

Factors involved in virulence variation of *Sclerotinia minor* and formulation improvement

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Abstract

Dandelion (*Taraxacum officinale*) is a widely distributed weed in turfgrass in North America. Standard chemical herbicides have been banned from use in urban environments in most regions in Canada since 2007 which hard-pressed the need to discover environmentally friendly methods for weed management. Sarritor[®] is a fungus-based (*Sclerotinia minor*) bioherbicide targeting dandelion and other broadleaf weeds in turfgrass. *Sclerotinia minor* is a soil-borne fungus that invades and lyses plant tissues and produces sclerotia as a resting, survival, reproductive structure. Sclerotia formation rarely occurred when the Sarritor[®] bioherbicide was used to control weeds on turfgrass. However, *S. minor* is known to be a serious pathogen of lettuce, peanut, and sunflower crops. Disease in lettuce and other crops starts below the soil surface by infecting the roots and root crowns and the fungus proceeds to decay entire lettuce plants and release numerous sclerotia back into the farmers' soil. In order to explore the factors involved in sclerotia development, different quantities of substrate biomass and infection position were compared on dandelion and lettuce plants. Results indicate that sclerotia development was related to substrate biomass, infection site and the plant species. Not all broadleaf species are equally susceptible to the *S. minor* bioherbicide. Dandelions are susceptible, plantains less susceptible and turf grasses, fortunately, are not harmed by *S. minor*. Oxalic acid (OA) is a key infection factor for *S. minor* and oxalic oxidase (OxO) is known to catalyze oxalate to hydrogen peroxide and carbon dioxide. Studies have shown increasing OxO activities in susceptible plants led to the reduction in the level of disease caused by *S. minor* and related pathogens. Dandelion, broadleaf plantain, narrowleaf

plantain and Kentucky bluegrass were assayed for OxO to examine the relationship between OxO activities in the host plants and susceptibility to *S. minor*. Dandelion (susceptible) had the highest level of OxO activity followed by the two plantains, while Kentucky bluegrass (resistant) had the lowest OxO activity. The standard Sarritor[®] formulation was ground barley grits (1.4 mm-2.0 mm) colonized by *S. minor*. Barley is an easily acquired substrate, but the grinding process is costly and time-consuming for laboratory production of bioherbicide product. Several alternatives to barley: millet, couscous and rice were studied as alternative substrates for the production of *S. minor* bioherbicide. Viability and virulence of these granular formulations were evaluated on agar media, on detached leaves and in field test plots. Millet was determined to be the ideal substitute among the tested substrates to replace barley. The millet formulation was as efficacious as barley formulation and much easier to produce.

Résumé

Le pissenlit officinal (*Taraxacum officinale*) est une mauvaise herbe populaire qui a colonisé en Amérique du Nord. Tous les correspondant herbicides chimiques sont bannis depuis 2007 pour leur utilisation sur la pelouse dans le but d'encourager l'utilisation des techniques plus écologiques pour la gestion des mauvaises herbes. Sarritor[®] est un bioherbicide développé à partir d'un champignon *Sclerotinia minor* dans le but de détruire certaines plantes comme le pissenlit et les autres mauvaises herbes à feuilles larges. *Sclerotinia minor* est un champignon qui s'attaque et lyse les tissus des hôtes et qui produit des sclérotés comme structures reproducteur et de survie. Les sclérotés se produisent rarement sur le système gazon, mais arrivent sur la laitue, l'arachide, le tournesol, etc. Afin d'explorer les facteurs impliqués dans le développement de sclérotés, différentes biomasses et inoculation d'endroits ont été testés sur le pissenlit et la laitue. Les résultats indiquent que le développement des sclérotés est lié à la biomasse de *S. minor*, l'emplacement et les espèces végétales. *S. minor* mineures variations de virulence sur les mauvaises herbes à feuilles larges. L'acide oxalique (OA) est le facteur clé de l'infection sécrété par *S. minor*. L'oxalate oxydase (OxO), une enzyme agit comme bouclier en catalysant l'acide oxalique. Des études démontrent que l'augmentation de l'activité de l'oxalate oxydase chez les plantes sensibles augmente leur résistance contre *S. minor*. Les expériences qui impliquent OxO ont été conduites sur les plantes suivantes: le pissenlit officinal, le plantain majeur, le plantain à feuilles lancéolées et les herbes communes. Le pissenlit (sensible) renferme la plus haute activité d'oxalate oxydase suivi par les plantains. Les herbes communes (résistant) montrent le niveau le moins élevé de

l'activité de cet enzyme. Le substrat norme pour Sarritor[®] est 1.4 mm-2.0 mm gruau d'orge infecté par *S. minor*. Malgré que l'orge est un ingrédient facile à acquérir, le processus de fabrication du gruau avec cette céréale est assez cher et longue. Les alternatives d'orge: le millet, le couscous et le riz, ont été examinés comme les substrats alternatifs pour produire du bioherbicides de *S. minor*. En plus, ils ont été utilisés comme formulation de *S. minor* sur la gélose, les feuilles détachées et sur le champ. Le millet se retrouve à être le substitut idéal parmi les trois grains étudiés. Le millet a été aussi efficace que la formulation d'orge et beaucoup plus facile à produire.

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List of Acronyms

ANOVA	analysis of variance
AW	water activity
BIC	Bayesian Information Criterion
cAMP	cyclic adenosine monophosphate
CWDE	cell wall degrading enzyme
DR	damage rate
DW	dew point
GLMM	generalized linear mixed model
HR	hypersensitive response
LP	lipid peroxidation
MAPK	mitogen-activated protein kinase
MRS	modified Richard's solution
OA	oxalic acid
OxO	oxalic oxidase

PCD	programmed cell death
PDA	Potato dextrose agar
PKA	protein kinase
RCBD	randomized complete block design
RCD	randomized complete design
RH	relative humidity
ROS	reactive oxygen species
SOD	superoxide dismutase

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Chapter 1

Introduction

Sclerotinia minor Jagger is a soil borne necrotrophic fungus characterized by irregular black resting structures. It is plant pathogen with a broad host range. In recent years, it has been studied as a biocontrol agent targeting dandelion and other broadleaf weeds in turfgrass. Biological weed control becomes promising since chemical herbicide have been banned on turfgrass.

