop on the spears as purple lesions which are unsightly, making the spears unmarketable. In the summer and fall infections on the 'fern' cause premature defoliation resulting in reduced carbon assimilation, decreased levels of stored carbohydrates during the winter, and reduced spear production the following year.

Removal of crop residue harbouring the pathogen and reducing sandblasting through hedgerows and cover crops are the only useful cultural practices to decrease disease severity, but these are limited in their effectiveness. Chemical control through numerous fungicide applications is often relied upon. As asparagus is a perennial crop, crop rotation is not an option. Biological control has limited effectiveness, and as the pathogen is wind-borne and can reproduce on dead non-host tissue, quarantines to stop the spread do not work.

While current cultivars of asparagus have superior resistance to *S. vesicarium* than some previously popular cultivars, none are known to have exceptional resistance in the spear. Other species in the genus *Asparagus*, including *A. virgatus* and *A. densiflorus*, are highly resistant (Bansal et al. 1986) yet interspecific crosses to breed resistance into *A. officinalis* cultivars face incompatibility barriers (Marcellan and Camadro 1999). Whether these other species have greater resistance due to horizontal or vertical resistance compared to *A. officinalis* is unknown. Screening asparagus cultivars for resistance by surveying spears and ferns in the field has produced inconsistent results (Foster 2018). Fern architecture may affect disease severity by

affecting the availability of free water and humidity required for propagule germination and hyphae growth respectively (Broadhurst 1993).

Determining which cultivars are the most resistant to *S. vesicarium*, and if the mechanisms of resistance differ among cultivars, will assist plant breeders to build resistance into new cultivars. A bioassay for detached spears, where factors affecting resistance can be carefully controlled, would be useful for both of these needs.

The primary objective of this work was to create a detached spear bioassay to determine cultivar resistance to *S. vesicarium* that is consistent with disease reaction in the field. A second objective was to determine factors that affect resistance and determine mechanisms of resistance among cultivars. Factors that affect resistance in the field also require investigation to assist in breeding for resistance.

2. Literature Review

2.1 Asparagus production in Ontario

Asparagus originated in the Mediterranean where the young shoots have been consumed by locals for millennia. This monocot perennial vegetable crop is in the family Asparagaceae in the order Asparagales (Govaerts, 2021). During the summer, what is known colloquially as a fern emerges. These ferns are composed of a stem over 2 meters in height and approximately 2 cm in diameter with many thin branches with side branches emerge from the stem. Closely spaced clusters of cladodes, which are a modified stem with length of 2 cm and width of 1 mm, cover the branches. The plant is dioecious with the female plants producing inedible red berries, although production in Ontario is of all male hybrid cultivars. In the fall, the cladodes senescence and photosynthate translocates to the crown that remains dormant during the winter, under the soil surface. In the spring, new shoots emerge from the crown. If these are harvested before becoming woody they make a wonderful and widely consumed vegetable (Agriculture and Agri-Food Canada 2018).

In 2018, Canada produced 8,518 tonnes of asparagus worth $38 million on 1,931 hectares (Statistics Canada 2020). Ontario was responsible for 70% of the production, Quebec 20%, and British Columbia 5% (Statistics Canada 2020). White asparagus and canned asparagus are common in some areas of the world, especially Europe and Australia, but in Canada, production and consumption is almost exclusively fresh green spears (Agriculture and Agri-Food Canada 2018).

In Ontario, production is concentrated around Simcoe in Norfolk county on the sandy soils in that region. Asparagus spears may be deformed in heavy and rocky soils and the risk of

asparagus root diseases is reduced in well-drained sandy soils (Agriculture and Agri-Food Canada 2018). No-till is common practice for asparagus production in Ontario as tillage will damage asparagus roots and increase the risk of root diseases, both reducing long-term yields (Agriculture and Agri-Food Canada 2018). Due to this, perennial weeds should be controlled prior to planting an asparagus field (Fritz et al. 2013).

The cultivars grown in Ontario are all male-hybrids developed through breeding with a supermale (YY) parent. Male plants produce higher yields compared to female plants, produce a higher ratio of #1 spears, and do not expend energy producing seeds, which also leads to the problem of volunteer asparagus plants (Sinton and Wilson 1997).

Asparagus is a perennial crop and plantings may stay productive for 15-20 years. New fields may be started by seed, transplanted seedlings, or crowns. Specialized growers produce crowns that are planted 15-20 cm deep. Harvest begins in the third year of establishment (Agriculture and Agri-Food Canada 2018).

Asparagus spears, the new shoots, begin to emerge in the spring as soil temperatures rise. The speed at which bud break occurs varies among cultivars and the speed increases as temperatures increase (Ku 2007). In Ontario, harvest generally begins once night temperatures stay above 10°C. Harvesting takes place for approximately six weeks on well-established crops, although the harvest season will be shorter for newer plantings. If newly emerged spears are less than 10 mm in diameter the harvest should end for the year as further cuttings will overly-weaken the plants causing significant reduction in future yields (Fritz et al. 2013).

During the harvest season, new spears emerge each day and will grow to a picking height of 23 cm in less than a week. Daily harvest may be required to avoid overly long spears when

warm weather causes an increase in the growth rate. Spears are graded into size groups based on diameter which is measured 25 mm from the butt. Canada Slender has the thinnest spears at 8-9 mm, and Canada Jumbo is for spears thicker than 19 mm (Canadian Food Inspection Agency 2011). Asparagus spears may be degraded if they are too dirty, misshapen, crushed, if there is insect damage or presence, or there are “seeds" (the first emergence of branches from under the bract) in the tip. The harvested crop may be rejected entirely due to too much white colouration at the base of the spear, or there are spread tips, broken spears, decay, and freezing damage. The presence of symptoms of purple spot on spears may result in a grading reduction, or if severe, rejection (Canadian Food Inspection Agency 2011). As new spears may be harvested every day during the spring, applying pesticides to control diseases or insects during the harvest season is usually not possible. Immediately after the last harvest is an ideal time to apply a post-emergence herbicide, nitrogen fertilizer, and fungicides and insecticides as required (Fritz et al. 2013).

With harvesting complete, the spears are allowed to grow into large ferns so that they may accumulate photosynthate for immediate use and then for over-winter storage in the crown to produce spears the following spring. Ideally, ferns will senesce and translocate carbohydrates to the crown before the first frost in the fall. The timing of the senescence in response to cool fall temperatures and shortening day length varies among cultivars, as does winter hardiness and timing of spring emergence (Panjtandoust and Wolyn 2016; Landry and Wolyn 2011). Cultivars that release dormancy early and have reduced freezing tolerance through changes in concentrations of various molecules and water are at increased risk of being damaged by late spring freezing (Panjtandoust and Wolyn 2016; Landry and Wolyn 2011). Simple sugars, including sucrose, glucose, and fructose are the source of energy for shoot production (Landry

and Wolyn 2011). A cover crop, such as rye, may be sown in August or September and killed with a herbicide such as glyphosate shortly before spears begin to emerge (Myers 2012). In the spring, overwintering fern debris will be mowed, or if pathogens are overwintering in the debris it could be removed, burned, or buried (Fritz et al. 2013).

2.2 Disease resistance in plants

There are many types of genetic resistance to plant diseases that vary depending on the plant-microbe relationship. Resistance to pathogens can be determined by genes that allow the plant to detect the presence of pathogens (Boschi et al. 2017) or pathogen-produced molecules (Horvath et al. 2012), detect pathogen-induced perturbations within the plant (Kim et al. 2016), produce molecules that are toxic to the pathogen (Li et al. 2011), degrade the pathogen produced toxins (Partridge-Telenko et al. 2011), and boost (Wang et al. 2018) or modify the defense response (Namukwaya et al. 2012). Disease resistance has been improved in plants by altering the requirements, such as temperature, for the expression of resistance genes (Rinaldo et al. 2017), or by creating plants that produce siRNA to inhibit gene expression in the pathogen (Govindarajulu et al. 2015). Plants can also have susceptibility genes (S-genes) that when removed from the genome of the plant, resistance is increased (Pessina et al. 2016; Murphy et al. 2018).

Resistance to pathogens can be described as either horizontal or vertical (Vander Plank 1963). Horizontal resistance is incomplete but is stable as it is based on many genes and it is not specific against races of a pathogen. Vertical resistance is based on a gene-for-gene relationship where the plant has a single gene coding for a product that recognizes a pathogen associated molecular pattern (PAMP) on a product originating from the pathogen. The drawback is that

vertical resistance can exert a strong selection pressure for strains to emerge that are not affected by the vertical resistance gene. Current asparagus cultivars have horizontal resistance to *S. vesicarium*, and the type of resistance other asparagus species have is unknown (Bansal et al. 1988; Bansal et al. 1992).

Systemic acquired resistance (SAR) is a whole-plant non-specific defense response to infection. SAR relies upon the accumulation of salicylic acid (SA) to signal a defense response throughout the plant. The SAR defense response is composed of many mechanisms and varies among plant species (Gozzo & Faoro 2013). Plants may also defend against infection by biotrophs through a hypersensitive response where plant cells quickly die around the area of infection to block further expansion into the plant by the pathogen (Balint-Kurti 2019).

2.3 *Stemphylium vesicarium* host range and distribution

Stemphylium vesicarium is a fungus in the family Pleosporaceae and order Pleosporales (Centre for Agriculture and Bioscience International 2021). Researchers have identified *S. vesicarium* as a pathogen on a wide range of crop and weed species; the host range includes approximately 150 plant species (Farr and Rossman 2021). *Stemphylium vesicarium* has been identified as a plant pathogen on all continents. The most important crops that are damaged by the pathogen include asparagus (Suzui 1973), pears (Ponti et al. 1982), onions and garlic (Rao and Pavgi 1975), and parsley (Koike et al. 2013). Both pathogenic and non-pathogenic isolates of the fungus have been found to colonize and sporulate on many dead non-host plant species (Kohl 2013). The pathogen can infect dead weeds and grasses, and can colonize and sporulate on necrotic rye, a common cover crop for asparagus production (Foster et al. 2019). Isolates of *S. vesicarium* do not lose pathogenicity while in a saprophytic stage on dead tissue of non-host

plant species (Rossi et al. 2005). Researchers were able to isolate *S. vesicarium* from nearly half of symptomless onion leaves from an infested field (Misawa and Yasuoka 2012). This pathogen cannot be eradicated from an area once it is present as it can sporulate on dead non-host material, be carried into fields on the wind, and is polycyclic, producing abundant conidia over several cycles in a growing season.

2.4 Life cycle

The teleomorph *Pleospora herbarum* (Wallr.) Simmons reproduces through the production of ascospores in asci within pseudothecia. The pseudothecia form on both host plants and dead non-host-tissues as temperatures drop in autumn. The pseudothecia stay dormant until a combination of warmth and free water in the spring cause the turgor pressure in the pseudothecia to rise and expel the ascospores held within. The release of the ascospores can be modelled by summing the degree days with a base of 5°C on days with precipitation. By 200 degree days, 50% of ascospores will be released, and 90% will be released by 400 degree days in German asparagus fields (Bohlen-Janssen et al. 2018b). Not all pseudothecia erupt during the first warm and wet spring day, some will open intermittently and others will erupt on subsequent wet and warm days (Bohlen-Janssen et al. 2018b). Ascospores begin to be released months prior to first symptoms appearing on onions. Conidia are quickly produced in the spring, mainly on newly infected plants and colonized plant debris. Conidia have been found on the surface of pseudothecia and on overwintered mycelia (Bohlen-Janssen *et al.* 2018b). Low levels of conidia can be found in onion fields along with the first ascospores in the spring (Gossen et al. 2020). The concentration of *S. vesicarium* conidia increases quickly as the ascospore levels drop and high numbers of conidia are found throughout the summer (Gossen et al. 2020; Bohlen-Janssen

et al. 2018b). During the growing season, the number of airborne conidia correlated with yellow mottle lesions characteristic of *S. vesicarium* lesions on onion (Gossen et al. 2020).

The start of the asparagus season and ascospore release both depend upon the weather, but the beginning of ascospore release will predate the first asparagus harvest (Bohlen-Janssen *et al.* 2018b). Ascospores are believed to be the primary inoculum on asparagus, but this has not been proven. Purple spot lesions on asparagus spears appear the same whether caused by ascospores or conidia (personal observation). Infections on asparagus may not be a major source of conidia; conidia production may be substantial on dead non-host tissue in or around the field.

Conidia production has been shown to be more than 20 times higher on many dead grasses than on lesions found on dead pear leaves or fruit (Rossi et al. 2005). Ascospores were larger when formed on pear leaves and fruit than on grasses and were also fewer in number (Rossi et al. 2005). Based on the delay between ascospore release and symptoms appearing on pear, it is thought that the ascospores first infect dead leaves on the orchard floor, then conidia produced on the plant material on the floor infect the pear leaves or fruit. (Rossi et al. 2005)

2.5 Propagule germination

Stemphylium vesicarium conidia require free moisture to germinate; very high humidity alone is not sufficient (Llorente and Montesinos 2002). Once conidia have germinated, free water is not required for the infection process to continue although low humidity will inhibit infection (Llorente and Montesinos 2002). Periods of low humidity stop the growth of hyphae and inhibit new infections from occurring (Llorente and Montesinos 2002).

The germination rate for conidia depends on temperature. When a solution of conidia suspended in sterile deionized water is placed on water agar plates at 20-30°C more than 95% of

conidia germinate within 4 hours, at 15℃ there is 70% germination at 4 hours, which plateaus at 90% around 6 hours, and at 5℃, germination reaches 75% at 24 hours. Germ tube growth rates increase over time. Growth rates are highest around 25-30℃ and diminish significantly as temperatures decrease. The number of lesions caused by conidia inoculated on spears was highest at 20℃ and modelled to be highest at 22℃. The mycelial growth rate on agar was fastest at 25℃ (Bohlen-Janssen et al. 2018a).

The germination speed and rates for ascospores follow the same pattern of conidia regarding temperature. Germ tube growth rates are constant at any given temperature, with growth rates slowest at 5℃ at 3.27 μm/h and highest at 25℃ at 27.07 μm/h (Bohlen-Janssen et al. 2018b).

2.6 *Stemphylium vesicarium* and asparagus

Stemphylium leaf spot on asparagus was first reported in Japan in 1973 with the author reporting the causal pathogen as *S. botryosum* (Suzui 1973). In 1982, purple spot was first described in Michigan, U.S. as a disease caused by *S. vesicarium* (Lacy 1982). *Stemphylium botryosum* and *S. vesicarium* cannot be reliably differentiated based on morphology. A DNA marker was found in 2016 which allowed for genetic testing to prove that most samples collected from asparagus purple spot lesions were *S. vesicarium* and not *S. botryosum* (Graf et al. 2016) and the molecular markers have been further improved (Foster et al. 2019). Isolates from asparagus and onion in Ontario were confirmed to be *S. vesicarium* (Foster et al. 2019).

The lesions that develop from *S. vesicarium* infection are only a few millimeters in size, are elliptical in shape, and are vertically orientated (Lacy 1982). The lesions are light brown but are surrounded by a several mm wide purple halo due to accumulation of anthocyanin around the

lesion. A single spear may have several hundred lesions if conditions are apt for infection (Lacy 1982).

Stemphylium lesions cause two problems for asparagus growers; the lesions on the spears can reduce marketability of the spears and the lesions on the cladodes cause premature defoliation leading to a possible substantial yield reduction in subsequent growing seasons (Wilson et al. 2001; Menzies et al. 1992).

The pathogen was initially reported to only infect through wounds on asparagus spears (Lacy 1982) but has since been shown to produce appressoria for infection through stomata (Falloon 1987). On onion, *S. vesicarium* has been shown to use terminal and intercalary appressoria to penetrate epidermal cells as well as to penetrate through stomata (Suheri and Price 2000). Direct epidermal penetration has not been recorded on asparagus (Falloon 1987).

Stemphylium vesicarium produces at least two host-specific toxins that are involved in pathogenesis (Singh et al. 1999). Applying the isolated toxins alone caused damage to leaves from susceptible pear trees but did not damage leaves of resistant pear or non-host leaves. *Stemphylium vesicarium* strains known to be non-pathogenic to pear were applied in conjunction with the isolated toxin and this allowed the strain to infect the usually susceptible pear leaves but not the resistant pear leaves.