Weed biocontrol represents using living organism-derived products covered with favorable formulation to suppress the biomass of target weeds (Bailey, 2010, 2014). Therefore, *S. minor* (the biocontrol agent), target plants and coverage formulation of *S. minor* are respectively studied in order to improve the weed control progress of *S. minor*.

Sclerotinia minor is a soil borne fungus that produces sclerotia as resting structure to defend stress or unfavorable environmental conditions (Willetts and Bullock, 1992). Sclerotia rarely

occur when applied on turfgrass. The potential reason for this phenomenon is that the fungus has not enough plant biomass to utilize before it detects the stress. Rarely has research studied on *S. minor* pathogenicity and sclerotia development so that the reason why sclerotia rarely occur on turfgrass system is unclear.

Dandelion (*Taraxacum officinale*), broadleaf plantain (*Plantago major*) and narrowleaf plantain (*Plantago lanceolata*) can be suppressed by *S. minor*. Although *S. minor* shows good command of dandelion, it does not easily infect the other two weeds. *Sclerotinia spp.* secrete oxalic acid (OA) in the process of infection and sclerotia initiation and is shown to be related to infection and pathogenicity of *S. minor*. Oxalic oxidase (OxO), an OA-catalyzed enzyme, was found in wheat and transferred into several plants successfully to resist *S. minor* and its relative *Sclerotinia sclerotiorum* (Dong et al., 2008; Livingstone et al., 2005; Papapostolou and Georgiou, 2010). OxO can react with OA and produce hydrogen peroxide (H₂O₂) and induce the hypersensitive reactions in plant. Therefore, OxO level in plant seems to be another hint to test resistance ability to *S. minor*.

Barley is currently been used formulation of *S. minor*. However, to make the 1.4 mm-2.0 mm barley grits, barley needed to be ground and sorted through metal screens, a time-consuming procedure. A superior formulation would be desirable. Several grains have previously been used as bioherbicide formulations (Auld et al., 2003). Preferred formulations are presumed to be

economic, cheap, applicable and easy to acquire. Storage period is also an important factor for formulations of bioherbicides. In general, economic, applicable and storage-benefit formulations need to be developed.

Chapter 2

Objectives and hypotheses

Objectives

- 1) To determine if sclerotia production is related to the initial soil location and biomass of *S. minor* bioherbicide substrate as well as infected plant species;
- 2) To determine if the oxalic oxidase content is related to the susceptibility variation among dandelion, broadleaf plantain, narrowleaf plantain and Kentucky bluegrass.
- 3) To find a new ideal formula to replace barley grits for *S. minor* bioherbicide substrate.

Hypotheses

- 1) Sclerotia formation is related to initial soil location and biomass of *S. minor* substrate and infected plant species.
- 2) Kentucky bluegrass, the non-susceptible plant, possesses superior resistance to infection followed by broadleaf plantain, narrowleaf plantain and dandelion. Dandelion would have the lowest

oxalic oxidase activity among the four plants.

3) At least one of the tested grains is better than barley grits as the *S. minor* bioherbicide formulation substrate.

Chapter 3

Literature Review

3.1 *Sclerotinia minor* as a crop plant pathogen and bioherbicide

3.1.1 General introduction

Sclerotinia minor is a soil borne necrotrophic fungus characterized by small (0.5–2.0 mm), irregular black firm sclerotia, a survival structure that germinates by eruptive growth of mycelium that invades and colonizes susceptible plant tissues (Dillard and Grogan, 1985; Willetts and Wong, 1980). *S. minor* infects plant tissues rapidly via upper roots, crowns and other parts touching the soil in cool and humid conditions. White mycelia grow on plant tissues causing wilt, collapse, and formation of small black sclerotia on the lysed plant tissue.

Sclerotinia minor could invade susceptible plants at any growth stage in applicable field conditions by plant-to-plant contact (Subbarao, 1998). After several days' development, white fluffy

mycelia aggregate to resting structures and melanize to small mature black sclerotia. Sclerotia can persist in the soil or germinate, releasing hyphae that are able to spread in favorable environments starting a new disease cycle. Apothecia and ascospores are rarely found in the natural environment and are insignificant for dispersal and persistence (Lumsden, 1979). Research in California found that no ascospores appeared in the field on lettuce (*Lactuca sativa* L.) (Hao and Subbarao, 2005), although hyphae are the effective agent for spread and then aggregate as sclerotia in the soil (Adams, 1987; Subbarao, 1998).

Sclerotinia minor sclerotia, the resting structure, can survive four to five years in the soil under natural conditions, but mycelia can only survive for several days without a host (Adams and Ayers, 1979). In another study, sclerotia displayed rapid decline and most sclerotia could not survive into the next year (Alexander and Stewart, 1994). These contrasting reports suggest different factors may influence sclerotia survival.

Survival and generation of *S. minor* sclerotia are interrelated with temperature, soil moisture, soil position, nutrition, pH and natural enemy activities. The optimum temperature for mycelia growth is between 22°C to 25°C while mycelia can survive from 0°C to 30°C. Sclerotia formation is favored from 12°C to 24°C. It has been reported that sclerotia were formed at -1 to -43 bars on cornmeal agar, but sclerotia survive better in dry soil (-1.5 MPa) than in moist soil (-0.033 MPa) since eruptive germination occurs mostly in moist soil (-1/3 bars) (Imolehin et al., 1980).

Sclerotia position in the soil also influences the persistence and survival of sclerotia (Imolehin et al., 1980). Only when placed within a 2 cm range of a taproot or less than 8 cm below the soil can sclerotia be virulent. Evidence shows that nutrition also influences the growth condition of the fungus. Nutritional requirements of *S. minor* are similar to those for *S. sclerotiorum* and *S. trifoliorum* with favorable carbon sources including glucose, galactose, lactose and potassium nitrate as nitrogen sources. *S. minor* grows over a broad pH range, both on acidic and alkaline media with the optimum range of 6.0-6.6. Anti-fungi microorganisms also influence the survival of sclerotia (Willettts and Wong, 1980). A total of 46 fungi, 2 insects, 1 mite and 1 snail are known to impede *S. minor* (Adams, 1989; Adams and Ayers, 1979; Coley-Smith and Cooke, 1971). Some of these natural enemy were reported as the main attributed components for a suppressive soil and some have been developed as registered biofungicides for the control of *S. minor* (Jones and Stewart, 1997; Ridgway et al., 2001).

Sclerotinia minor has been reported to infect plant hosts in 21 families, 66 genera and 94 species from class Angiospermae mainly through eruptive and myceliogenic germination of sclerotia (Melzer et al., 1997). Although the range of host is not as wide as *S. sclerotiorum*, *S. minor* is a cosmopolitan pathogen reported in North America, South America, Asia, Europe, Africa and Australia (Melzer et al., 1997). In North America, it has been reported in Quebec (Chivers, 1929), Ontario (Melzer and Boland, 1994), British Colombia (Toms, 1964) (Toms, 1964), Alberta (Kohn, 1976), New York (Chivers, 1929; Jagger, 1920), Washington (Gardner, 1973), Texas

(Woodard and Newman, 1993) and California (Patterson and Grogan, 1985).

Sclerotinia minor does not result in destructive economic damage for most susceptible plant species but it has been reported that *S. minor* causes several prevalent diseases and economic loss on lettuce in North America (Willetts and Wong, 1980). In Canada, *S. minor* was first reported on lettuce in 1984, causing over 75% loss in Ontario (Jarvis, 1985). Diseases caused by *S. minor* have also been reported in Quebec (Reeleder and Charbonneau, 1987).

The grass family (Poaceae) is not susceptible to *S. minor*. On the other hand, *S. minor* can infect several main broadleaf weed species on turfgrass system, including common dandelion (*Taraxacum officinale* Wiggers) and prickly lettuce (*Lactuca serriola* L.) in the Asteraceae family, broadleaf plantain (*Plantago major* L.) and narrow-leaved plantain (*Plantago lanceolata* L.) in Plantaginaceae family and white clover (*Trifolium repens* L.) and black medic (*Medicago lupulina* L.) in the Fabaceae family.

3.1.2 *Sclerotinia minor* as a biocontrol agent

Sclerotinia spp. have attracted the attention of researchers for their potential ability to control a number of broadleaf weeds. *S. sclerotiorum* was the first one tested as a biological control agent to control spotted knapweed (*Centaurea maculosa*) and Canada thistle (*Cirsium arvense*) with good results (Bourdôt et al., 1995; Brosten and Sands, 1986). However, the potential risk of ascospore

development and aerial dispersal restricted future development as a biocontrol agent (Bourdôt et al., 2001). Alternately, tests with *S. minor* isolate (IMI 344141), isolated from a lettuce field in Sherrington, Quebec in 1983, performed better than *S. sclerotiorum* and *S. trifoliorum* on common dandelion. Continued research on this isolate fashioned a turfgrass broadleaf weed bioherbicide named Sarritor[®], with *S. minor* (IMI 344141) as the active ingredient. Sarritor[®] works best at 15-24°C with high relative humidity (RH). Studies displayed that exposed broadleaf weeds were killed within 7 days, twice faster than the chemical herbicide, KillexTM. Statistically there was no difference between the effect of 2,4-D and *S. minor* on dandelion control (Abu-Dieyeh and Watson, 2007c; Schnick et al., 2002). Besides, *S. minor* shows similar virulence on different dandelion genotypes (Abu-Dieyeh, 2006).

As a bioherbicide in lawn and turfgrass systems, *S. minor* targets broadleaf weeds. Broadleaf weeds interfere with the aesthetic standard of turfgrass system by damaging the quality and quantity of turfgrass and cause visual uniformity disruption due to difference of leaf shapes and widths. Dandelion and broadleaf plantains are widely distributed broadleaf weeds in Canada and are the main targets of Sarritor[®]. Dandelion is a common perennial invasive weed that was introduced from Eurasia and is now widely distributed across Canada (Stewart-Wade et al., 2002). The wide distribution of dandelion is due to its strong reproductive and dispersal abilities (Abu-Dieyeh and Watson, 2007b). Dandelions develop taproots, which are long enough to reach below the level of competing grass roots (Loomis, 1938). At the same time, dandelion has strong regeneration abil-

ity to generate new roots and shoots in very short time after being segmented. These morphological traits instill a competitive advantage over turfgrass and make it difficult to manually remove or mow the plant. Sarritor[®] provides good control of dandelion with no significant variation among different biotypes (Abu-Dieyeh and Watson, 2007a). Plantains are additional broadleaf targets. Broadleaf plantain is a perennial herb characterized by broad egg-shaped leaves with several main veins. Narrowleaf plantain, also a perennial, has lanceolate leaves with three to five strong parallel veins. Both species are less susceptible than dandelion to *S. minor* infection, especially narrowleaf plantain. However, the reasons for these differences in susceptibility are not clear.