The necrosis-inducing host-specific toxins of the closely related *Alternaria alternata* requires apple trees to have certain S-genes for the toxins to be effective (Saito et al. 2001). It is not known if susceptibility to infection in *A. officinalis* is due to the presence of S-genes that are absent in closely related highly resistant species, or if *A. officinalis* is missing genes that confer resistance.

2.7 Infection on asparagus

Stemphylium vesicarium only infects asparagus through open stomata and wounds (Falloon 1987). Stomata are in the epidermis and are composed of two guard cells whose shape changes from oval to kidney-shape when their osmotic pressure is high. An aperture exists between guard cells only when they are engorged. By controlling the osmotic pressure in the guard cells plants can control the diffusion of gases between the plant and the air to optimize plant function. The signaling mechanisms used to actively control the stomata is debated, though there is strong evidence that abscisic acid plays a role in decreasing solute concentration, and thus osmotic pressure in the guard cells, causing stomata closure (Buckley 2019). It is very common for microorganisms to penetrate plants through open stomata and many plants have developed a system to close stomata in response to the detection of pathogen-associated molecular patterns (PAMPs) regardless of other factors that may dictate an opening of the stomata (Melotto et al. 2017). Some pathogens are known to produce secondary metabolites that induce stomata to open (Melotto et al. 2006).

While the plant is signaling to the stomata to stay closed in response to the detection of PAMPs, it is possible the stomata could inadvertently open temporarily due to a 'wrong-way-reaction' (Buckley 2019). A wrong-way-reaction occurs when the water status of epidermal cells surrounding the stomata drops causing the epidermal cells to shrink in size. When epidermal cells on both sides of the guard cells of the stomata shrink, they pull the guard cells apart from each other, creating a slightly open stoma. Reclosing the stoma by reducing the osmotic pressure in the guard cells requires active effort by the plant and it may take a number of minutes to return to the closed state (Buckley 2019).

A wrong-way-reaction may occur in asparagus fields after a heavy rain. First, the plant absorbs excessive water from the wet soil as a result of root pressure, causing a passive inflation of epidermal cells and an active increase in osmotic pressure in guard cells. When the sandy soil dries quickly after the rain, the epidermal cells passively shrink back to their original size as turgor pressure falls, pulling open nearby stomata that cannot reclose until the osmotic pressure is actively lowered (Buckley 2019).

High levels of purple spot often occur shortly after rain. This has been thought to be a result of rains causing the release of ascospores or conidia and providing the moisture required for propagules to germinate (Falloon et al. 1987). The increase could also be related to stomata opening due to wrong-way-reactions and all three factors may be involved.

For lesions to appear on asparagus spears at harvest time, a series of events must have occurred in the three to four-day period between spear emergence and harvest. First, conidia or ascospores must land upon the spears. Next there must be a continuous period of free water for the spores to germinate. The length of the wetness period required for a high rate of germination depends on temperature; a four-hour period of free water will only be enough for a few percent of conidia to germinate at 5°C, around 50% at 10°C, and over 90% at temperatures over 15°C. An eight-hour wetness period at 5°C will be sufficient for about a quarter of conidia to germinate, and an eight-hour wet period at 10°C will result in nearly 90% of conidia to germinate (Bohlen-Janssen et al. 2018a). Due to moisture, and possibly nutrients, exuding from wounds, the presence of wounds may allow germination to occur even when the environment is not providing free-water (Bohlen-Janssen et al. 2018b). As the spores germinate, the hyphae spread and attempt to enter the plant through open stomata and wounds. After successfully penetrating

the spear, lesions, and the accumulation of anthocyanins around the lesion, become visible after approximately 24 hours. If an event that causes wounding or the stomata to open occurs after the spear surface is colonized and more than 24 hours before harvest, a high level of infection will be seen at harvest. If such an event occurs after this critical period, spears may show few lesions at harvest time, but many additional lesions may begin to appear in storage. These postharvest lesions will lack the "purple spot" as the level of sucrose, which is required to induce the genes responsible for the biosynthesis of anthocyanin, falls rapidly within hours of harvest (Solfanelli et al. 2006). Even when spears are stored at 0°C, sucrose levels fall by 50% within 24 hours of harvest (Verlinden et al. 2014) Anthocyanin production is also light-dependent, so if sucrose levels could be artificially maintained during an experiment on detached spears, purple spots should only form under light and not under a dark treatment (Tao et al. 2020; Warnasooriya et al. 2011).

2.8 Management of *S. vesicarium* on asparagus

Since *S. vesicarium* is an airborne plant pathogen that can survive as a saprophyte, keeping it out, or removing it from a farm, is difficult. Resistance to *S. vesicarium* would be the ideal way to reduce or eliminate both Stemphylium leaf spot and purple spot, however no modern cultivar shows high levels of resistance (Foster 2018). Disease management during the fern phase currently relies heavily upon numerous fungicide applications. Growers may spray based on time, scouting, or based on a TOM-cast forecast model (Foster and McDonald 2018).

Incorporating asparagus residue into the soil significantly reduces disease severity in the following year (Johnson 1990). Pseudothecia survive for months post-burial but the life cycle is disturbed because the ascospores cannot become airborne (Johnson 1990). However, tillage is

rarely practiced in asparagus production as it causes an increase in the incidence of root-borne diseases, damages storage and feeder roots, and increases the likelihood and severity of sandblasting which promotes infection of spears by *S. vesicarium* (Agriculture and Agri-Food Canada 2018).

High winds in the spring can cause sandblasting of spears, usually causing minimal damage to the spears, but these micro-wounds can be a point of entry for *S. vesicarium*, greatly increasing disease incidence and severity. High lesion numbers can be found after wet weather as the water can trigger both ascospore release and propagule germination on the plant (Falloon et al. 1987). No-till, cover crops, hedgerows, moist-soil surfaces, and high organic matter (less sandy) soils can help reduce the amount of sandblasting that occurs in the spring (Agriculture and Agri-Food Canada 2018).

2.9 Management on pear

A substantial amount of research has been conducted on *S. vesicarium* in relation to pear trees where it causes the disease brown spot. There are resistant pear cultivars, but due to local demand for certain historic cultivars that are susceptible, controlling the pathogen through other means has been investigated.

Similar to asparagus production, removing crop residue during the fall or winter in pear orchards has been shown to reduce disease incidence and severity in the following year (Llorente et al. 2010). This reduction in disease occurs as crop residue harbours a significant amount of primary inoculum. If initial inoculum is not present or very low, the populations of propagules will be reduced for the entire growing season.

Control of brown spot on pears through fungicide use may require 15-25 sprays per year (Llorente and Montesinos 2006). Foliar application of $CaCl_2$ has been shown to reduce disease incidence on pear fruit (Toselli et al. 2012). This does not seem to be related to changes in fruit calcium concentration (Toselli et al. 2012) but may be due to inducing host resistance or drying the surface of the fruit to reduce conidia germination.

As *S. vesicarium* is known to colonize and sporulate on non-host dead tissue on the orchard floor (Rossi et al. 2005), applying biological control agents to the floor has been attempted, resulting in moderate success (Rossi and Pattori 2009; Llorente et al. 2010). Attempts to apply biological controls onto pear trees have produced poor results (Ponti et al. 1993). The uptake of biological control may also be hampered by incompatibility with a fungicide program.

Comparisons of transcriptomes before and after inoculating pear leaves from resistant and susceptible cultivars show a number of possible mechanisms of resistance. Compared to the susceptible cultivar, the resistant cultivar had higher constitutive production of phytoalexins and enzymes that thicken the extracellular surface, and also a higher production of reactive signaling molecules and fungicidal compounds (Pereira et al. 2015). A quantitative trait locus associated with the susceptibility of pear to *S. vesicarium* infection has been discovered, but the exact gene within the region has not yet been identified (Cappai et al. 2018).

2.10 Screening for resistance

Detached leaf assays to assess disease severity have been shown to correlate with full plant resistance in maize (Aregbesola et al. 2020), apple (Abe et al. 2010) and tomato (Foolad et al. 2015). However, experiments have also shown that different soybean cultivars may retain or lose their resistance to pests in detached leaves (Michel et al. 2010). Inconsistent results for

measurements of resistance in whole strawberry plants and detached leaves have been reported (Miller-Butler et al. 2018). Results from detached leaf assays for blight resistance in chestnut have been reported to show no relationship with disease severity of stem cankers (LaBonte et al. 2017). A study on citrus canker and citrus bacterial spot showed strong correlation between attached and detached leaves for both lesion count and pathogen population 14 days post-inoculation, however, a very high pathogen population survived on one of the resistant cultivars (Francis et al. 2010). The key mechanisms that confer resistance vary among plant-microbe relationships and some of these may be more affected by detachment than in other cases.

Differences in resistance to *S. vesicarium* among *A. officinalis* cultivars have been found in previous screens, albeit the disease is still a serious concern for growers of the most resistant cultivars (Bansal et al. 1986; Bansal et al. 1988; Bansal et al. 1992). Screens have measured resistance on spears and fern stems by determining the necrotic surface area, on the fern by measuring defoliation, and by length of lesions, and lesion count on spears (Bansal et al. 1986; Foster 2018; Johnson and Lunden 1986). Other asparagus species including *A. virgatus* and *A. densiflorus* that were included in these screens showed a very high level of resistance while *A. verticillatus* was susceptible (Bansal et al. 1986; Bansal et al. 1992). Interspecific crosses to breed resistance into *A. officinalis* cultivars face incompatibility barriers (Marcellan and Camadro 1999).

An interaction between cultivars and isolates of *S. vesicarium* has been recorded within *A. officinalis* and across *Asparagus* taxa (Bansal et al. 1992). Researchers have also stated that isolates collected on one host-species may or may not be pathogenic on other host-species (Foster 2018)

A 1988 study by Bansal et al. where attached spears were inoculated while in a greenhouse found differences in resistance among the asparagus cultivars. The authors state that the results agree with a previous field study looking at the spears of many of the same cultivars (Bansal et al. 1988). A subsequent study by the same research group assessed disease in the greenhouse based on the amount of necrotic surface area on inoculated spears and measured premature fern defoliation on cultivars in the field that were infected by naturally present *S. vesicarium* inoculum (Broadhurst 1993). The results for cultivar resistance between the field and greenhouse did not match, and this disagreement was attributed to differences in plant architecture. The author argued that cultivars with a short and compact fern will retain free moisture after a wetting event, providing a conducive environment for successful infection by *S. vesicarium*, and this effect would not be realized through studying spear resistance alone (Broadhurst 1993).

Wounding a section of a spear may induce resistance in the rest of the spear due to defense signals triggered by damage-associated molecular patterns from the wounding or from potentially faster detection of the pathogen at the wound (Chassot et al. 2008). Conversely, wounding could weaken the defenses by draining limited resources or removing cutin based defence signals (Ziv et al. 2018). Another possibility is that the cuticle could block detection by the spear of elicitors from the pathogen and if the cuticle is removed or damaged, the spear may then quickly detect the pathogen and activate a faster defense response (L'haridon et al. 2011). In these experiments, the effect of wounding may be undermined as the act of harvesting triggers a damage response and the cuticle of spears could be damaged during transportation.

The most popular asparagus cultivars all produce spears containing anthocyanins, even in the absence of infection, although when spears are grown in the dark to produce white asparagus, no pigments are produced in the spear (Dong et al. 2019). The titular 'purple spots' do not arise upon infection in pigmentless cultivars, but the lesions are still visible, even without the purple halo. Visual inspection of asparagus plots for purple spot must be conducted by analyzing individual spears. In the field, tiny physical wounds on spears may look similar to small lesions caused by *S. vesicarium* as both may become encircled with anthocyanins (Falloon et al. 1987). Viewing plots at a distance may confound purpling caused by purple spot with purpling related to the way that different cultivars respond to cold weather or sandblasting; both can cause high expression of anthocyanin in some cultivars (Dong et al. 2019; K. Wall, personal communication, Oct. 27, 2020).

Asparagus spears are coated with a fragile hydrophobic self-cleaning wax that will not survive harvest without special care (Voigt and Gorb 2009). This wax may affect germination rate, defense response activation, or number of conidia and thus could affect the number of lesions that form.

Young pear leaves and fruit (Montesinos et al. 1995) and young apple leaves (Abe et al. 2010) were found to be much more susceptible to infection than their older counterparts. As the cells in asparagus spears are very young when they first emerge from the soil and are first exposed to *S. vesicarium* their resistance to infection may be lower compared to older, 23 cm spears and this difference in resistance may be unequal among cultivars.

In Arabidopsis, transcription studies have shown the expression of phenylalanine ammonia-lyase (PAL) to be light dependent (Zeier 2004). PAL not only initiates the production

of various defensive molecules, but also triggers the accumulation of salicylic acid (SA) which itself induces the production of further defense molecules including pathogenesis related protein PR-1. Plants infected in the dark did not mount a systemic defense response. Light has also been shown to affect many aspects of pathogenesis in plant pathogens (Kim et al. 2011).

By withholding light during incubation any notable differences among cultivars for these light-dependent defenses should be apparent. Light can also cause the stomata to open, and open stomata are a key route of infection, however this possible effect of light can be bypassed by viewing the effect of light on infection of wounded surfaces.

Acibenzolar-S-methyl (ASM) is a synthetic analog of salicylic acid which can be used to protect plants from pathogens by triggering systemic acquired resistance (SAR) in treated plants (Sakata et al. 2020). The product is not directly toxic to plant pathogens. SAR involves the direct activation of pathogenesis related (PR) genes and is normally triggered as a reaction to infection by pathogens (Gozzo & Faoro 2013). Applying ASM to spears before inoculation and comparing to untreated spears could demonstrate a difference in the strength of SAR between cultivars as expressed by susceptibility to the disease. Treatment with ASM in combination with periodic light or continuous dark treatments would indicate if PR genes triggered through SAR are light-dependent or not (Zeier 2004).

2.11 Conclusion and objective

There are many gaps in the literature about resistance in asparagus to *S. vesicarium* particularly which factors affect resistance in the field and lab and what mechanisms of resistance are present in non-susceptible asparagus species. Previous surveys of resistance to *S. vesicarium* in field asparagus have shown differences between both the spears and ferns of

cultivars at one location that were not seen at another Ontario location (Foster et al. 2019). Reasons need to be found for the interaction among cultivar, location, and year, not only as resistance assays require comparable field results, but also to best understand the environmental factors that support disease development. Inconsistent results from field surveys are due to unknown, uncontrolled factors that affect the plant-microbe relationship.

In a controlled environment, factors including cultivar, lighting, location on the spear, spear size, presence of wounds or epicuticular wax, isolate, and treatment with acibenzolar-S-methyl (ASM), may affect the number of lesions that form on detached asparagus spears and these factors may interact with each other. The susceptibility of each cultivar may also change by unequal degrees when the spears are detached from the plant and this can only be determined if both field and assay results are consistent.

The primary objective of this work was to establish a reliable bioassay for determining the resistance of asparagus cultivars to *S. vesicarium* that was consistent with ratings in the field. The second objective was to determine the mechanisms that affect the number of lesions that form, in both the field and lab. If the primary objective fails, then a method to gather consistent results from attached spears in the field is required as a method is needed to determine which asparagus cultivars are the most resistant. This work will be useful to quickly and reliably screen asparagus breeding lines for resistance as part of the overall breeding program to incorporate resistance to *S. vesicarium* into Ontario asparagus.

3. Methods

3.1. Disease assessment in the field

Surveys for purple spot were conducted at an asparagus plot naturally infested with *S. vesicarium*, at the University of Guelph Simcoe Research Station (42.854261, -80.268423). The plot was planted in 2007 and cultivars were arranged in a randomized complete block design with four replicates for each of Guelph Millennium, Guelph Eclipse, Jersey Giant, and Gijnlim. Guelph Millennium and Guelph Eclipse were developed by the University of Guelph asparagus breeding program and Gijnlim and Jersey Giant were from Limgroup B.V. and Rutgers University breeding programs, respectively. The fourth block did not contain Guelph Eclipse and there was a noticeable amount of die-off of Jersey Giant in blocks one and two. The asparagus plot was on a silt loam soil (23% sand, 73% silt, 4% clay, pH 7.4 and 1.7% OM). Block one was the northernmost and the highest as the field sloped slightly down towards block four. The wind was predominantly from the west, and as the plots in that direction were also mostly asparagus, and the treeline was 200 m away, the wind could be fairly strong.

Field surveys for purple spot were conducted six times in both 2019, between 20 May and 10 June, and in 2020, between 21 May and 14 June (Table 2 & 3 for dates). The numbers of purple spot lesions were counted on spears that would be graded as number one (defect and damage-free spears with a tight head and diameter over 8mm) if lesions were ignored, an hour prior to their 7:30 am harvest. Inspected spears ranged from 21-27 cm pre-harvest and had a diameter between 12-17 mm. Usually 10 spears of each cultivar from each of the four blocks were surveyed on a sampling date, but that number was lower on some days for some cultivars.

Temperature, rain, humidity, and wind speed data gathered by the Delhi CS weather station were analyzed to determine any relationships with results obtained from the field (Government of Canada 2022).

3.1.1. Field inoculation

In 2020, spears ranging in length from 6-9 cm were inoculated in the field on five days, 21 May, 24 May, 26 May, 1 June, and 4 June, just before sunset. A suspension of conidia was prepared as for the bioassay inoculations, as described below. An aluminum shield with a 1x3 cm opening was attached to the spray bottle to hang 10 cm in front of the nozzle. The shield was placed flat on the side of the spear and the spear was sprayed twice consecutively at the same location. The shield controlled the amount of spray landing on each spear, but the spray did roll down the spear. The individual spears were marked by placing a coloured rock at the base, then covered with a 20 cm can that was wetted on the inside by a water spray. At 8:00 am the following morning the can was removed, and 64 hours post inoculation the spears were harvested and the lesions on the whole spear were counted. Nontreated spears, without mock treatment, within the height range of those that were inoculated were also harvested and were assessed as a control.

The number of lesions that resulted from inoculation in the field was calculated by subtracting the mean number of lesions forming on each cultivar due to natural infection on non-inoculated spears from the mean number of lesions on the inoculated spears of each cultivar. The lesions resulting from natural inoculation were counted on spears that emerged at the same time as those that were inoculated.

3.2. Controlled environment bioassays

3.2.1. Preparation of inoculum

Fresh conidia suspensions were prepared for each inoculation from one-week old colonies of *S. vesicarium* grown on a V8 agar medium (800ml H_2O, 200mL V8 [Campbell Soup Co., Etobicoke, ON], 15g agar [Fisher Scientific, Mississauga, ON], 3g $CaCO_3$ [Sigma-Aldrich, Oakville, ON]) in Petri dishes (90 mm dia ×15 mm, Fisher Scientific, Fair Lawn, NJ) sealed with Parafilm (Bemis Company, Inc., WI) under blacklight (15W) set to provide a 12 hour photoperiod at approximately 22°C. The conidia suspensions were prepared by adding 10ml of sterile water and one drop of Tween-80 [J.T Baker Inc., Philipsburg, NJ, USA] to each Petri dish. The surface of the agar was gently scraped with a scalpel to free the conidia and the resulting suspension was poured through four layers of cheesecloth miniwipes [Fisherbrand] to isolate the spores. The suspension was diluted to 1×10^5 spores/mL after using a Neubauer hemocytometer to estimate conidia concentration. Three isolates, OA03, NA61, and OA101, were used during the study as described below.

3.2.2. Plant material

Asparagus spears for the controlled environment assay were harvested during the asparagus season (mid-May to mid-June in Ontario). Number one grade spears (minimum 140 mm in length and 8 mm width) were harvested, trimmed to 23 cm, placed upright in a cooler containing 2 cm of water, and brought to the lab for immediate surface disinfection and inoculation. Spears were disinfected by submerging in a 0.5% sodium hypochlorite bath for 30 seconds then washing

in a water bath and air-dried for an hour. The same four cultivars as in the field trial, Guelph Millennium, Guelph Eclipse, Jersey Giant, and Gijnlim, were tested in all experiments.

3.2.3. Inoculation and incubation

The spears were trimmed to remove 0.5cm from the base, laid flat, and sprayed with the conidia suspension until runoff, approximately 1mL per spear. The spears were then placed vertically in test tube racks in two clear plastic boxes with 2 cm of water in the bottom and walls sprayed with water to ensure a sufficient spear wetness period (at least four hours for 90% germination) that is vital for the infection process. Spears were arranged by cultivar diagonally in 4 x 10 test tube racks to ensure they were evenly spread across the boxes. The closed boxes were moved into one of the various available growth chambers (not recorded) that were set to 100% humidity and 20°C. When the experiments required growth chambers to provide a light treatment, spears were divided among two to four boxes to be split among growth chambers. The temperature in the growth chambers was steady but the humidity fluctuated between 70 to 100%. A very high humidity was obtained within the closed box within the growth chamber. Besides ASM and light treatments, which could only be applied to all the spears within a box, spears with different treatments were evenly divided among boxes. The details of each set of experiments are described below. The number of spears available varied by date and the number available for each treatment combination naturally decreased in experiments with a high number of treatments. Spears per treatment combination per box ranged from three to ten. On most days, experiments included over 150 spears. Non-inoculated spears were included in the bioassay as a control for naturally present *S. vesicarium*; one bioassay replication conducted on 30 May 2019 (not shown) was discarded due to a high number of lesions on the controls.

3.2.4. Assessment of lesion numbers

The area in which lesions were counted varied as factors such as wounding and location were included in experiments, as described below. Lesions large enough to be seen with the naked eye were counted 72 hours post-inoculation. Clusters of lesions were never too dense to count the separate lesions.

3.3 Experiments and variables

There were six experiments conducted under controlled environment conditions over two years. Each experiment was repeated. The experiments and variables are summarized in Table 1. The variables were cultivar, isolate, wounding, lighting during incubation, location on the spear, and application of acibenzolar-S-methyl. Experiment four used only small spears and experiment five used only spears with an intact epicuticular wax.

Table 1: Summary of controlled environment experiments

Exp #	Treatments	Assessment of lesions by location and wound status:
1	**Isolate:** OAO3 or NA61; **Lighting:** alternate light or continuous dark; **Dates:** 20 and 23 May 2019; **Cultivar**[1]	**Wound:** 5-7 cm from the base; **Unwounded:** rest of spear
2	**Isolate:** OAO3 or NA61 or OA101[2]; **Lighting:** alternate light or continuous dark; **Cultivar; Dates:** 3, 10, and 13 June 2019	**Wounded top:** 5-7 cm from top; **Wounded bottom:** 5-7 cm from the base; **Unwounded:** rest of spear
3	**Spears were wounded at top or bottom or were unwounded**; **Isolate:** Mixture of NA61 and OA101; **Lighting:** continuous dark; **Cultivar; Dates:** 24 and 26 May 2020	**Wounded or unwounded top:** 5-7 cm from top; **Wounded or unwounded bottom:** 5-7 cm from the base; **Unwounded middle:** band 7.5-17.5 cm from the top
4	All **small spears; Isolate:** Mixture of NA61 and OA101; **Lighting:** continuous dark; **Cultivar; Dates:** 29 May and 1 June 2020	**Wound:** 3.5-5.5 from the top; **Unwounded:** rest of the spear.
5	All spears with **epicuticular wax** intact; **Isolate:** Mixture of NA61 and OA101; **Lighting:** alternate light or continuous dark[3]; **Cultivar; Dates:** 7 and 9 June 2020	**Unwounded top:** band 2.5-5 cm from the tip; **Unwounded middle:** band 6-18.5 cm from the tip.
6	**Acibenzolar-S-methyl:** 0 or 20mg L^{-1}; **Isolate:** Mixture of NA61 and OA101; **Lighting:** alternate light or continuous dark; **Cultivar; Dates:** 14 and 18 June 2020	**Unwounded top:** band 2.5-5 cm from the tip; **Unwounded middle:** band 6-18.5 cm from the tip.

[1]Cultivars: Guelph Millennium, Guelph Eclipse, Jersey Giant and Gijnlim.

[2]Only replicate two of experiment two (10 June 2019) included OA101.

[3]Only continuous darkness was applied in replicate two of experiment 5 (9 June 2020)

3.3.1 Isolate

A number of *S. vesicarium* isolates used in these trials were collected in previous years and subcultured on detached spears and V8 agar in the lab during storage. A preliminary investigation was conducted to determine which isolates could produce an adequate number of conidia and have an adequate level of virulence. Except for isolate KK01, the isolates were named based on the province where they were collected (O for Ontario, N for Nova Scotia) and host (O for onion, L for leek and A for asparagus). The tested isolates were KK01 from onion in Keswick, Ontario in 2018, OO69 from onion in the Holland Marsh, Ontario, in 2016, OL05 from leeks in the Holland Marsh, Ontario in 2018, OA46 from asparagus near Gilbertville, Ontario in 2013, OA03 from asparagus in Hemlock, Ontario in 2012, and NA61 from asparagus in Canning, Nova Scotia in 2014 (Foster 2018; Stricker 2021) Isolates OA03 and NA61 were selected. These two produced a similar number of lesions on wounded surfaces, but OA03 caused very few infections on unwounded asparagus spears. New isolates were collected in 2019 from the asparagus plot at the Simcoe Research Station and one was used for this work. A new isolate, OA101, was used in one 2019 bioassay and it was mixed with NA61 in all 2020 bioassays.

3.3.2 Wounding

The effect of wounding was determined in nine of the thirteen experimental runs (Table 1). The spears were wounded minutes before they were sprayed with the conidia suspension. A thin flexible cover with an opening 1×2 cm was placed lengthwise over the spear and the exposed area was lightly stroked seven times using cheesecloth miniwipes [Fisherbrand] to create wounds uniform in size and severity. Puncturing the spears with a pipette tip was trialed,

but the yes/no infection results are inherently less precise than lesion counts on wounds created by stroking the surface unless a very large number of punctures were created.

Despite great effort to create uniform wounds, on several dates there was a failure to produce visible wounds seemingly caused by an abnormally resistant spear surface. Most of these failures to wound, 17 out of 21 occurrences, happened on Jersey Giant spears. No data were collected from failure-to-wound sites.

In experiment three, spears were either wounded once near the top, once near the bottom, or not wounded to see if these treatments may affect lesion development on unwounded sections of a spear.

3.3.3 Lighting

The growth chambers where the spears were incubated were either in continuous dark or had a photoperiod of 12 hours light (Standard F54T5/80/HO/PS, 325 μmol/m2/second).

3.3.4 Location

Location along the spear, near the tip or in the middle, was taken into account in a number of experiments, both for lesion counts on unwounded surfaces and at wounds.

3.3.5 Spear size

Cultivar resistance was evaluated on spears measuring 23cm in length for all experiments except experiment four where spears measured 8-10cms in length.

3.3.6 Epicuticular wax

The resistance of cultivars was evaluated on spears with an intact epicuticular wax in experiment five, unlike in all other experiments where the fragile wax surface was mostly

incidentally removed during harvest and transportation to the lab. Spears for experiment five were carefully collected and transported from the field so as not to disturb the integrity of the fragile epicuticular wax that covers the asparagus spear. These spears were inoculated with the conidial spray whilst upright in test-tube racks rather than lying flat.

3.3.7 Acibenzolar-S-methyl

Acibenzolar-S-methyl (ASM), known to directly stimulate host resistance, was assessed in experiment six to determine if it would interact with the light treatment. A solution of ASM (Sigma Aldrich) was suspended in distilled water at a concentration of 20mg L^{-1} and sprayed onto half of the spears until runoff (~1ml per spear) 3-5 minutes before inoculation; spears not receiving the ASM treatment were sprayed with distilled water until runoff. After inoculation, spears treated with ASM were randomly placed into two boxes, one for each light treatment, and spears not treated with ASM were also randomly divided between two boxes so that there were four boxes in total. Spears were incubated under each lighting regime, 12-hour photoperiod or continuous darkness.

3.3.8 Spear weight, stomatal density, and germination rate

All spears harvested in 2019 were weighed in bunches by cultivar and block after spears were trimmed to 23 cm in the field. Most bunches contained ten spears, but some had fewer due to lack of number one spears available for harvest.

The density of stomata on each cultivar and location on the spear was assessed. Clear nail polish was applied to 20 spears from each of the four cultivars at two locations: 2-4 cm from the top and 11-13 cm from the top. The nail polish was peeled off and mounted on slides. The stomata were counted in a single field of view at 40x magnification.

The germination rate of conidia on spears was assessed. Spears were randomly selected and removed from experiment two, 24 hours post-inoculation. Spears were taken from all cultivars for both isolates OA03 and NA61. Clear nail polish was applied either at a wound or on an unwounded area in the middle of the spear. The nail polish was peeled off, mounted on slides, and lactophenol cotton blue was applied. Conidia were counted as germinated if the germ tube was longer than the conidium. For germinated conidia, the length of the longest germ tube was measured.

3.4 Statistical analysis

Statistical analysis was conducted with SAS v.9.4. PROC GLIMMIX was used for mixed model analysis of variance. Tukey-Kramer's test at $P \leq 0.05$ was utilized for means separation when the ANOVA was significant. All field data had date*block as a random effect and cultivar as the fixed effect. There was a significant interaction between date and cultivar for the field survey data. All bioassay data were analyzed with date as a random effect and cultivar as a fixed effect, with all other factors added as fixed effects when included. All controlled environment experiments were repeated on a second date except for testing isolate OA101 as the repetition was discarded because of lesions on controls, and light was tested on only one date in experiment five. Experiments were conducted as RCBD with blocks as repetitions over time. When included, light and ASM treatments were applied as whole plot treatments and all other factors as split-plot treatments. For comparison reasons, lesion counts for bioassays are presented as full-spear equivalents, where the mean number of lesions and standard error are multiplied by a factor to make the data lesions/$50cm^2$.

PROC UNIVARIATE showed that all lesion count data followed a lognormal distribution. To include observations with no lesions in the lognormal analysis, all observations were increased by one, and one was then subtracted from all calculated means. The only lesion count data set without any zeroes, and thus not requiring a plus one transformation for lognormal analysis, were the data collected from field inoculations.

4. Results

The significance of main effects and interactions found in the six controlled environment assays were compiled in Table 2.

Table 2: Effects and interactions, with associated P values, tested for in controlled environment assays.

Effect	Exp 1 (Appendix Table 6)	Exp 2 (Appendix Table 8)	Exp 3 (Appendix Table 10)	Exp 4[1] (Appendix Table 12)	Exp 5[2,3] (Appendix Table 13)	Exp 6 (Appendix Table 14)
cultivar	**0.0008**	<.0001	<.0001	**<.0001**	**<.0001**	<.0001
wound	<.0001	<.0001	**<.0001**	**0.0182**	-	-
cultivar*wound	0.5043	0.0021	0.0975	0.1664	-	-
isolate	<.0001	<.0001	-	-	-	-
cultivar*isolate	0.4112	0.3990	-	-	-	-
wound*isolate	0.0315	0.9006	-	-	-	-
cultivar*wound*isolate	0.1689	**0.0404**	-	-	-	-
light	0.0015	0.0009	-	-	.0001	0.0719
light*cultivar	0.5998	0.1908	-	-	**0.0006**	0.0235
light*wound	<.0001	0.0318	-	-	-	-

light*cultivar*wound	0.7201	0.1321	-	-	-	-
light*isolate	0.0080	0.1749	-	-	-	-
light*cultivar*isolate	0.9454	0.8756	-	-	-	-
light*wound*isolate	**<.0001**	**0.0005**	-	-	-	-
light*cultivar*wound*isolate	0.8897	0.9077	-	-	-	-
location[4]	-	-	-	-	<.0001	0.0019
location(wound)[4]	-	**<.0001**	<.0001	-	-	-
cultivar*location	-	-	-	-	**0.0010**	0.0046
cultivar*location(wound)	-	0.0976	**0.0007**	-	-	-
location*isolate(wound)	-	0.9742	-	-	-	-
cultivar*location*isolate(wound)	-	0.5523	-	-	-	-
light*location	-	-	-	-	0.3634	0.1348
light*location(wound)	-	0.2383	-	-	-	-
light*cultivar*location	-	-	-	-	0.2039	**0.0137**
light*cultivar*location(wound)	-	0.4068	-	-	-	-
light*location*isolate(wound)	-	0.4488	-	-	-	-
light*cultivar*location*isolate(wound)	-	0.9373	-	-	-	-

ASM	-	-	-	-	-	0.1761
cultivar*ASM	-	-	-	-	-	0.1799
ASM*location	-	-	-	-	-	0.6697
cultivar*ASM*location	-	-	-	-	-	0.653
light*ASM	-	-	-	-	-	0.136
light*cultivar*ASM	-	-	-	-	-	0.1228
light*ASM*location	-	-	-	-	-	**0.0493**
light*cultivar*ASM*location	-	-	-	-	-	0.3324

Significant factors and interactions are in bold, significant factors and interactions that are subsumed by larger interactions are underlined.
[1]All spears in experiment four were small, 8-10 cm in length, rather than spears 23 cm in length used in all other experiments.
[2]Only in experiment five did spears tested have the epicuticular wax intact.
[3]Light treatment was included in only one of the two blocks for experiment five.
[4]Location, top or middle of the spear, was recorded for only wounded surfaces in experiments two and three and unwounded surfaces in experiments five and six. A nested factorial design was used for location nested within wounding for experiments two and three due to the oversight of location not being studied on the otherwise studied unwounded areas in these experiments.