Sarritor[®] does not infect turfgrass and has been demonstrated through human health and environmental toxicology that it is not pathogenic or toxic to non-target organism (Health Canada, 2010). Besides, tests on 250 field plots have shown that product could settle down on the turf but the active ingredient, *S. minor*, neither moves off the application site nor result in disease on nearby susceptible plants. Sclerotia rarely occur in the turfgrass system although the reason for the absence is not known.

3.2 Factors involved in *Sclerotinia minor* sclerotia formation

Sclerotinia minor forms sclerotia on lettuce, peanut (*Arachis hypogaea* L.) and sunflower (*Helianthus annuus* L.) (Phipps and Porter, 1982; Porter and Beute, 1974; Sedun and Brown, 1989)

while this situation rarely happened on broadleaf weeds with the *S. minor* bioherbicide in turf-grass. These phenomena have not been clearly explained in previous studies but the involving factors have been investigated on *S. minor* and other *Sclerotinia* species.

In general, sclerotia are estimated to grow when mycelia are cut, torn or grow against the sides of the culture vessels, or come in contact with a variety of chemical barriers (Willettts and Bullock, 1992). Various endogenous and exogenous factors have been reported to affect sclerotia growth and development. Exogenous factors that affect sclerotia formation include environmental factors (temperature, RH and light) and nutrition factor. Sclerotia form only within 12°C to 24°C and sclerotia grow most at 12°C with -1 to -43 bar solute potential (Imolehin et al., 1980)). Although *S. minor* grows better in dark, light is believed to promote sclerotial formation, not only because of forming several reaction oxygen species (ROS) but also link with melanin formation of sclerotia, an important process of sclerotia formation. Nutrition sources including carbon and nitrogen supply also affect sclerotia formation (Chet and Henis, 1975). Few references are reported about nutrition sources for *S. minor*. However, its relative, *Sclerotinia rolfsii*, has been reported not forming sclerotia without glucose and thiamine and few occurrences of sclerotia without NO₃, K and PO₄ (Wheeler and Sharan, 1965). Besides, sucrose, maltose, lactose, D-mannose, D-glucose, and D-fructose are all good supporters of sclerotia formation in *S. sclerotiorum* (Wang and le Tourneau, 1971).

The endogenous factors that affect sclerotial development could be explained by a ROS-induced sclerotial metamorphosis theory. This theory believes that sclerotial differentiation and development are induced when cells are in hyperoxidant state (overwhelming oxidative stress) induced by ROS. Factors that could increase or decrease oxidative stress directly or indirectly affect the sclerotial differentiation (Georgiou et al., 2006). Studies on two antioxidants in fungi, ascorbic acid and beta-carotene proved this theory. Evidence shows that exogenous ascorbic acid and beta-carotene can cause concentration-dependent reductions on sclerotial development on *S. minor* and *S. sclerotiorum* (Georgiou and Petropoulou, 2002; Georgiou et al., 2001). More directly, increasing superoxide radicals (Papapostolou and Georgiou, 2010) will raise the oxidative stress benefiting sclerotia formation. Ions often bind with certain proteins that have influence on superoxidation. Iron is proved to promote sclerotial development since it prompts lipid peroxidation (LP) and anticipates the formation of ROS. Other metal ions are often interacting with superoxide dismutase (SOD) as scavengers of ROS. Copper is another ion that was found to affect sclerotial formation in the early time. It has been reported playing a role in certain protein that work as a repressor in sclerotia formation (Chet and Henis, 1968). Later study shows that copper, work with zinc ion, helps to reduce sclerotial formation on *S. sclerotiorum* since they interact with protein as an CuZnSODs-similar protein for clearing superoxidation (Veluchamy et al., 2012).

OA and pH have been considered the two most important factors involved in sclerotia formation. Whereas, an OA-mutant of *S. sclerotiorum* shows no ability to produce sclerotia even growing in a

lower pH environment (Rollins and Dickman, 1998). This indicates a more sophisticated pathway exists. With more involved endogenous factors being found, a series of studies indicate that a MAPK (mitogen-activated protein kinase) pathway of negatively mediated by cAMP (cyclic adenosine monophosphate) and Rap-1 protein are related to sclerotia development (Chen and Dickman, 2005; Chen et al., 2004; Harel et al., 2005; Jurick Ii et al., 2004; Rollins and Dickman, 1998, 2001). The MAPK gene is only expressed under an acidic environment showing that pH is also a regulating factor in this pathway. Although there is no clear evidence showing this pathway is related to the ROS-induced sclerotial metamorphosis theory, one study shows that cAMP is increased when *S. sclerotiorum* growing with the presence of caffeine (an antioxidant) (Rollins and Dickman, 1998). Until now, the entire signaling pathway of sclerotia development is not fully understood. New factors related to pathogenicity and sclerotia development have recently been reported including proteins, transcripts that may help to explain the pathway, or a new related pathway (Duan et al., 2013; Fan et al., 2016; Yu et al., 2012, 2016).

3.3 Factors involved in susceptibility variations of turfgrass species to

Sclerotinia minor

Little is known on potential factors that affect *S. minor* virulence on different host plants. Oxalic acid (OA) has been studied as an important character in pathogenicity of *S. minor*. OA is secreted during infection of plant tissues by pathogens of Sclerotiniaceae including *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, *Sclerotium cepivorum* and pathogens from other families, such

as *Poria placenta* (Ritschkoff et al., 1995), *Septoria musiva* (Liang et al., 2001), *Endothia parasitica* (Bennett and Hindal, 1989; Havir and Anagnostakis, 1985), and *Penicillium oxalicum* (Ikotun, 1984).

OA is secreted via hyphae of the pathogen and helps to degrade cell walls through cell-wall-degraded enzyme (CWDEs) acidification and as a chelator to bond calcium divalent cations, which deregulates the cell walls of host plants (Guimaraes and Stotz, 2004). Evidence shows a pH-dependency way for virulence and infection of *S. sclerotiorum* (Xu et al., 2015). By secreting OA, pathogens are able to create an acid environment for infection.