4.1 Field assessment of spears

Differences were found among the four cultivars in all six assessments of purple spot on asparagus spears in 2019 and in four of the six assessments in 2020 (Tables 3, 4). Pooling across all dates was not possible due to an interaction with cultivar (Appendix Table 1). There were three groups of dates that could be pooled wherein date did not interact with cultivar, one with seven dates (20, 23, 27 and 30 May 2019, and 26 May 2020, 14 June 2020 and 26 May 2020 field inoculation) one with three dates (3, 10 June 2019, and 24 May 2020), and another with four dates (21 May 2020, 7 and 9 June 2020, and field inoculation of 21 May 2020). The former two pooled groups exhibited a pattern where Jersey Giant had more lesions than the other three

cultivars, although the size of the difference between Jersey Giant and the others varied between the two pooled groups (Table 5)(Appendix Table 2, 3). The low level of infection of 9 June may explain why the prevailing pattern of infection was not seen. On 21 May, 7 June, and the field inoculation of 21 May, the deviation from the common pattern was most likely related to weather events as discussed below.

Table 3: Mean number of lesions per spear by date in 2019 field assessments.

Cultivar	20 May (30-40 spears)	23 May (26-37 spears)	27 May (17-38 spears)	30 May (27-37 spears)	June 3 (30-40 spears)	10 June (26-40 spears)
Jersey Giant	3.8 a[1] (0.79)[2]	3.2 a (0.80)	1.2 a (0.33)	1.9 a (0.33)	1.9 a (0.49)	0.5 a (0.13)
Guelph Millennium	1.1 b (0.33)	0.8 b (0.32)	0.3 b (0.16)	0.3 c (0.15)	1.3 ab (0.36)	0.1 b (0.10)
Guelph Eclipse	1.0 b (0.35)	0.7 b (0.34)	0.2 b (0.16)	1.2 ab (0.29)	1.1 ab (0.36)	0.1 ab (0.12)
Gijnlim	0.6 b (0.24)	1.4 b (0.45)	0.2 b (0.14)	0.6 bc (0.20)	0.7 b (0.26)	0.1 b (0.10)

[1]Means followed by the same letter indicate no significant difference within columns using Tukey-Kramer's test at $p \leq 0.05$.
[2]Standard error in parentheses.

Table 4: Mean number of lesions per spear by date in 2020 field assessments.

Cultivar	21 May (6-25 spears)	24 May (44-85 spears)	26 May (30-40 spears)	7 June (12-16 spears)	9 June (20-25 spears)	14 June (17-22 spears)
Jersey Giant	6.4 ns[1] (1.70)[2]	1.4 a[3] (0.31)	3.8 a (0.62)	6.3 ab (1.44)	0.5 ns (0.19)	3.0 a (0.60)
Guelph Millennium	7.1 (0.97)	0.9 ab (0.23)	1.1 b (0.28)	12.7 a (2.71)	0.8 (0.21)	0.6 b (0.23)
Guelph Eclipse	10.1 (1.72)	0.5 b (0.19)	1.5 b (0.38)	5.5 ab (1.38)	0.9 (0.23)	0.9 b (0.32)
Gijnlim	9.4 (1.40)	0.8 b (0.21)	1.0 b (0.25)	4.9 b (1.33)	0.4 (0.17)	0.8 b (0.26)

[1] Ns - not significant at $P \leq 0.05$
[2] Standard error in parentheses.
[3] Means followed by the same letter represents no significant difference within columns using Tukey-Kramer's test at $P \leq 0.05$.

Table 5: Mean number of lesions on asparagus spears in field assessments pooled across dates as appropriate.

Cultivar	Seven days[1] (206-271 spears) Calm weather (Appendix Table 2)	Three days[2] (103-162 spears) Calm weather (Appendix Table 3)	7 June 2020 (9-10 spears) Field Inoculation	Four days[3] (46-78 spears) High winds or rain (Appendix Table 4)
Jersey Giant	3.3 a[4] (0.27)[5]	1.2 a (0.16)	29.5 a (10.69)	2.2 b (0.48)
Guelph Millennium	1.0 b (0.12)	0.7 b (0.12)	11.5 ab (4.40)	4.7 a (0.63)
Guelph Eclipse	1.1 b (0.14)	0.5 b (0.12)	14.6 ab (5.56)	3.8 ab (0.62)
Gijnlim	1.0 b (0.12)	0.5 b (0.10)	3.8 b (1.38)	2.9 ab (0.47)

[1] Includes 20, 23, 27 and 30 May 2019, 26 May 2020, 14 June 2020, and field inoculations on 26 May.
[2] Includes 3, 10 June 2019, and 24 May 2020.
[3] Includes 21 May 2020, 7 and 9 June 2020, and field inoculation of 21 May 2020.
[4] Means followed by the same letter represents no significant difference within columns using Tukey-Kramer's test at $P \leq 0.05$.
[5] Standard error in parentheses.

Environmental factors during the infection period (Tables 6, 7) may explain some of the differences seen among dates (Table 3, 4). In 2020, field surveys conducted on 21 May and 7

June produced results very different than those on other days. Heavy rain ended 60 hours before the assessment on 21 May. There was high wind and the soil surface was dry during the critical period before the assessments on 7 and 9 June, suggesting that there was some sandblasting of the spears. A dry environment not conducive to propagule germination nor inoculum release from pseudothecia explains the low number of lesions on 9 June.

There were two other surveys, 23 May 2019 and 14 June 2020, where spears were exposed to high wind, yet the infection pattern and the disease severity did not appear to be affected. Spears surveyed on 23 May 2019 were subject to high winds 62 hours before inspection. Significant infection could have occurred post-high winds, particularly considering the two nights following the high winds were very humid, allowing for dew and thus propagule germination and hyphal growth while stomatal defences were not undermined. Spears surveyed on 14 June 2020 were exposed to winds, but the soil was very recently wetted, likely keeping the amount of sand picked up by the wind to a minimum.

Table 6: Weather during critical period (20-72 hours pre-inspection), 2019

	20 May	23 May	27 May	30 May	3 Jun	10 Jun
Rain (mm)	0	0	12.0	10.4	8.9	0
Highest wind (kmh)	17	26	20	11	13	13
Wet soil surface	No	No	Yes	Yes	Yes	No
Sandblasting	No	Likely	Maybe	No	No	No
Wrong-way-reaction	No	No	Maybe	Maybe	Maybe	No

Table 7: Weather during critical period (20-72hours pre-inspection), 2020

	21 May	24 May	26 May	7 June	9 June	14 June
Rain (mm)	19.9	3.9	0	0	0	0
Highest wind (kmh)	15	15	19	26	26	26
Wet soil surface	Yes	Yes	No	No	No	Yes
Sandblasting	No	No	Maybe	Likely	Likely	Maybe
Wrong-way-reaction	Likely	No	No	No	No	No

4.2 Field inoculation

Three of the five field inoculations produced usable results. The other two did not produce lesions, or lesion numbers were very low on both inoculated and non-inoculated spears. There were no differences among the cultivars for the numbers of lesions in the 21 May assessment (Table 8). There was an interaction between inoculation date and cultivar resistance (Appendix Table 5) that is likely due to weather during the critical infection period. Guelph Eclipse had fewer lesions than Jersey Giant on 26 May, and on 7 June, Gijnlim had fewer lesions than Jersey Giant.

Table 8: Mean number of lesions per spear in 2020 field inoculation trials. Lesion numbers from the non-inoculated controls were subtracted from the means of the spears

Cultivar	21 May	26 May	7 Jun
Jersey Giant	1.4 ns[1] (1.00)[2]	10.6 a[3] (2.31)	29.5 a (10.69)
Guelph Millennium	5.2 (2.94)	5.6 ab (1.27)	11.5 ab (4.40)
Guelph Eclipse	4.0 (2.67)	3.9 b (0.97)	14.6 ab (5.56)
Gijnlim	2.9 (1.59)	4.9 ab (1.08)	3.8 b (1.38)

[1] Ns - not significant at $P \leq 0.05$.
[2] Means number of lesions per spear based on 8 – 12 spears and standard error in parentheses.
[3] Means followed by the same letter represents no significant difference within columns using Tukey-Kramer's test at $P \leq 0.05$.

The field inoculations for 26 May and 7 June (Table 8) had a pattern of resistance across cultivars, where Jersey Giant had more lesions than other cultivars, not significantly different to surveys of natural infection conducted across six days following calm weather (Table 5)(Appendix Table 3). No significant differences were found among cultivars for the field inoculation on 21 May, similar to the results found in the field surveys from natural infection following wet or windy events on 21 May and 9 June (Table 5).

4.3 Controlled environment bioassay

4.3.1 Cultivar

Table 9: Effect of cultivar on number of lesions forming on whole, full-sized spear equivalents[1].

	Exp. 1[2,3]	**Exp. 2[4]**				**Exp. 3[5]**		**Exp. 4[6]**	**Exp. 5[7]**		**Exp. 6[8]**			
Cultivar		Wounded		Unwounded							Continuous dark		12-hour photoperiod	
		OA03	NA61	OA03	NA61	Top	Middle		Top	Middle	Top	Middle	Top	Middle
Guelph Millennium	12.2 a[9] (1.87)[10]	97.1 a (24.69)	159.1 a (40.31)	0.60 f (0.49)	5.8 de (2.11)	51.6 a (17.47)	29.5 abc (10.06)	13.5 a (3.35)	43.4 a (5.50)	12.1 bc (1.62)	15.5 ab (9.79)	17.7 ab (11.11)	9.5 abc (6.24)	5.7 cde (4.00)
Guelph Eclipse	11.3 a (1.87)	80.1 ab (22.27)	184.7a (50.96)	1.0 ef (0.69)	5.6 de (2.29)	41.0 ab (13.96)	22.5 abcd (7.79)	14.9 a (3.99)	44.3 a (6.53)	15.8 b (2.41)	19.5 ab (12.51)	21.0 a (13.38)	3.4 def (2.68)	9.2 abcd (6.20)
Jersey Giant	5.6 b (0.96)	34.0 bc (9.61)	129.7 a (35.87)	0.37 f (0.47)	1.7 ef (0.92)	37.3 abc (12.84)	4.8 e (2.01)	7.3 b (2.07)	39.4 a (5.63)	6.6 cd (1.06)	5.8 cde (4.12)	10.3 abc (6.88)	2.0 ef (1.78)	1.8 f (1.67)
Gijnlim	8.4 ab (1.33)	13.2 cd (3.77)	73.1 ab (19.16)	0.5 f (0.50)	3.5 ef (1.43)	13.1 cde (4.72)	13.5 bcde (4.83)	3.7 c (1.10)	10.1 bc (1.45)	4.1 d (0.67)	2.9 ef (2.33)	7.9 bcd (5.31)	2.1 ef (1.85)	2.8 ef (2.28)

[1] The area size where lesions were measured varied for some treatments and experiments, all areas were standardized to lesions per 50cm^2 (area of a full-sized spear) to allow for consistent comparisons.

[2] Factors include Cultivars: Guelph Millennium, Guelph Eclipse, Jersey Giant, and Gijnlim. Isolates: OA03, NA61. Wounding: rubbing the spear surface (1x2cm) with cheesecloth to damage surface vs no wounding. Location: near spear tip vs spear middle. Lighting: 12-hour photoperiod vs. continuous darkness. ASM: treated vs not treated with acibenzolar-S-methyl. Lesions counted 72 hours post-inoculation. Spears measured 10cm in experiment four and 23cm in all other experiments. Spears in experiment five had an intact epicuticular wax unlike in all other experiments.

[3] Experiment one tested factors: Cultivar, isolate, wounding, light. Interaction with cultivar: none.
[4] Experiment two tested factors: Cultivar, isolate, wounding, location, light. Interaction with cultivar: wounding x isolate.
[5] Experiment three tested factors: Cultivar, wounding, location. Interaction with cultivar: location
[6] Experiment four tested factors: Cultivar, wounding. Interaction with cultivar: none.
[7] Experiment five tested factors: Cultivar, location, light. Interaction with cultivar: location, light (not shown here see Table 11)
[8] Experiment six tested factors: Cultivar, location, light, ASM. Interaction with cultivar: light x location.
[9] Means followed by the same letter within an experiment represents no significant difference Tukey-Kramer's test at $P \leq 0.05$.
[10] Standard error in parentheses.

Exp 1: The factors were cultivar, light, wounding, and isolates (Table 2). There were significant differences among the cultivars and no interaction between cultivar and light, wounding, or isolate, so the main effects of cultivar were examined. Guelph Millennium and Guelph Eclipse had similarly high numbers of lesions, Jersey Giant had the fewest, and Gijnlim did not differ from the other cultivars (Table 9).

Exp 2: The factors were cultivar, light, wounding, location, and isolates (Table 2). A three-way interaction was observed among cultivar, wound, and isolate (Table 2). OA03 caused few lesions on unwounded surfaces and cultivars did not differ (Table 9). On wounded surfaces OA03 produced different lesion numbers among the cultivars, however no differences were observed with the NA61 isolate (Table 9). Guelph Millennium and Guelph Eclipse had more lesions than Jersey Giant and Gijnlim (Table 9). Light and location did not interact with cultivar (Table 2).

Exp 3: The factors were cultivar, wounding, and location (Table 2). A cultivar x wound location interaction was observed for lesion number (Table 2). Jersey Giant had fewer lesions at the wounds in the middle of the spear compared to wounds at the top of the spear while this difference was not seen for other cultivars (Table 9). At the top, Guelph Millennium, Guelph Eclipse, and Jersey Giant had a similar numbers of lesions, while Gijnlim had fewer lesions than Guelph Millennium and Guelph Eclipse (Table 9). At the middle, Guelph Millennium, Guelph Eclipse, and Gijnlim had a similar number of lesions, while Jersey Giant had fewer lesions than Guelph Millennium and Guelph Eclipse (Table 9).

Exp 4: The factors were cultivar and wounding, with all spears averaging 10cm in height unlike all other experiments where spears measured 23cm (Table 2). Cultivar was a significant factor

(Table 2). Guelph Millennium and Guelph Eclipse did not differ for lesion number but had more lesions than the other cultivars (Table 9). Jersey Giant had more lesions than Gijnlim (Table 9).

Exp 5: The factors were cultivar, light, and location, with all spears having a fully intact epicuticular wax unlike all other experiments (Table 2). Cultivar only interacted with inoculation location on the spear (Table 2). All cultivars have fewer lesions at the middle of the spear compared to the top, however, this effect was greater for Jersey Giant than other cultivars (Table 9). Guelph Millennium and Guelph Eclipse did not differ for lesion number at both inoculation sites (Table 9). Gijnlim had fewer lesions than all other cultivars at the top and was equal to Jersey Giant at the middle location (Table 9). Jersey Giant had a similar number of lesions to Guelph Millennium and Guelph Eclipse at the top, but significantly fewer at the middle (Table 9). There was no light and cultivar interaction (Table 2).

Exp 6: The factors were cultivar, light, location, and ASM treatment (Table 2). A cultivar x light x inoculation location interaction was observed (Table 2). The same pattern of lesion number by cultivar was seen as in previous experiments (Table 9) although light had no effect on lesion count at the middle section of Guelph Eclipse unlike all other cultivars. Light did have an effect on lesion number for the top section of Guelph Eclipse, also different from all other cultivars (Table 9). This three-way interaction is not seen in other experiments, and neither is an interaction between cultivar and light nor light and location (Table 2).

Overall: Guelph Millennium and Guelph Eclipse had similar lesion numbers in most experiments. Gijnlim regularly had fewer lesions than Guelph Millennium and Guelph Eclipse. Jersey Giant's response to inoculation was heavily influenced by inoculation location on the spear; when comparing resistance among cultivars at the middle of the spear, or whole spears

(mainly composed of “middle” surface area), Jersey Giant performed similarly to Gijnlim, but when comparing cultivars based on lesion number near the spear tip, Jersey Giant had more lesions than Gijnlim and was comparable to Guelph Millennium and Guelph Eclipse. There was no cultivar and location interaction in experiment two (P=0.0976)(Appendix Table 8) but Jersey Giant had 420% more lesions at the top location than at the middle location, while the average increase in lesions at the top over the middle for the other three cultivars was 120% (Table 12).

4.3.1.1 Cultivar, spear size, and epicuticular wax effects

Relative susceptibility to *S. vesicarium* was mostly consistent for cultivar across all six experiments (Table 9), including experiment four which contained only small spears and experiment five where all spears had an intact epicuticular wax. While the effect of size and wax on lesion development cannot be determined with these experiments, the effect of these factors did not appear to interact with cultivar.

4.3.2 Wounding and isolate

Table 10: Effect of wounding and isolate of *S. Vesicarium* on number of lesions on full-sized spear equivalents1.

	Exp. 1[2,3]				**Exp. 2[4]**				**Exp. 2**		**Exp. 3[5]**	**Exp. 4[6]**
	Continuous dark		12-hour photoperiod		Continuous dark		12-hour photoperiod		Continuous dark	12-hour photoperiod		
	NA61	OA03	NA61	OA03	NA61	OA03	NA61	OA03	OA101		NA61& OA101	NA61 & OA101
Wounded	165.9 a[7] (35.80)[8]	80.7 a (17.57)	84.1 a (15.39)	4.2 b (0.92)	192.5 a (44.28)	110.9 ab (25.59)	86.70 b (20.49)	16.8 c (4.19)	492.9 a (97.36)	287.0 a (65.19)	21.8 a (5.76)	10.6 a (2.53)
Unwounded	4.3 b (1.11)	0.1 c (0.23)	1.6 b (0.48)	0.1 c (0.20)	7.9 c (2.38)	1.1 d (0.55)	1.8 d (0.78)	0.3 d (0.36)	73.6 b (20.80)	3.7 c (1.50)	6.5 b (1.86)	7.2 b (1.80)

[1] The area size where lesions were measured varied for some treatments and experiments, all areas were standardized to lesions per 50cm^2 (area of a full-sized spear) to allow for consistent comparisons.
[2] Factors include Cultivars: Guelph Millennium, Guelph Eclipse, Jersey Giant, and Gijnlim. Isolates: OA03, NA61, OA101. Wounding: rubbing the spear surface (1x2cm) with cheesecloth to damage surface vs no wounding. Location: near spear tip vs spear middle. Lighting: 12-hour photoperiod vs. continuous darkness. Lesions counted 72 hours post-inoculation. Spears measured 10cm in experiment four and 23cm in all other experiments.
[3] Experiment one tested factors: Cultivar, isolate, wounding, light. Interaction with wounding: light x isolate.
[4] Experiment two tested factors: Cultivar, isolate, wounding, location, light. Interaction with wounding: light x isolate, cultivar x isolate (not shown here, see table 9)
[5] Experiment three tested factors: Cultivar, wounding, location. Interaction with wounding: none.
[6] Experiment four tested factors: Cultivar, wounding. Interaction with wounding: none.
[7] Means followed by the same letter within experiments (and isolate for OA101) represents no significant difference using Tukey-Kramer's test at $P \leq 0.05$.
[8] Standard error in parentheses.

Exp 1: The factors were cultivar, light, wounding, and isolates (Table 2). A three-way interaction was observed among wounding, lighting, and isolate treatments (Table 2). The effect of lighting on lesion formation was stronger for isolate OA03 than NA61 when on wounded surfaces (Table 10). On unwounded surfaces, isolate OA03 caused merely 0.1 lesions to form on average both under periodic light and continuous dark (Table 10). On wounded surfaces in the light, unlike in the dark, NA61 caused more lesions than OA03 (Table 10). Otherwise, wounding greatly increased the number of lesions that formed in an area compared to unwounded areas and isolate NA61 caused more lesions on wounded surfaces than did OA03 (Table 10).

Exp 2: The factors were cultivar, light, wounding, location, and isolates (Table 2). There were two three-way interactions that included wounding. The other two variables were cultivar x isolate (Table 9) and lighting x isolate (Table 10) (Table 2). The former interaction is due to the larger differences among cultivars for OA03 than for NA61 when wounds were present. For the later interaction, NA61 caused more lesions than OA03 in the dark when unwounded, and in the light when wounded (Table 10). Otherwise, wounding dramatically increased lesion formation, similar in magnitude to that seen in experiment one (Table 10). Isolate OA101 was included on a single date in experiment two and it followed the same pattern regarding wounding and lighting as did NA61, where both wounding and darkness additively caused an increase in lesion count, however the difference in the dark between wounded and unwounded was less than expected as the maximum possible number of lesions per area was reached (lesions covered ~100% of surface area) for the wounded areas in the dark (Table 10).

Exp 3: The factors were cultivar, wounding, and location (Table 2). The effect of wounding did not interact with cultivar and location (Table 2). Many more lesions formed at the wounded areas

than unwounded areas, but the relative difference was much smaller than that seen in experiments one and two (Table 10).

Exp 4: The factors were cultivar and wounding, with all spears averaging 10cm in height unlike all other experiments where spears measured 23cm (Table 2). There was no wounding by cultivar interaction (Table 2). More lesions formed at the wounded areas than unwounded areas, but the relative difference was much smaller than that seen in experiments one and two (Table 10).

Overall: All experiments showed that more lesions formed on wounded spear surfaces compared to unwounded surfaces. Isolate OA03 caused few lesions on unwounded surfaces which can at least partially be attributed to the lower rate of germination for OA03 conidia on unwounded surfaces compared to NA61 (Appendix Table 2). The number of lesions formed by isolate OA03 was affected by the factors of light, wounding, and cultivar to a greater extent than NA61 was affected.

4.3.2.1 Presence of wounds

In experiment three, the presence of wounds on an inoculated spear had no effect on the number of lesions that formed on unwounded sections (Appendix Table 11) and there was no interaction between the presence of wounds and cultivar (Appendix Table 11).

4.3.3 Lighting

Table 11: Effect of light on number of lesions on whole, full-sized spear equivalents[1].

	Exp. 1[2,3]				**Exp. 2[4]**				**Exp. 2**	
	Wounded		Unwounded		Wounded		Unwounded		OA101	
	NA61	OA03	NA61	OA03	NA61	OA03	NA61	OA03	Wounded	Unwounded
Dark	165.9 a[7] (35.80)[8]	80.7 a (17.57)	4.3 b (1.11)	0.1 c (0.23)	192.5 a (44.28)	110.9 ab (25.59)	7.9 c (2.38)	1.1 d (0.55)	492.9 a (97.36)	73.6 b (20.80)
Light	84.1 a (15.39)	4.2 b (0.92)	1.6 b (0.48)	0.1 c (0.20)	86.70 b (20.49)	16.8 c (4.19)	1.8 d (0.78)	0.3 d (0.36)	287.0 a (65.19)	3.7 c (1.50)

[1] The area size where lesions were measured varied for some treatments and experiments, all areas were standardized to lesions per 50cm^2 (area of a full-sized spear) to allow for consistent comparisons.
[2] Factors include Cultivars: Guelph Millennium, Guelph Eclipse, Jersey Giant, and Gijnlim. Isolates: OA03, NA61. Wounding: rubbing the spear surface (1x2cm) with cheesecloth to damage surface vs no wounding. Location: near spear tip vs spear middle. Lighting: 12-hour photoperiod vs. continuous darkness. ASM: treated vs not treated with acibenzolar-S-methyl. Lesions counted 72 hours post-inoculation. Spears in experiment five had an intact epicuticular wax unlike in all other experiments.
[3] Experiment one tested factors: Cultivar, isolate, wounding, light. Interaction with light: isolate x wounding.
[4] Experiment two tested factors: Cultivar, isolate, wounding, location, light. Interaction with light: isolate x wounding.

Table 11 (Continued): Effect of light on number of lesions on whole, full-sized spear equivalents[1].

	Exp. 5[5]				**Exp. 6[6]**			
	Cultivar				ASM No		ASM Yes	
	Guelph Millennium	Guelph Eclipse	Jersey Giant	Gijnlim	Middle	Top	Middle	Top
Dark	12.9 ab (3.05)	31.6 a (9.14)	8.8 bc (2.14)	3.4 d (0.96)	8.6 bc (5.68)	10.9 b (7.03)	20.2 a (12.58)	7.0 bc (4.73)
Light	6.4 bc (1.62)	5.4 bc (1.59)	3.9 d (1.07)	0.2 e (0.29)	3.6 d (2.75)	4.7 cd (3.39)	4.8 cd (3.42)	2.6 d (2.11)

[5] Experiment five tested factors: Cultivar, location, light. Interaction with light: cultivar qaz.

[6] Experiment six tested factors: Cultivar, location, light, acibenzolar-S-methyl. Interaction with light: ASM x location, and location x cultivar (not shown here, see table 13)

[7] Means followed by the same letter within experiments (and isolate for OA101) represents no significant difference using Tukey-Kramer's test at $P \leq 0.05$.

[8] Standard error in parentheses.

Exp 1: The factors were cultivar, light, wounding, and isolates (Table 2). There was a three-way interaction among lighting, isolate, and wounding treatments because isolate OA03 caused very low lesion numbers, 0.1 lesions on average, per unwounded spear and because light decreased lesion formation by OA03 but not NA61 when wounded (Table 2) (Table 11).

Exp 2: The factors were cultivar, light, wounding, location, and isolates (Table 2). There was a three-way interaction among lighting, isolate, and wounding treatments because isolate OA03 caused very low lesion numbers, on average less than 1 lesion per unwounded spear, as in experiment one, and also light had a stronger effect on OA03 than NA61 at reducing lesion formation (Table 2). When lesions did form, there were fewer lesions in the light than in the dark for all isolates except for OA101 at the wound, likely due to a physical lack of space for more lesions restricting the effect of light on lesion count (Table 11).

Exp 5: The factors were cultivar, light, and location, with all spears having a fully intact epicuticular wax unlike all other experiments (Table 2). More lesions formed in continuous dark than periodic light (Table 2). Light did not interact with cultivar nor location in experiment five (Table 2).

Exp 6: The factors were cultivar, light, location, and ASM treatment (Table 2). There was a three-way interaction with ASM, light, and location (Table 2). Fewer lesions formed on spears exposed to periodic light regardless of ASM treatment or location but the effect of ASM was stronger in the dark at the top than at the middle or anywhere in the light treatment (Table 11).

Overall: All experiments showed that spears incubated with periodic light have fewer lesions, ranging from 45-95% fewer, than spears incubated in continuous dark.

4.3.4 Location

Table 12: Effect of location of wounds, top or middle of the spear, on number of lesions forming on full-sized spear equivalents[1].

	Exp 2.[2,3]				Exp 3.[4]			
	Guelph Millennium	Guelph Eclipse	Jersey Giant	Gijnlim	Guelph Millennium	Guelph Eclipse	Jersey Giant	Gijnlim
Top	178.3 a[5] (45.15)[6]	183.0 a (50.53)	151.1 a (41.77)	48.4 bc (12.97)	51.6 a (17.47)	41.0 ab (13.96)	37.3 abc (12.84)	13.1 cd (4.72)
Middle	86.6 ab (22.05)	80.9 ab (22.50)	29.1 c (8.26)	20.2 c (5.57)	29.6 abc (10.06)	22.5 abc (7.79)	4.8 d (2.01)	13.5 bcd (4.83)

[1] The area size where lesions were measured varied for some treatments and experiments, all areas were standardized to lesions per 50cm^2 (area of a full-sized spear) to allow for consistent comparisons.
[2] Factors include Cultivars: Guelph Millennium, Guelph Eclipse, Jersey Giant, and Gijnlim. Isolates: OA03, NA61. Wounding: rubbing the spear surface (1x2cm) with cheesecloth to damage surface vs no wounding. Location: near spear tip vs spear middle. Lighting: 12-hour photoperiod vs. continuous darkness. Lesions counted 72 hours post-inoculation.
[3] Experiment two tested factors: Cultivar, isolate, wounding, location, light. Interaction with location: none.
[4] Experiment three tested factors: Cultivar, wounding, location. Interaction with location: cultivar.
[5] Means followed by the same letter within experiments represents no significant difference using Tukey-Kramer's test at $P \leq 0.05$.
[6] Standard error in parentheses.

Exp 2: The factors were cultivar, light, wounding, location, and isolates (Table 2). The location on the spear of the wounded areas did not interact with other tested factors for lesion numbers (Table 2). There was a location x cultivar interaction in experiment three and five because there was a difference in lesion numbers at the two locations for Jersey Giant, but not for the other cultivars. There were five times more lesions forming at wounds close to the tip of Jersey Giant spears compared to those wounds near the middle of the spear (Table 12). The effect of wounding on other cultivars was half as strong, and not significantly different, although there was a 9.76% chance of obtaining this result without a cultivar x location interaction in experiment two (Appendix Table 8).

Exp 3: The factors were cultivar, wounding, and location (Table 2). There was a location by cultivar interaction (Table 2). Jersey Giant had many more lesions at the top than at the middle of the spears while others did not, similar to experiment two (Table 12). There were nearly double the number of lesions at the top than at the middle for Guelph Millennium and Eclipse as well, however these differences were not significant.

Table 13: Effect of location on the spear on number of lesions forming on full-sized spear equivalents[1].

	Exp 5.[2,3]				**Exp 6.**							
					Continuous dark				12-hour photoperiod			
	Guelph Millennium	Guelph Eclipse	Jersey Giant	Gijnlim	Guelph Millennium	Guelph Eclipse	Jersey Giant	Gijnlim	Guelph Millennium	Guelph Eclipse	Jersey Giant	Gijnlim
Top	43.4 a (7.74)	44.3 a (8.58)	39.4 a (7.51)	10.1 bc (1.99)	15.5 ab (9.79)	19.5 ab (12.51)	5.8 cde (4.12)	2.9 ef (2.33)	9.5 abc (6.24)	3.4 def (2.68)	2.0 ef (1.78)	2.1 ef (1.85)
Middle	12.1 bc (2.29)	15.8 b (3.17)	6.6 cd (1.41)	4.1 d (0.92)	17.7 ab (11.11)	21.0 a (13.38)	10.3 abc (6.88)	7.9 bcd (5.31)	5.7 cde (4.00)	9.2 abcd (6.20)	1.8 f (1.67)	2.8 ef (2.28)

[1] The area size where lesions were measured varied for some treatments and experiments, all areas were standardized to lesions per 50cm^2 (area of a full-sized spear) to allow for consistent comparisons.
[2] Factors include Cultivars: Guelph Millennium, Guelph Eclipse, Jersey Giant, and Gijnlim. Location: near spear tip vs spear middle. Lighting: 12-hour photoperiod vs. continuous darkness. ASM: treated vs not treated with acibenzolar-S-methyl. Lesions counted 72 hours post-inoculation. Spears in experiment five had an intact epicuticular wax unlike in experiment six.
[3] Experiment five tested factors: Cultivar, location, light. Interaction with location: cultivar.
[4] Experiment six tested factors: Cultivar, location, light, acibenzolar-S-methyl. Interaction with location: light x cultivar, and light x ASM (not shown here, see Table 11).
[5] Means followed by the same letter within experiments represents no significant difference using Tukey-Kramer's test at $P \leq 0.05$.
[6] Standard error in parentheses.