Another pathogenic strategy involves the enhancement of the suppressive ability on resistance reactions of host plants. OA can directly depress oxidative burst (Cessna et al., 2000), which is the first step of plant defense to decrease the infection of pathogens and induce damage to plant cells. Another result shows that OA is related with detoxifying the older hyphae from high calcium concentration at the site of infection in later stages (Heller and Witt-Geiges, 2013). These results indicate that oxalic acid concentrations in the early stage of infection stay below the toxic level.

Oxalic oxidase (OxO), a protein secreted by the plant, was purified from barley (*Hordeum vulgare*) seedlings and is also known as a homopentameric and glyco-protein germin from wheat

(Lane et al., 1993, 1992; Sugiura et al., 1979). OxO is able to catalyze OA to hydrogen peroxide (H_2O_2) and carbon dioxide (CO_2), promoting defensive ability of host plants (Chiriboga, 1966; Lane et al., 1993). This reaction is crucial for plant defense since H_2O_2 is largely produced. H_2O_2 is a key factor related with oxidative burst and hypersensitive response (HR) for plant defense. Researchers also found that OxO activity increased when facing biotic and abiotic stress on wheat and was induced to defend against the infection of barley (*Hordeum vulgare*) by *Erysiphe graminis* (Dumas et al., 1995). Moreover, a OxO-related gene has been transferred into other susceptible plants and OxO was expressed successfully to resist the pathogen. By transferring OxO genes, resistance to *S. minor* was enhanced on peanut (Livingstone et al., 2005) and resistance to *S. sclerotium* was also increased on soybean (*Glycine max*), sunflower (*Helianthus annuus*), oilseed rape (*Brassica napus*) and tobacco (*Nicotiana tabacum*) (Berna and Bernier, 1997; Donaldson et al., 2001; Dong et al., 2008; Hu et al., 2003). OxO activity level is thought to be a metabolic host plant signal of stress and it has been regarded as a pathogen-inducible enzyme. OxO affects pathogenicity mainly in two ways: OxO degrades OA levels by producing H_2O_2 in order to induce HR in the host plant and by cross-linking of plant cell walls; and secondly, OxO could increase the pH value to moderate the infection process.

Recent studies also show that the infection may also relate to small proteins secreted by the fungi (Lyu et al., 2016; Yu et al., 2012; Zhu et al., 2013). These proteins interact with host plant cells, disturb normal plant processes and lead to cell death. Host plants are also weakened by

this interaction and the relocation ability of the protein. This series of interactions would help hinder biotrophic fungi to survive on living cells. However, *S. sclerotiorum* (and *S. minor*) would benefit from this interaction due to its necrotrophic mode. Another recent reported protein, *Ss-Rh1*, is necessary for early infection and may increase the adhesions with host cells and promote appressorium formation (Yu et al., 2016).

3.4 Selection of a bioherbicide formulation

Bioherbicide formulation is to improve an array of traits of the biocontrol agents and make the agents fitted for commercial use. Thus, the function of bioherbicide's formulation is similar to the formulation of a chemical herbicide (Boyette et al., 1991). To be specific, this requires the formulation being compatible to biological agents, prolonging the storage period, enhancing the efficacy of biocontrol agents, reducing damages and forming the physical appearance for easily applying in the field (Bothast et al., 1993; Greaves and MacQueen, 1990; Leggett and Gleddie, 1995). The ideal formulation of any herbicides has long shelf-life, relative ease of application, efficacy and low cost (Auld et al., 2003). Bioherbicide formulation selection has been considered as one of the main difficulties for developing bioherbicide because of the requirement for long storage time and rapid establishment of bio-agents (Fravel and Lewis, 1992). Therefore, most of studies on formulations of bioherbicide are focus on increasing storage period and reducing shelf period requirement (Green et al., 1998).

There are two main pathways for bioherbicide formulation: liquid formulation and solid particles. Both types of formulations have been used on biological weed control agents. This review will include formulations that have been used for research use and commercialized formulations.

The main types of liquid formulation include water and adjuvant. Water is often used to mixed with biocontrol agents during the early stage of study. However, it is inferior to other advanced formulations in terms of increasing dew period and improving the efficacy of biocontrol agents. Adjuvants are used for enhancing or modifying the traits of an active ingredient in order to reduce adverse factors (Green et al., 1998). Tween[®] and oil emulsion are two simple adjuvant that have been examined for a series of bioherbicides including *Colletotrichum truncatum*, *Xanthium spinosum* and *Exserohilum monoceras* (Auld, 1993; Boyette, 1994; Zhang and Watson, 1997). The emulsions could spread on foliar surfaces, which facilitates the infection by biocontrol agents during the early stage (Auld et al., 2003). More complicated adjuvants have been developed as simple emulsions, but these did not last for long period in the field tests (Klein et al., 1995). Invert emulsion was developed to reduce the evaporation and dew dependence but this method is not economics due to the high demand of oil (Auld et al., 2003; Quimby et al., 1999). Auld (2002) found a water-in-oil-in-water (WOW) emulsion that significantly decreased the dew reduction. However, this method has not been applied in the bioherbicide industry.

Solid formulations are categorized by natural ingredient solid and synthetic solid as formulations.

Natural ingredient are mainly grains. Several common grains have been used as formulations directly including rice, barley, millet and wheat. The formulation of Sarritor[®] is 1.4 mm -2.0 mm grinded barley granules as media, which is easy to acquire and the size is great for quick germination of the biocontrol agent in the field. Another way to formulate is using different materials to create man-made “shields” for the fungus. The ingredient and shape of shields vary depending on different procedures. Walker and Connick Jr (1983) created a procedure using calcium alginate to cover fungi. Another way is that they developed a process that imitated pasta production, encapsulating *Alternaria cassiae*, *A. crassa*, *Colletotrichum truncatum*, and *Fusarium lateritium* with flour and kaolin clay and ground into granules respectively (Connick et al., 1991). Several other methods have also been used to make a capsule to protect the biocontrol organism. The shield or capsule has to own the traits to prolong the storage life of fungi and also has a good permeability for fungi to access targets easily.