Exp 5: The factors were cultivar, light, and location, with all spears having a fully intact epicuticular wax unlike all other experiments (Table 2). The location of unwounded areas of spears interacted with cultivar in experiment five (Table 2). All cultivars had more lesions near the top than at the middle, however the location effect was stronger in Jersey Giant than other cultivars (Table 13).

Exp 6: The factors were cultivar, light, location, and ASM treatment (Table 2). The effect of location on the spear was largely not seen in this experiment, in contrast to the other three experiments that included location (Table 13). There was a three-way interaction, with the only difference found for Gijnlim under continuous darkness (Table 2)(Table 13). Location also had a three-way-interaction with light and ASM in experiment six (Table 16). Spears kept in continuous darkness had fewer lesions at the middle when treated with ASM while there was no effect of ASM on spears at other locations and light conditions (Table 16).

Overall: In three of four experiments examining location on the spear, more lesions appeared near the top of spears than at the middle of spears. In experiments three and five, the effect of location along the spear was significantly larger for Jersey Giant than other cultivars; in experiment three, the only significant effect of location was in Jersey Giant. This larger effect of location along the spear on Jersey Giant can also be seen in experiment two as there is only a 9.76% chance of obtaining the results of experiment two if there were no interaction occurring (Appendix Table 8). The effect of location along the spear appears to be the same in experiments with wounded or unwounded spears.

4.3.5 Acibenzolar-S-methyl

The effect of the application of acibenzolar-S-methyl (ASM) was evaluated in a four-way factorial with cultivar, light, and location on the spear. There was a three-way interaction between ASM application, light treatments, and location (P=0.0493) (Table 2). The application of ASM reduced lesion formation at the middle in the dark, but not for other location and light combinations (Table 14).

The highest number of lesions was found on spears at the middle, that received no ASM, and were kept in the dark. Regardless of location, spears without ASM treatment had more lesions when kept in continuous darkness compared to the 12-hour photoperiod. Spears treated with ASM and kept in continuous darkness had a similar number of lesions compared to spears not treated with ASM that were kept under a 12-hour photoperiod. The effect of the photoperiod light treatment on reducing lesion numbers was stronger than the ASM treatment. Applying ASM to spears in the light had no significant effect. There was no ASM by cultivar interaction (Appendix Table 14).

Table 14: Average number of lesions (whole spear equivalent) by location, application of acibenzolar-S-methyl, and light treatments[1].

Exp. Six[2,3,4]	Continuous darkness		12-hour photoperiod	
Acibenzolar	Middle	Top	Middle	Top
No	20.2 a[5] (12.58)[6]	10.9 b (7.03)	4.8 cd (3.42)	4.7 cd (3.39)
Yes	8.6 bc (5.68)	7.0 bc (4.73)	3.6 d (2.75)	2.6 d (2.11)

[1] The area size where lesions were measured varied for some treatments and experiments, all areas were standardized to lesions per 50cm^2 (area of a full-sized spear) to allow for consistent comparisons.
[2] Factors include Cultivars: Guelph Millennium, Guelph Eclipse, Jersey Giant, and Gijnlim. Location: near spear tip vs spear middle. Lighting: 12-hour photoperiod vs. continuous darkness. ASM: treated vs not treated with acibenzolar-S-methyl. Lesions counted 72 hours post-inoculation.
[3] Tested factors: Cultivar, location, light, acibenzolar-S-methyl. Interaction with ASM: light x location.
[4] Means of 116 – 128 spears.
[5] Means followed by the same letter represents no significant difference using Tukey-Kramer's test at $P \leq 0.05$.
5 Standard error in parentheses.

4.3.6 Spear weight, stomatal density, and germination rate

In 2019, the season spear weight averages were 20.8, 18.0, 17.8, and 16.8 grams per spear for Guelph Eclipse, Gijnlim, Guelph Millennium, and Jersey Giant, respectively. These weights did not correlate with lesion formation in the lab or the field. Particularly high or low average weights on a given day for a cultivar did not correlate with any change in how the cultivar performed in that day's experiment. The average weight of spears of Jersey Giant was lower than expected due to fewer spears available for this cultivar necessitating using some thinner spears.

The cultivars had a similar number of stomata per 0.05mm^2 and there was no interaction between cultivar and location (Appendix Table 15). Stomatal density was five times higher at the top of the spears than in the middle of the spear (Table 15).

Table 15: Stomata per 0.05mm^2, top and middle of spear by cultivar

Cultivar	Location	
	Top	Middle
Guelph Millennium	56.6 a[1] (2.18)[2]	11.0 b (2.23)
Guelph Eclipse	52.0 a (2.39)	10.2 b (2.39)
Jersey Giant	49.2 a (2.28)	9.5 b (2.45)
Gijnlim	47.1 a (2.28)	10.4 b (2.23)

[1] Means followed by the same letter represents no significant difference using Tukey-Kramer's test at $P \leq 0.05$.
[2] Standard error in parentheses.

The germination rate of conidia was assessed on detached spears inoculated in controlled environments. The germination rate was not affected by cultivar (Appendix Table 16) or light (Appendix Table 17), but the percent that germinated was affected by wounding and isolate which interacted with each other (Appendix Table 16). There was a significant difference in percent germination for isolate OA03; the germination rate was 250% higher on wounded surfaces compared to unwounded surfaces. There was no difference in percent germination between wounded and unwounded surfaces for NA61 (Table 16). Isolate NA61 had higher percent germination in both wounded and unwounded spears.

Table 16: Germination rate (%) of two *S. vesicarium* isolates on wounded and non-wounded spears

Isolate	Wounded[1]	Non-wounded[2]
NA61	90.1 a[3] (4.17)[4]	79.3 ab (6.16)
OA03	65.9 b (3.77)	19.6 c (5.03)

[1] Means of 246-247 conidia in this column.
[2] Means of 114-120 conidia in this column.
[3] Means followed by the same letter represents no significant difference using Tukey-Kramer's test at $P \leq 0.05$.
[4] Standard error in parentheses.

The length of germ tubes that emerged from germinated conidia was not affected by cultivar, wounding, or isolate (Appendix Table 18).

5. Discussion

The research demonstrated that inoculating spears in the field will provide results that best match results from natural infection, provided that weather during a critical infection period is considered. Under controlled environment conditions, detached spears of Guelph Millennium and Guelph Eclipse generally developed more lesions than Jersey Giant while Gijnlim had the fewest lesions. Field results were affected by weather; Guelph Millennium and Guelph Eclipse had the most lesions, and Gijnlim the least when spears were exposed to heavy rains or sandblasting. However, Jersey Giant had many more lesions than the other cultivars when grown under calm weather (wind <10km/h and no rain). The study demonstrated that inoculation of spears in the field could be used to determine cultivar resistance in both dry and calm conditions or wet and windy conditions.

A bioassay for detached asparagus spears was developed which consistently showed differences in resistance to *S. vesicarium* among asparagus cultivars. The effect of a number of important factors was discovered in the controlled environment experiments. Wounding and lighting had the greatest influence on the number of lesions that developed. Overall, the greatest

numbers of lesions were observed in continuous darkness and on wounded areas of the spear. The application of acibenzolar-S-methyl (ASM), which induces host resistance, reduced the number of lesions on the middle section of the spear, in continuous darkness, but had no effect under alternating light and dark. Three of four experiments that included location on the spear showed more lesions near the tip than near the base. Isolates of *S. vesicarium* varied in aggressiveness depending on cultivar, wounding, and lighting. Spear size, the presence of the epicuticular wax, and creating wounds outside of the monitored area to cause a wound response across the rest of the inoculated spear, all appear to be unimportant.

Overall, Gijnlim had the highest level of resistance. Guelph Millennium and Guelph Eclipse were nearly identical in resistance and had the most lesions of all cultivars. There were many more lesions in Jersey Giant in the apical section of the spear compared to the middle of the spear. Near the tip, Jersey Giant had a similar number of lesions as Guelph Millennium and Guelph Eclipse but at the spear middle the number of lesions was low in Jersey Giant and was similar to Gijnlim.

5.1 Comparing field and lab results

Comparing results between the field and lab requires acknowledging the differences between the environments. In the field, propagules may be both ascospores and conidia with a level of genetic and phenotypic diversity that exceeds that of the conidia grown in the lab. In the lab, spears are inoculated with only *S. vesicarium* while in the field, spears, and *S. vesicarium*, are exposed to a wide variety of microbes that may affect the plant-pathogen relationship of interest (Abdullah 2017). It is important to acknowledge that detachment of the spear causes a rapid and dramatic change in sugar concentrations (Solfanelli et al. 2006), activates countless

molecular level changes including those for disease response, slows spear growth, and will affect water pressure and stomatal conductance. Environmental conditions such as light intensity, quality, and duration, free water and humidity, and ambient temperature can be controlled in the lab, but not in the field. Spear growth in the field complicates the effect of location along the spear and healing of wounds on attached spears necessitates a more exact approach. Depositing an equal amount of conidia along the vertical attached spear is difficult due to runoff and due to the uneven growth post-inoculation.

The difference seen among cultivars between calm and active weather, where Jersey Giant is less affected than other cultivars by active weather, could be attributed to 1) Jersey Giant being resistant to the effects of active weather, 2) weather shifting disease pressure away from tip towards spear middle, or 3) all cultivars but Jersey Giant possessing resistance to infection that is undermined by active weather.

The evidence for resistance to the effects of active weather is that Jersey Giant appears to have a thicker cuticle compared to other cultivars. A thicker cuticle may protect against low-speed sandblasting and may also reduce the chance of stomata opening due to a wrong-way-reaction by restricting the temporary expansion of epidermal cells during periods of high soil moisture. The thicker cuticle may exert increased inward pressure as the epidermal cells return to their normal size. In soybeans, sandblasting caused an increase in the number of bacterial spot lesions formed on the plants, although the strength of the effect varied greatly among cultigens; one cultigen that had half the number of lesions compared to another cultigen when both were unwounded had twice the number of lesions as the other when both were sandblasted, similar to

the way that Jersey Giant was much less affected by active weather than other cultivars (Pohronezny et al. 1992).

The evidence for weather shifting disease pressure away from the tip to towards the middle was found in the detached spear bioassays. Jersey Giant was far more susceptible to infection near the spear tip than at the spear middle, and this effect of location along the spear was much stronger for Jersey Giant than the other cultivars. Relative to other cultivars, Jersey Giant had high infection under calm conditions and lower infection during active weather. In the field under calm conditions, stomata may be the primary point of infection and stomatal density is much higher near the spear tip than at the middle and thus the apparent susceptibility to infection in a calm field was based primarily upon resistance near the tip. Sandblasting should create wounds across the spear evenly. This would result in equal points of infection at the top and middle and thus resistance at the middle will be greater represented in total lesions forming on a spear. A wrong-way-reaction could be similar despite the higher stomatal density near the spear tip as stomatal density may no longer be a limiting factor during a wrong-way-reaction. Neighbouring open stomata could be infected by the same propagule and will form what appears to be a single lesion due to overlapping. There is no evidence that the other cultivars have resistance that is undermined by active weather, yet it is a possible explanation.

The results from field surveys and bioassays certainly contrast by cultivar. There could be several explanations which may explain the gap between these results. One explanation relies upon differences in resistance by location along the spear among cultivars in the field and bioassay. A particularly susceptible tip in Jersey Giant would explain the effect of weather on resistance among cultivars, however it would not explain why Jersey Giant has similar resistance

to Guelph Millennium and Guelph Eclipse near the tip in the bioassay. A possible explanation is that the effect of location is affected by the detachment of spears. It is known that detaching spears causes a rapid decline in sugar concentration and that spears have lower concentrations of sugars nearer the tip than towards the base (Solfanelli et al. 2006). The greater susceptibility of the spear tip in Jersey Giant may be due to a greater gradient of sugars across the spear compared to other cultivars. In the field, other cultivars, possibly with a more subtle gradient, may have sufficient sugars at the tip to grant full resistance, and this gradient may only become pertinent to resistance as sugars fall post-harvest. A stronger gradient in Jersey Giant may be apparent for disease resistance even pre-harvest. Together this can explain why Jersey Giant has more lesions in the field under calm conditions, fewer lesions under active weather in the field relative to other cultivars, relatively mediocre resistance in the bioassay at the top, and relatively good resistance in the bioassay at the middle.

The similar lesion numbers seen across cultivars in active weather and at the middle of spears in the bioassay could be due to active weather shifting the proportion of infections on the spear away from the stomata rich-tip towards the middle combined with the cultivar by location interaction found in the bioassay also existing in the field. The similarity could also be a coincidence if Jersey Giant is simply more resistant to the effect of weather than other cultivars, because of a thicker cuticle, and the effect of detachment is unequal among cultivars.

The differences in relative resistance among cultivars is not related to disease pressure as inoculated spears with high lesion numbers show results similar to those found through natural infection under calm conditions. Temperature likely plays no direct role in relative resistance

among cultivars as temperature in the field fluctuated greatly and no effect of this was seen in the field surveys or in detached experiments using spears grown under various temperatures.

An unequal effect of detachment not explainable by sugars, could be related to wounding response or stomatal control. In the bioassay, wounding demonstrated that there was no relationship between number of lesions and the increased density of stomata near the tip. That is, there were more lesions on wounded sections at the middle of the spear than wounded sections near the tip of the spear. In the field, stoma may act differently than in the bioassay, possibly resulting in increased numbers of lesions to form near the tip where stomata density is high.

Small spears could either be akin to the top half of a full spear (based on age), or act like a miniature full spear (based on sugar gradient). Age and sugar related resistance has been widely observed between countless plant species and all types of pathogens (Hu and Yang 2019; Bolouri Moghaddam and Van den Ende 2012; Horsfall and Dimond 1957). The pattern of resistance in Jersey Giant suggests that small spears act like larger spears. If the effect of location was due to age alone, the study on short, young, spears should have returned results similar to those seen at the young tops of older spears, that is Jersey Giant should develop a similar number of lesions as Guelph Millennium and Guelph Eclipse. However, based on the results for Jersey Giant relative to other cultivars, small spears were similar to full spears. That is, small spears do not equal the top half of full spears, nor the middle of full spears, but fall in between. In terms of age, small spears are similar to tops, but the gradient of sugars may be greater between the tip and base of small spears than it would be for the top half of a full-size spear.

It is unfortunate that no cultivar showed very high or low resistance to light or acibenzolar-s-methyl treatments and that the best traits (relatively high resistance at spear tip,

high resistance in bioassay and field) were all found to already exist in a single cultivar, Gijnlim. However, this work will be useful when testing a much more diverse group of breeding lines in the future. The finding that the number of lesions that form is dependent on a number of factors, especially wounding, lighting, and location on the spear, will help future researchers in finding quantitative trait loci.

5.2 Speculation of timing of weather

The trials assessing purple spot symptoms in the field found that cultivar performance relative to one another was inconsistent across dates. It appears that certain acute weather events within a critical period, usually 20-75 hours before counting lesions, affect the resistance of some cultivars much more than others. Following dry and calm weather, few lesions form on the spears, but more develop on Jersey Giant than the other three cultivars. After a dry and windy period causing sandblasting or a heavy rain perhaps causing a wrong-way-reaction, an increased number of lesions are formed and resistance across cultivars resembles the bioassay results where Guelph Millennium and Guelph Eclipse had more lesions than Jersey Giant and Gijnlim.

As both microwounds and wrong-way-reactions (Buckley 2019) are short-lived, no effect should be seen unless the spear surface is colonized by *S. vesicarium* prior to the weather event. Low temperatures greatly reduce germination speed and success rate; an eight-hour wetness period at 5°C will be sufficient for about a quarter of conidia to germinate, an eight-hour wet period at 10°C will result in nearly 90% of conidia to germinate, and a four-hour wet period at 20-30°C results in more than 95% of conidia germinating (Bohlen-Janssen et al. 2018a). The rate of infection is modelled to be highest at 22°C (Bohlen-Janssen et al. 2018b). If the weather event occurs less than a day before harvest there will not be time for a visible lesion to form.

Therefore, the critical period for weather to affect resistance among cultivars begins when *S. vesicarium* has colonized the spear surface and ends approximately one day before harvest. Depending on temperature, which determines the rate of spear growth, this critical period is approximately 20-75 hours before inspection, with the 75-hour frame decreasing as the temperature during this window is above average and thus spear growth rate is above average.

As the deposition of propagules onto spears varies greatly between days and hours, the availability of free water required for germination is inconsistent, and the spears are only in the field for a short time, it is possible that spears could be exposed to weather that undermines resistance but due to a lack of established hyphae no effect is seen. If propagules are first deposited on spears during sandblasting or were previously deposited but were yet to germinate prior to the sandblasting, and germination and infection only or mostly occurs soon after the wounds heal, the pattern of lesions among cultivars will appear as if the sandblasting never occurred. This may be what occurred for spears surveyed on 23 May 2019 as the high winds occurred 62 hours pre-inspection, easily allowing enough time for new infections to create visible lesions by the time of inspection. The two very humid nights, and probable dew formation, following the wind certainly would have supported further germination, hyphae growth, and infection. Following the high-winds on 20 May 2019, temperatures fell to 4℃, which would reduce germination and infection by the conidia and there would be low disease development to show the potential wounding. Similarly, spears surveyed on 9 June 2020, were likely exposed to sandblasting but had few lesions. These likely had few germinated propagules on their surface because there was no rain and the relative humidity was always below 90% while the spears were in the field.

Field inoculation tests were consistent with weather interacting with cultivar resistance. Inoculating spears with a high level of conidia and providing free water at sunset preceding high humidity nights resulted in high numbers of lesions. Inoculated spears surveyed on 21 May, 2020 were inoculated with sufficient time for the conidia to germinate before the likely wrong-way-reaction occurred. On this date, both inoculated and non-inoculated spears showed the same pattern of resistance that is different from that seen during calm weather.

Spears inoculated for the 7 June survey showed a pattern of resistance similar to that seen during calm weather, while uninoculated spears surveyed that day showed a pattern similar to that seen as a result of active weather. The reason this occurred is that sandblasting likely happened 24 hours prior to inspection/44 hours post-inoculation. During these 44 hours there was a much higher level of germinated propagules on the surface of the inoculated spears compared to the non-inoculated spears, and a lot of infection took place during this time. The sandblasting allowed for a temporary high infection rate on both groups of spears, but as so many infections already took place on the inoculated spears, this burst of infection was a fraction of all lesions seen on these spears, while it caused almost all infections on non-inoculated spears.

Overall, it appears that when germinated conidia are able to take advantage of acute weather events, an interaction between weather and cultivar resistance occurs. Research by Foster (2018) had shown cultivar resistance to change by year and location, meaning there was a need to test asparagus in a controlled environment. The current research provides a possible explanation for the interaction between cultivar and environment and could explain why differences were seen based on both location and year.

5.3 Bioassay design

Purple spot of asparagus is difficult to manage and host resistance would be the most effective and economical method to reduce disease in future years. This research identified methods to conduct a bioassay under controlled environment conditions to identify some, but not all, of the components of disease resistance. To acquire consistent results, there must be free water for at least three hours, humidity sufficient to maintain free water, and temperatures between 20 and 30℃, for the *S. vesicarium* propagules to germinate and infect, and for the spear to not decompose during the 72-hour incubation period. If temperatures are too far below the 20℃ used in this study, *S. vesicarium* development will slow, and if the temperatures are much higher, spears will decompose too rapidly. At a temperature between 15-30℃, almost all conidia will germinate within three hours in the presence of free water on the spear surface. Very high humidity, over 90%, is required throughout the experiment to slow spear decomposition and sustain hyphae growth. During data collection, the location on the spear, whether upper or lower half, must be recorded and should be consistent. Factors that affect resistance such as wounding and lighting need not be included in the bioassay but must be consistent.

Based on the variability seen within cultivars and between repetitions, 100 spears per cultivar would be sufficient to see significant differences among cultivars in the detached spear bioassay. Preliminary screens using 20 spears per cultivar should be able to identify the most susceptible lines or cultivars if there is a sizable difference in resistance among cultivars.

In the bioassay, spears were placed standing in distilled water, allowing ready uptake of water to maintain cell expansion and cellular turgor which allows for stomatal opening (Di Stasio 2010). Previous screens have stated that wounds are required for infection on detached spears but

not for attached spears (Johnson and Lunden 1986; Lacy 1982; Foster 2018). These screens used a misting chamber (Johnson and Lunden 1986), moist paper towel (Lacy 1982), or spears were laid horizontally above a pool of water (Foster 2018) to maintain moisture levels in the spears rather than standing them directly in water. The inability of the spears to take up water in these screenings likely caused stomata to stay closed during incubation. Detached asparagus spears allowed to imbibe water continue to lengthen and increase in mass during storage while those kept only in a humid or dry environment stop elongating and decrease in mass (Heyes et al. 1996). For cut roses, stomatal conductance is higher on stems standing in water than for those stored without free-water (Cruz-Guzman et al. 2018). Perhaps bioassay results would more closely mirror field results during calm weather if the spears were placed standing in distilled water, or water containing a solute, to allow water/nutrient uptake but with the humidity reduced to 70% for several hours after inoculation to promote stomatal closure.

There was no interaction between cultivar and whether lesions were counted on wounded or unwounded surfaces. Wounding does have value however in that it facilitates using isolates that produce few conidia or perform poorly on unwounded surfaces. Differences among cultivars in stomatal or surface-based resistance may also be bypassed to uncover other resistance mechanism through wounding.

Creating a bioassay for detached spears that can maintain the resistance that is undermined in the field by certain acute weather events, particularly in cultivars other than Jersey Giant, may not be possible. Detaching the spears causes many profound changes which evidently affects an important defense mechanism, at least in some cultivars. Detachment affects the growth rate, respiration rate, causes sugar concentrations to fall greatly, the need for stomata

to open is reduced, and the detachment injury will induce defense systems in the plant, although this response may already be active from the harvesting of earlier spears from the same plant (Verlinden et al. 2014). Inoculating attached spears in the field or in pots may be the only way to obtain useful results for resistance.

Stemphylium vesicarium can penetrate asparagus spears through both open stomata and wounds. Many plant species have developed defenses against microbes entering through stomata such as closing stomata in response to the detection of pathogen-associated molecular patterns (PAMPs) by receptors in the plant's outer surfaces (Melotto et al. 2017). Creating wounds on the spears would show the level of resistance that can occur without any stomatal defense. If a cultivar had an above average stomatal defense, this should be evident when comparing lesions forming on wounded and unwounded surfaces across cultivars. However, it is likely that the stomatal defense present in attached spears is either partially or fully impaired in detached spears. Wounding dramatically increased the number of lesions formed by removing barriers to penetration and providing free water and nutrients from cell leakage.

Previous work by Foster (2018) looked at the length of lesions originating at wounds to determine resistance on detached spears, but results were inconsistent. This bioassay produces consistent results by carefully controlling important factors like light and accounting for location along the spear. In this bioassay the number of lesions was counted rather than lesion length. This change was made as the cultivars tested all appeared to have very similar size lesions that did not expand at a noticeable rate, thus the chief concern was decreasing the number of lesions rather than their size. The bioassay developed through the current study included resistance that prevented infection, a very important characteristic, which is more relevant to assessing

resistance that occurs when infection is guaranteed when high numbers of conidia are applied to a wound.

5.4 Other bioassay findings

Creating equal wounds by rubbing the spear with cheesecloth was not always successful and almost all of the dozen failed attempts to wound (no wound visible three days post-inoculation) occurred on Jersey Giant. This apparently damage resistant surface could cause Jersey Giant spears to acquire fewer micro-wounds in the field and during harvest and transport. However, the increase in the number of lesions formed due to wounding Jersey Giant spears was similar to the other cultivars. This means that either Jersey Giant acquires the same number of micro-wounds regardless of the apparently stronger cuticle, or that the micro-wounds are not important and most infections occur through stomata.

No interaction was found between wounding and light for NA61 (Appendix Table 7; Appendix Table 9). This is noteworthy as it shows the effect of the light treatment is not related to causing stomata to close as the effect of stomatal closure is minimal in the presence of wounds. The similar effect of location on wounded and unwounded spears is also notable as it provides evidence that the effect of location is not based in the density of stomata which are at a greater concentration of near the tip.

There was a three-way ASM by location by light interaction in experiment six. Treatment with SAR-triggering ASM reduced the number of lesions in the dark at the middle of the spear. There was no effect of ASM in the light which is likely due to the defense response that ASM triggers being already active. This shows that at least part of the SAR response normally requires light for activation. The effect of ASM applied in the dark is weaker than the effect of providing

light compared to keeping spears in continuous darkness. This difference may be due to applying ASM only at inoculation and not throughout incubation, while the light was present at intervals during the infection process. The weak response could also be due to an insufficient dose of ASM, or the presence of light-dependent pathogenesis related genes which are not activated through SAR such as those triggered by jasmonic acid (Xie et al. 2011). Overall, important PR genes were light-dependent, but these genes appear to be uniform across cultivars.

There was no interaction between ASM and cultivar. As ASM was expected to act upon the spears in a manner similar to light, and as there was no interaction of light regime and cultivar, this was expected.

In experiments two, three, and five, location on the spear was an important factor, particularly for Jersey Giant more than the other cultivars. This location effect could be related to sugar levels which change dramatically in the first few hours postharvest (Irving and Hurst 1993) and steadily decrease in concentration when measured from the base to the tip of the spear (Verlinden et al. 2014). If the sugar levels of Jersey Giant are less uniform across the spear, or are particularly low near the tip or high near the base compared to others, this would be evidence of a relationship between sugars and lesion formation.

The surface of asparagus spears is covered with a crystalline epicuticular hydrophobic wax (Voigt and Gorb 2009). This wax is very delicate; the gentlest touch will completely remove the wax. This possible preformed defense could play a role in restricting penetration of epidermal cells, restrict attachment, or play a role in signaling a defense response (Wang et al. 2020). Resistance amongst cultivars in experiment five where all spears had intact an epicuticular wax did not appear to be different than in experiments where there was no special

care taken to keep the delicate wax intact. Due to a shortage of spears, the effect of the wax on lesion development was not compared within a single experiment, however, lesion counts were at a level similar to other experiments indicating the effect of wax is not large.

Differences among the aggressiveness of the isolates of *S. vesicarium* were detected. Isolate OA03 appeared to be similar to the other isolates tested except that wounding was required for lesion formation. However, OA03 may be most susceptible to a number of plant defenses. The presence of light, which is required for spears to activate some of their PR-genes, had a greater effect on reducing lesion formation for OA03 than for other isolates (Table 11). In the 2019 experiments, the lesion counts varied among cultivars for OA03 at the wounded area but were not significantly different for NA61 at the wounded area, showing OA03 to be more sensitive to resistance. The germination rate for both NA61 and OA03 was lower on unwounded surfaces than on wounded surfaces, but the effect was much greater for OA03 than on NA61 (Table 16). The low germination rate alone does not explain the difference in lesion production, but resistance involving the cuticle might be more effective against OA03 than NA61.

Germination and germ tube growth rates among isolates were not affected by cultivar and did not correlate with resistance. The concentration of stomata was five times higher near the tip of the spears than at the middle. There was no indication of substantial differences among cultivars. The differences in stomatal density between the top and middle of spears appeared to match the change in elongation in the surrounding epidermal cells.

Resistance ranking among cultivars was not affected if conditions were modified to cause more lesions to form. In the controlled environment studies, lesion counts changed between inoculation dates, but the relative resistance among cultivars was consistent. On attached spears

in the field, the weather-related resistance trends were consistent on days with high and low lesion counts.

5.5 Future directions

There are a number of directions for future research that could be pursued to determine more about the mechanisms of resistance. It appears that weather events are undermining stomatal defenses, either by forcing stoma open or creating wounds, and that there is a difference in the quality of stomatal defense among cultivars. Several additional experiments are required in the field to investigate the role of stomatal defence and any cultivar differences.

The effect of sandblasting on attached spears in the field could be investigated in detailed experiments. Wounds will quickly heal in the field (Johnson and Lunden 1986), so these wounds need to be made at the time that spears are inoculated, or preferably, several hours post-inoculation, so that the propagules will have germinated, and the hyphae are ready to invade the wounds. The act of post-inoculation wounding must not disturb the hyphae and it must allow for the cuticle to resist wounding as the cuticle could possibly resist a weak sandblasting, and this resistance could vary among cultivars. The most suitable method of wounding is to artificially sandblast, preferably at a high (14km h^{-1}) and low speed (10km h^{-1}(half the kinetic energy of 14km h^{-1})). Sandblasting spears at 14km/h for 0.4s has been shown to cause wounding more severe than typically observed in the field (Johnson and Lunden 1986). Whole spears could be inoculated, and after several hours sections of the spears could be marked and either sandblasted at high or low speed, or not at all. An interaction among cultivars due to sandblasting should be evident.

Staggering field inoculation over time without any wounding could also be used to determine how cultivar resistance to *S. vesicarium* changes in response to natural sandblasting and heavy rain events. If newly emerged spears were inoculated every 24 hours starting 48 hours prior to heavy rain or sandblasting, and ending 24 hours after the event, the effect of the event would be clear. It is necessary to begin inoculating many hours in advance of the rain or sandblasting as short-lived open wounds and open stomata may only be taken advantage of by hyphae that are already present.

Samples taken from asparagus spears in the field or lab appear to have all of the stomata closed. These samples however were all taken from spears exposed to *S. vesicarium*. If the stomata are closed due to the detection of PAMPs, this closure could be reversed by applying certain chemicals, such as oxalic acid or coronatine, although these chemicals may also affect resistance in other ways (Guo and Stotz 2010). Applying a chemical to open stomata on 13-16 cm spears in the field that were inoculated 10 hours previously may show a cultivar's resistance to *S. vesicarium* with stomatal control removed, perhaps similar to results from a wrong-way-reaction. Closely monitoring stomatal conductance over time on both attached and detached spears may yield valuable information.

In the lab, penetration on surfaces not purposefully wounded likely occurs through a mix of microwounds and open stomata. There are chemicals that could be used to force open stomata, such as oxalic acid, coronatine, or applying light with an abscisic acid scavenger (Garcia-Mata and Lamattina 2007). If these were used most infections should occur through the stomata. An interaction between chemical application and resistance across cultivars could show that not all

cultivars keep stomata shut equally during incubation and differences in lesion number could be recorded.

Plant defense responses are affected in many ways by sugar content (Bolouri Moghaddam and Van den Ende 2012). Sugars are used by plants as defense signals and affect the regulation of the production of defense molecules (Bolouri Moghaddam and Van den Ende 2012). Individual plants with abnormally high or low sugar levels may gain resistance to certain “low or high-sugar diseases" (Horsfall and Dimond 1957). Some rusts and powdery mildews do not infect otherwise susceptible plants that are kept in the dark, unless sugar is applied to the leaves, and early blight does not infect tomato plants with no fruit load while plants whose leaves were removed become highly susceptible (Horsfall and Dimond 1957). Anthocyanin, which accumulates around sites of infection by *S. vesicarium* in non-anthocyanin free asparagus and gives the disease the name purple spot, depends upon sucrose availability to induce the genes responsible for its biosynthesis (Solfanelli et al. 2006). Purple spot lesion size appears similar between anthocyanin free or anthocyanin-containing asparagus lines (personal observation), in line with results seen in cedar-apple rust on apple trees (Lu et al. 2017), indicating that anthocyanins are not a major component of resistance to *S. vesicarium.*

Sugar levels in the spears are of interest as they may also explain some of the differences seen between the field and lab and the effect of location on the spear. The sugar levels change dramatically in the first few hours postharvest (Irving and Hurst 1993). If a mechanism of resistance that requires certain sugars is strongest in some cultivars, sugar levels are different among cultivars affecting the expression of mechanisms of resistance, or the postharvest drop in sugars is not uniform across cultivars, then field and lab results could diverge by cultivar. Other

researchers have shown sugar levels to steadily decrease when measured from the base to the tip of the spear (Verlinden et al. 2014). This may be related to a high number of lesions forming near the low-sugar tip, particularly for Jersey Giant.