Sarritor[®] is made by applying *S. minor* inoculation on 1.4 mm - 2.0 mm grinded barley granules. Although barley is easy to acquire and economic as media, natural barley diameter is too large to make formulations directly. To meet the requirement as inoculation size, grinding machine and metal screening have to be used to select granules in right size. This procedure delays the inoculation production and hinders the laboratory testing of the *S. minor* bioherbicide.

Chapter 4

Factors involved in sclerotic formation in *Sclerotinia minor*

4.1 Abstract

The reasons for sclerotia deficiency of *S. minor* when being applied on weeds as a bioherbicide in turfgrass are not clear and few studies investigate the potential reasons behind it. According to the different infection scenarios of *S. minor* in a cropping system versus turfgrass bioherbicide, fungal biomass, initial infection location and plant species (lettuce and dandelion) have been studied as potential factors involved in sclerotia formation. It is supposed that the substrate biomass, initial infection location of the fungus and plant species are three factors involved in sclerotia formation. The factorial experiment showed that the three factors are all related to sclerotia formation of *S. minor* and there are interactions among the three factors.

Keywords: Sclerotia formation, *Sclerotinia minor*, fungal biomass

4.2 Introduction

As mentioned in Chapter 3, numerous studies focus on the sclerotia development process of *S. sclerotiorum* while *S. minor* attracts little attention on this issue. Also, research studied on sclerotia development focus on the related gene and biochemical processes of the pathogen. However, no studies examine sclerotia formation from the perspective of an infection scenario.

In cropping systems, mycelia generate from sclerotia in the soil and invade lettuce roots and root crowns, causing plant collapse. Meanwhile the mycelia develop rapidly and the fungus forms numerous sclerotia in infected fields. Lettuce infected by *S. minor* below soil and lettuce head fallen into soil take sclerotia into soil. The fungus is then spread with tillage and other operation of farmers. In turfgrass where *S. minor* is used as bioherbicide, the fungus is applied to the broadleaf weeds only aboveground in a limited amount.

By comparing the pathogenicity in two systems, it is proposed that three factors may affect the sclerotia formation (Hao and Subbarao, 2005), the initiation location of fungus, the amount of substrate and the different susceptible plants.

4.3 Methodology and materials

4.3.1 Procedure of producing *Sclerotinia minor* inoculation

Barley (*Hordeum vulgare* L.) seeds were ground and sorted through metal screens to produce 1.4 mm to 2.0 mm barley grits. One hundred grams of the ground barley grits and fifty milliliter of water were combined in autoclaveable and breathable bags and autoclaved for 30 minutes at 103 kPa (kilopascals).

Sclerotinia minor (IMI 344141) was used in all of the experiments in this project. This isolate was collected from a diseased lettuce field in Sherrington, Quebec and the culture was stored as sclerotia at 4°C (Abu-Dieyeh and Watson, 2006). Sclerotia were disinfected by a modified method of Shaheen et al. (2010). Sclerotia were submerging in 70% ethanol for 30 seconds, 9% hypochlorite for three minutes and dried on sterilized filter paper. Between each step, sterilized deionized water was used to wash sclerotia twice. Dry sclerotia were placed on PDA plates in 9-cm petri dishes and incubated at 20°C ± 1°C in dark.

The *Sclerotinia minor* mycelia were used to produce the starter culture for inoculation of the grain substrates to formulate the bioherbicide following Abu-Dieyeh and Watson (2006): Five mm diameter mycelia discs were taken from the edge of five-day-old colonies and incubated in 100 ml MRS (Briere et al., 2000) in 250 ml Erlenmeyer flask on a rotary shaker at 100 rpm, 20°C

in dark for five days. The modified Richard's solution (MRS) recipe is as follows: 10g KNO₃, 10 g sucrose, 5 g KH₂PO₄, 2.5 g MgSO₄·7H₂O, and 0.02 g FeCl₃·6H₂O with 150 ml V-8 juice (Campbell Soup Company Inc.) per litre. Twenty-five milliliter flask cultures were homogenized and mixed with 100g of ground barley grits and 50 ml sterilized water in autoclavable bags. Bags were sealed before incubating at 20°C in the dark for four to five days. Bags were massaged every day in order to spread out fungus homogenously on grains. Formulas were then placed in a 0.5 mm mesh tray in sterile air cupboard and dried for twelve hours. The water activity was tested by HygroLab-3 water activity system (Rotronic AG, Bassersdorf, Switzerland)(accuracy ±0.03) at 23°C ± 2°C to maintain ca. 0.4 to 0.6 a_w (Abu-Dieyeh and Watson, 2006). Formulations were transferred to 17.7 cm×18.8 cm plastic zipper bags (Ziploc, Johnson and Son Ltee) and stored at -20°C fridge.

4.3.2 Plant growth

Dandelion were grown from seeds collected in the spring 2016 from fields on the Macdonald Campus of McGill University in St-Anne-de-Bellevue, Quebec. After drying for one day, seeds were stored at 4°C fridge. Stratification was used to dandelion before sowing in the 39 cm × 39 cm × 58 cm plastic pots to break the dormancy and synchronization: seeds were submerged in deionized water in 25 mL falcon tube. The tubes were stirred for four to five hours before depriving deionized water. Then seeds were placed in 4°C fridge for five days before sowing in soil. The plants were transferred into 85 cm × 85 cm × 95 cm plastic pots after the third leaf emerges. Soil contained one half gardening soil (Voila!®, S. Doubrias Inc.) and one half

ProMix soil (Promix BXt, Premier Horticulture Ltee). Plants were grown in a Conviron-E15 growth chamber (Controlled Environments Ltd., Winnipeg, MB, Canada) at 20°C with 12 light hours and 12 dark hours with photon flux density minimum of $350 \pm 50 \mu\text{mol m}^{-2} \text{s}^{-1}$. Plants were watered every two or three days according to the soil moisture.

Butterhead lettuce seeds were purchased from Stokes Ltd. Company. Seeds were pre-germinated in deionized water for four-eight hours before sowing in 85 cm × 85 cm × 95 cm plastic pots with soil [one half gardening soil (Voila!®, S. Doubrias Inc.) and one half ProMix soil (Promix BXt, Premier Horticulture Ltee)]. After seedlings emerged and the third leaf grew, plants in homogeneous growth conditions were kept in the growth chamber for three weeks. Potted dandelion and lettuce were placed into a Conviron-E15 growth chamber (Controlled Environments Ltd., Winnipeg, MB, Canada) at 20°C with 12 light hours and 12 dark hours with photon flux density minimum of $350 \pm 50 \mu\text{mol m}^{-2} \text{s}^{-1}$. Humidity was not controlled in the growth chamber. Plants were watered every two or three days depending on the growth condition.

4.3.3 Application of *Sclerotinia minor* on dandelion and lettuce seedlings

The experiment was a factorial design with three independent factors: 1) plant species, dandelion and lettuce, 2) bioherbicide biomass, 0.1 g/plant and 1 g/plant and 3) location of inoculation, on surface of soil, in soil or mixed of on surface and in soil (the total amount of substrate was twice as the other two groups). Each treatment had six replicates with a total number of 48 observations. Three-weeks-old lettuce seedlings and three-weeks-old dandelion were transferred to 85

cm × 85 cm × 95 cm pots. Hollow cube plastic models 40 mm × 40 mm × 35 mm were used to place around the aboveground plant for “on surface of soil” treatment and around the root for “in soil” treatment. For plants with inoculation both on the surface and in the soil, one model was placed aboveground and another was placed underground. Plants were placed in a Conviron-E15 growth chamber with same setting physical conditions mentioned above. Virulence tests were taken on barley inoculations before applying on plants: Six granules were randomly sampled and placed on PDA plates. They were kept in dark at 20°C for 48 hours. Diameters of colonies were measured. Inoculation was permitted to apply on plant if the colony diameter is over four centimeters 48 hours after incubating. Plants were watered every three days. Conditions including the germination of mycelia and plant health were recorded before treatment, 1 day, 3 days, 5 days and 7 days after treatment. The experiment was conducted twice.

4.3.4 Sclerotia isolation from soil

Soil from experimental pots was washed for five minutes with running tap water through two metal sieves, one metal sieve with 2.0 mm mesh and one sieve with 0.5 mm mesh. The soil in the bottom sieve was rinsed for two minutes to remove the suspended solids and washed again with running water for three minutes. Remaining soil was transferred into 200 ml conical tubes filled with 2.5M sucrose to 200 ml. Samples were centrifuged for 20 minutes at 2000 g at room temperature. The suspended part was transferred into a 50 mm sieve with 0.177 mesh and the pellet was discarded. The 50 mm sieve was emerged into water for five minutes for three times. The sieved material was transferred into a 9 cm petri dish and covered with water to release

sclerotia which were then counted under a dissecting microscope at $12 \times$ magnification.

4.3.5 Data analysis

SAS statistics package 9.4 was used to statistically analyze the collected data. Levene's test was used to test the homogeneity of variance. Three-way ANOVA were used to analyze the factors affecting sclerotia formation at $P=0.05$. The dependent variable is sclerotia number generated from each plant and in soil with three fixed effects, substrate biomass, fungus location and plant species. Tukey's test was used to compare the differences among groups at $P=0.05$.

4.4 Results

Mycelia emerged from the granules within 24h and plants were infected within 2 to 3 days. However, infection failures were discovered on several plants within the group of "in the soil" although the mycelia had contacted roots. Infected plants wilted after 3 to 5 days (Figure 4.2, 4.1).

Data from two replicates were pooled since the results were similar in each replicates. According to the data collected, three-way ANOVA revealed differences among different plant, different biomass and different locations. The interactions between two factors (plant $\times$ biomass, plant $\times$ location and biomass $\times$ locations) and interaction among three factors (plant $\times$ biomass $\times$ location) ($F_{2,132} = 6.42$, $P = 0.022$) were all significantly different. Since the interaction among three



Fig. 4.1 *Sclerotinia minor* application on dandelion on the soil.

factors is significantly different, main effects were not studied further independently. Therefore, it is concluded that the biomass, plant species and location are all factors in sclerotia initiations.

Table 4.1 Effect of biomass and infection location on the number of *Sclerotinia minor* sclerotia produced on dandelion.

Substract Biomass	Substract Location *	Mean Number of Sclerotia $\pm$ Std Error	Lower 95% CL	Upper 95% CL
0.1g	1	0.83 ± 0.32^d	0.17	1.50
	2	1.89 ± 0.44^c	0.97	2.81
	3	3.67 ± 0.53^b	2.55	4.79
1g	1	17.33 ± 1.19^a	14.82	19.85
	2	19.39 ± 1.85^a	15.52	23.26
	3	17.89 ± 1.33^a	15.08	20.70

* Location 1 represents applying *S. minor* inoculation on the surface, 2 represents applying *S. minor* inoculation in soil, 3 represents applying *S. minor* inoculation on the surface and in soil.

Table 4.2 Effect of biomass and infection location on the number of *Sclerotinia minor* sclerotia produced on lettuce.