An experiment could also be conducted where some spears are soaked in sugar solution (such as sucrose, fructose, or glucose) for a period between harvest and inoculation to boost sugar levels in the spears. The spears that were soaked could also be placed standing in sugar solution during the infection process to maintain increased sugar levels throughout the experiment. If these spears show fewer lesions than non-treated spears it would be evidence that sugar content in the spear post-harvest is important for resistance, and potentially in the field as well. Alternatively, a sugar treatment may increase lesion count by affecting defense signaling within the spear or by supporting the pathogen (Bezrutczyk et al. 2018).

An idea not explored in this work is the relationship between cultivar resistance and susceptibility to the host-specific toxins in *S. vesicarium*. Applying host-specific toxins produced by *S. vesicarium* to susceptible pear leaves showed the formation of invaginations in plasma membranes which would allow for the pathogen to enter the affected cells (Singh et al. 2000). Finding a cultivar with decreased susceptibility to the host-specific toxins could be a valuable mechanism of resistance, as other mechanisms of resistance, such as stomatal closing or maintaining high sugar content could have undesirable side-effects such as reduced carbon assimilation or change in taste. Resistance to host-specific toxins should also protect the fern.

By comparing the genomes of highly resistant asparagus species against susceptible species, a number of potential genes, selected by predicted gene function and known homologous genes, related to resistance or susceptibility could be found. By knocking out these

genes with CRISPR, or by silencing with siRNA, a gene may be found in the resistant cultivar that, if transferred to susceptible cultivars, would make them resistant. Conversely, finding a gene for susceptibility and removing it from the genome could make the cultivar resistant.

Lastly resistance could also be investigated on the fern following the work of Bansal 1988, Broadhurst 1993, and Foster 2018. Looking at surface area necrotic and defoliation over a long period of time may show some cultivars may have a slower disease progression or are less negatively affected by infection. There also appears to be a negative relationship between the severity of Asparagus rust and Stemphylium leaf spot on individual plants' ferns that may be fruitful to investigate at a molecular level including rtPCR.

6. Conclusions

In conclusion, this study evaluated the relative resistance of four asparagus cultivars, Jersey Giant, Guelph Millennium, Guelph Eclipse, and Gijnlim, to infection by *S. vesicarium* and found differences among the resistance of cultivars that was affected by heavy rainfall and sandblasting in the field and location along the spear in the bioassay. Cultivars performed consistently in detached spear controlled environment trials, but these results did not mirror field assessment results. Inoculating spears with conidia in the field resulted in the same pattern of lesions among cultivars that would be found in naturally infected spears in the field. A number of factors that influenced lesion development were discovered, and procedures for measuring resistance in both the field and lab were refined.

In the field, Jersey Giant was significantly more susceptible than the other three cultivars except when the plot was affected by high rain or wind, when all cultivars were infected to a similar degree. In the controlled environment studies, Gijnlim was consistently the least

susceptible and Guelph Millennium and Guelph Eclipse were the most susceptible and always nearly identical. Lesion formation on Jersey Giant was greatly influenced by location on the spear; near the tip there were high numbers of lesions, comparable to Guelph Millennium and Guelph Eclipse and at the middle of the spear there were low numbers of lesions, similar to Gijnlim. Unfortunately, no cultivar was found to possess exceptional resistance. More lesions developed in the dark than under a 12-hour daylength suggesting the presence of light-dependent SAR pathogenesis related resistance as previously found in other plant species (Kim et al. 2011, Zeier 2004).

The detached spear bioassay developed from these studies provides consistent results. Following inoculation, there must be free water on the spears for at least 4 hours, humidity over 90%, and a temperature between 15-25℃. This allows for infection and preservation of the spears in good condition during the 72-hour incubation period. A specific portion of the spear, upper one-third or lower two-thirds, should be selected for inoculation and the location on the spear must be consistent and recorded. Wounding prior to inoculation and the light regime (completely dark or 12 hours photoperiod) were found to affect the number of lesions but there were no differences among cultivars. Thus, these factors need not be included in the bioassay, although they must be consistent.

Inoculating attached spears in the field produced results matching field survey results when accounting for weather during a critical period. The large effect of weather on resistance was not replicated in the detached spear assay, thus the results of the assay do not fully encompass disease development that will occur in the field.

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Appendix

ANOVAS

Appendix Table 1: Estimates for lesion count on four cultivars naturally infected with *S. vesicarium* in the field

Covariance Parameter	Estimate	SE		
block*date	0.02224	0.008656		
residual	0.4369	0.01648		
Effect	Num DF	Den DF	F-Value	Pr > F
date	11	36	31.03	<.0001
cultivar	3	1402	26.85	<.0001
date*cultivar	33	1402	3.44	<.0001

Appendix Table 2: Estimates for lesion count on four cultivars infected with *S. vesicarium* in the field on seven select dates

Covariance Parameter	Estimate	SE		
block*date	0.02106	0.01123		
residual	0.4727	0.02404		
Effect	Num DF	Den DF	F-Value	Pr > F
date	7	23	55.34	<.0001
cultivar	3	770	46.52	<.0001
date*cultivar	21	770	1.46	0.0854

Appendix Table 3: Estimates for lesion count on four cultivars naturally infected with *S. vesicarium* in the field on three select dates

Covariance Parameter	Estimate	SE
Block*date	0.02487	0.01389
Residual	0.4993	0.02666

Effect	Num DF	Den DF	F-Value	Pr > F
date	2	9	10.93	0.0039
cultivar	3	510	9.51	<.0001
date*cultivar	6	510	1.31	0.2499

Appendix Table 4: Estimates for lesion count on four cultivars naturally infected with *S. vesicarium* in the field on four select dates

Covariance Parameter	Estimate	SE		
Block*date	0	.		
Residual	0.8473	0.07833		
Effect	Num DF	Den DF	F-Value	Pr > F
date	3	12	52.74	<.0001
cultivar	3	222	3.62	0.0139
date*cultivar	9	222	1.52	0.1403

Appendix Table 5: Estimates for lesion count on four cultivars inoculated with *S. vesicarium* in the field

Covariance Parameter	Estimate	SE		
Block*date	0	.		
Residual	1.7252	0.2348		
Effect	Num DF	Den DF	F-Value	Pr > F
date	2	8	9.71	0.0072
cultivar	3	100	1.75	0.1618
date*cultivar	6	100	2.40	0.0331

Appendix Table 6: Estimates for lesion count across cultivars of asparagus spears when tested by light, isolate, and wounding in experiment one.

Covariance Parameter	Estimate	SE		
date	0.007990	0.02374		
date*box*light	0	.		
residual	1.6001	0.1196		
Effect	Num DF	Den DF	F-Value	Pr > F
cultivar	3	354	5.75	0.0008
wound	1	354	600.41	<.0001
cultivar*wound	3	354	0.78	0.5043
isolate	1	354	126.15	<.0001
cultivar*isolate	3	354	0.96	0.4112
wound*isolate	1	354	4.66	0.0315
cultivar*wound*isolate	3	354	1.69	0.1689
light	1	4	60.76	0.0015
light*cultivar	3	354	0.62	0.5998
light*wound	1	354	27.24	<.0001
light*cultivar*wound	3	354	0.45	0.7201
light*isolate	1	354	7.12	0.0080
light*cultivar*isolate	3	354	0.12	0.9454
light*wound*isolate	1	354	27.69	<.0001
light*cultivar*wound*isolate	3	354	0.21	0.8897

Appendix Table 7: Estimates for lesion count across cultivars of asparagus spears when tested by light and wounding for NA61 in experiment one.

Covariance Parameter	Estimate	SE		
date	0	.		
date*box*light	0.02099	0.04049		
residual	1.1903	0.1290		
Effect	Num DF	Den DF	F-Value	Pr > F
cultivar	3	170	3.92	0.0097
wound	1	170	462.69	<.0001
cultivar*wound	3	170	0.15	0.9319
light	1	4	12.58	0.0239
light*cultivar	3	170	0.45	0.7204
light*wound	1	170	0.00	0.9473
light*cultivar*wound	3	170	0.28	0.8418

Appendix Table 8: Estimates for lesion count across cultivars of asparagus spears when tested by light, isolate, and wounding in experiment two.

Covariance Parameter	Estimate	SE		
date	0.08785	0.1037		
date*box*light	0.01855	0.03494		
residual	1.8328	0.1137		
Effect	Num DF	Den DF	F-Value	Pr > F
cultivar	3	521	15.11	<.0001
wound	1	521	745.55	<.0001

cultivar*wound	3	521	4.97	0.0021
isolate	1	521	86.53	<.0001
cultivar*isolate	3	521	0.99	0.3990
wound*isolate	1	521	0.02	0.9006
cultivar*wound*isolate	3	521	2.78	0.0404
light	1	5	49.12	0.0009
light*cultivar	3	521	1.59	0.1908
light*wound	1	521	4.63	0.0318
light*cultivar*wound	3	521	1.88	0.1321
light*isolate	1	521	1.85	0.1749
light*cultivar*isolate	3	521	0.23	0.8756
light*wound*isolate	1	521	12.39	0.0005
light*cultivar*wound*isolate	3	521	0.18	0.9077
location(wound)	1	521	50.66	<.0001
cultivar*location(wound)	3	521	2.11	0.0976
location*isolate(wound)	1	521	0.00	0.9742
cultivar*location*isolate(wound)	3	521	0.70	0.5523
light*location(wound)	1	521	1.39	0.2383
light*cultivar*location(wound)	3	521	0.97	0.4068
light*location*isolate(wound)	1	521	0.57	0.4488

light*cultivar*location*isolate(wound)	3	521	0.14	0.9373

Appendix Table 9: Estimates for lesion count across cultivars of asparagus spears when tested by light and wounding for NA61 in experiment two.

Covariance Parameter	Estimate	SE		
date	0.1270	0.1449		
date*box*light	0	.		
residual	1.5466	0.1344		
Effect	Num DF	Den DF	F-Value	Pr > F
cultivar	3	260	5.05	0.0020
wound	1	260	443.63	<.0001
cultivar*wound	3	260	2.03	0.1101
light	1	5	43.76	0.0012
light*cultivar	3	260	0.47	0.7007
light*wound	1	260	1.11	0.2932
light*cultivar*wound	3	260	0.62	0.6036
location(wound)	1	260	30.83	<.0001
cultivar*location(wound)	3	260	1.25	0.2927
light*location(wound)	1	260	2.23	0.1365
light*cultivar*location(wound)	3	260	0.85	0.4695

Appendix Table 10: Estimates for lesion count across cultivars of asparagus spears when tested by wounding in experiment three.

Covariance Parameter	Estimate	SE		
date	0.1142	0.1692		
residual	1.5413	0.09186		
Effect	Num DF	Den DF	F-Value	Pr > F
cultivar	3	563	14.56	<.0001
wound	1	563	109.02	<.0001
cultivar*wound	3	563	2.11	0.0975
location(wound)	1	563	20.38	<.0001
cultivar*location(wound)	3	563	5.74	0.0007

Appendix Table 11: Estimates for lesion count across cultivars of asparagus spears when tested by effect of wounding on non-wounded sections of spears in experiment three.

Covariance Parameter	Estimate	SE		
date	0.1623	0.2346		
residual	0.6159	0.04752		
Effect	Num DF	Den DF	F-Value	Pr > F
cultivar	3	336	14.75	<.0001
wounds	2	336	1.42	0.2420
wounds*cultivar	6	336	1.19	0.3119

Appendix Table 12: Estimates for lesion count across cultivars of asparagus spears when tested by wounding on small spears in experiment four.

Covariance Parameter	Estimate	SE

date	0.07521	0.1202		
residual	2.1542	0.1457		
Effect	Num DF	Den DF	F-Value	Pr > F
cultivar	3	437	17.60	<.0001
wound	1	437	5.62	0.0182
cultivar*wound	3	437	1.70	0.1664

Appendix Table 13: Estimates for lesion count across cultivars of asparagus spears when tested by location on the spear and wax intact in experiment five.

Covariance Parameter	Estimate	SE		
date	0.01754	.		
residual	0.6122	0.05370		
Effect	Num DF	Den DF	F-Value	Pr > F
cultivar	3	260	41.24	<.0001
location	1	260	138.35	<.0001
cultivar*location	3	260	5.56	0.0010
light(date)	2	260	68.61	<.0001
light*cultivar(date)	6	260	4.11	0.0006
light*location(date)	2	260	1.02	0.3634
light*cultivar*location(date)	6	260	1.43	0.2039

Appendix Table 14: Estimates for lesion count across cultivars of asparagus spears when tested by location on the spear and ASM and lighting treatments in experiment six.

Covariance Parameter	Estimate	SE
block	0.6582	0.9457

block*light	0.003300	0.01391		
block*chemical	0.01096	0.02474		
residual	0.7694	0.05135		

Effect	Num DF	Den DF	F-Value	Pr > F
cultivar	3	449	46.44	<.0001
location	1	449	9.76	0.0019
cultivar*location	3	449	4.41	0.0046
light	1	1	77.66	0.0719
light*cultivar	3	449	3.19	0.0235
light*location	1	449	2.24	0.1348
light*cultivar*location	3	449	3.59	0.0137
ASM	1	1	12.41	0.1761
cultivar*ASM	3	449	1.64	0.1799
ASM*location	1	449	0.18	0.6697
cultivar*ASM*location	3	449	0.54	0.653
light*ASM	1	449	2.23	0.136
light*cultivar*ASM	3	449	1.94	0.1228
light*ASM*location	1	449	3.89	0.0493
light*cultivar*ASM*location	3	449	1.14	0.3324

Appendix Table 15: Estimates for stomatal density of asparagus spears by cultivar and location

Covariance Parameter	Estimate	SE
residual	114.48	12.6041

Effect	Num DF	Den DF	F-Value	Pr > F
cultivar	3	165	2.06	0.1071
location	1	165	630.66	<.0001
cultivar*location	3	165	1.37	0.2540

Appendix Table 16: Estimates for germination rate across cultivars of asparagus spears when tested by wounding and *S. vesicarium* isolate

Covariance Parameter	Estimate	SE		
residual	202.31	67.4352		
Effect	Num DF	Den DF	F-Value	Pr > F
isolate	1	18	70.81	<.0001
cultivar	3	18	0.78	0.5189
cultivar*isolate	3	18	0.65	0.5922
wound	1	18	34.41	<.0001
isolate*wound	1	18	13.20	0.0019
cultivar*wound	3	18	0.97	0.4276
cultivar*isolate*wound	3	18	1.44	0.2643
location(wound)	1	18	0.07	0.7927
isolate*location(wound)	1	18	0.00	1.0000
cultivar*location(wound)	3	18	0.11	0.9504
cultivar*isolate*location(wound)	3	18	0.32	0.8106

Appendix Table 17: Estimates for germination rate of conidia on asparagus spears when tested by lighting, wounding, and *S. vesicarium* isolate

Covariance Parameter	Estimate	SE		
residual	158.37	38.4100		
Effect	Num DF	Den DF	F-Value	Pr > F
isolate	1	34	100.19	<.0001
light	1	34	0.62	0.4376
light*isolate	1	34	3.27	0.0796
wound	1	34	43.48	<.0001
isolate*wound	1	34	15.15	0.0004
light*wound	1	34	0.08	0.7767
light*isolate*wound	1	34	0.05	0.8266

Appendix Table 18: Estimates for germ tube length across cultivars of asparagus spears when tested by wounding and *S. vesicarium* isolate

Covariance Parameter	Estimate	SE		
residual	8932.77	546.68		
Effect	Num DF	Den DF	F-Value	Pr > F
isolate	1	534	0.24	0.6220
cultivar	3	534	0.16	0.9259
cultivar*isolate	3	534	0.29	0.8319
wound	1	534	2.76	0.0970
wound*isolate	1	534	1.80	0.1803
wound*cultivar	3	534	2.07	0.1028
wound*cultivar*isolate	3	534	0.20	0.8947