Substract Biomass	Substract Location *	Mean Number of Sclerotia $\pm$ Std Error	Lower 95% CL	Upper 95% CL
0.1g	1	4.78 ± 0.60^c	3.52	6.04
	2	1.50 ± 0.39^e	0.68	2.32
	3	3.78 ± 0.46^d	2.80	4.76
1g	1	43.67 ± 3.04^a	37.25	50.09
	2	25.67 ± 2.45^b	20.50	30.84
	3	28.53 ± 1.99^b	24.33	32.73

* Location 1 represents applying *S. minor* inoculation on the surface, 2 represents applying *S. minor* inoculation in soil, 3 represents applying *S. minor* inoculation on the surface and in soil.



Fig. 4.2 *Sclerotinia minor* application to lettuce on the soil surface.

In general, sclerotia were formed more on lettuce than on dandelion (Table 4.1,4.2). However, sclerotia number generated from lettuce and dandelion have different trends responded to fungal biomass and location. When *S. minor* was applied on dandelion, sclerotia number increased with

the increase of substrate biomass, with almost no sclerotia formed in 0.1g *S. minor* application and around 20 sclerotia formed with 1g *S. minor* application (Table 4.1). There were no differences on sclerotia formation among the different inoculation locations. However, "in soil" groups generated slightly more sclerotia than the on the surface groups. This indicates that there was no interaction between fungal biomass and location on dandelion. When *S. minor* was applied on lettuce, sclerotia number also increased with increasing substrate biomass on both location groups (Table 4.2). However, the increase rates are different for two location groups. The sclerotia number of in soil groups grows from 3 to 22.5 while the on soil groups grow from 8 to over 40. The on soil groups grow more sclerotia than in soil group on lettuce for both 0.1g-biomass level and 1g-biomass level. This indicates the interaction between substrate biomass and location on lettuce.

4.5 Discussion

Results indicate that substrate biomass of *S. minor* is a factor that influences the number of sclerotia produced. However, it is not a simple linear relation, between substrate biomass and sclerotia number. Locations of fungal inoculum and plants are both involved in sclerotia production. Variation to sclerotia production on dandelion and lettuce has been noted (Abu-Dieyeh and Watson, 2006; Imolehin et al., 1980).

The effect of inoculum location on sclerotia production could be explained in several ways. The

first is the root system differences. In the experiment, dandelion and lettuce roots show susceptibility differences to *S. minor*. Dandelion plants with under soil application were less susceptible to *S. minor* than lettuce while plants received *S. minor* on the surface were similar susceptible. When dandelion was inoculated by *S. minor* substrate in soil, mycelia developed around the roots but no infections was observed 7 days after the inoculation (Figure 4.3).



Fig. 4.3 Mycelia of *Sclerotinia minor* on roots but not infecting the dandelion seedling 7 days after *S. minor* application.

Lettuce and dandelion show differences in sclerotia formation. Lettuce grows a large number of

sclerotia while dandelion rarely grows sclerotia in the field. In the experiment, sclerotia number increased as inoculum biomass increased on dandelion. The sclerotia number decreased on lettuce with biomass decrease.

An interesting phenomenon is that some of the sclerotia were produced on the surface of barley grits instead of on plant tissues. More sclerotia grew on the barley grits on 1g-biomass group than in 0.1g-biomass group. This could be explained by the relative deficiency of susceptible tissue and relative large amount of nutrition in barley grit.

Chapter 5

Factors involved in susceptibility variations of turfgrass species to *Sclerotinia minor*

5.1 Abstract

Turfgrass species display different susceptibilities to *S. minor*. Oxalic acid (OA) is known as a key factor involved in the pathogenicity of *Sclerotinia* species while oxalate oxidase (OxO) is an plant-generated enzyme to clear OA and defer fungal infection. It is supposed that OxO activity of different turfgrass plants affects the susceptibility. The OxO activities of three susceptible weeds, dandelion (*Taraxacum officinale*), broadleaf plantain (*Plantago major*), narrowleaf plantain (*Plantago lanceolata*) and a non-susceptible grass, Kentucky bluegrass (*Poa pratensis*) were studied. It is hypothesized that Kentucky bluegrass has the highest OxO activity followed by narrowleaf plantain, broadleaf plantain and dandelion. Conversely, the result shows that dandelion

had the highest OxO activity followed by broadleaf plantain and narrowleaf plantain, Kentucky bluegrass had very low OxO activity.

Keywords: Susceptibility, *Sclerotinia minor*, oxalic acid, oxalate oxidase, broadleaf weed

5.2 Introduction

When *Sclerotinia minor* is used as bioherbicides, it shows the susceptibility variation on different broadleaf weeds and it does not infect species of grass family (*Poaceae*). Specifically, *S. minor* shows high efficacy to infect dandelion while broadleaf plantain and narrowleaf plantain are less susceptible. OA is known to benefit fungal infection in many ways: 1) it activates several infection related enzyme, e.g. cell wall degrading enzymes (CWDEs); 2) it depresses the defense of the host plant; 3) it provides a lower pH environment where the fungus benefits for infection while plant is weakened. OxO could clear OA by transferring it into hydrogen peroxide and carbon dioxide. Evidence shows that transferring OxO-related gene into susceptible plant can increase the resistance to *S. sclerotiorum* on oilseed rape (Dong et al., 2008), sunflower (Hu et al., 2003), soybean (Donaldson et al., 2001) and the resistance to *S. minor* on peanut (Livingstone et al., 2005). This indicates the potential relationship between OxO activities in different plants and susceptibility to *S. minor*.

5.3 Methodology and materials

5.3.1 Plant growth

Seeds of dandelion, broadleaf plantain and narrowleaf plantain were collected from field on the Macdonald Campus in Saint Anne-de-Bellevue and stored at 4°C. Stratification was used to break the dormancy. Seeds were sown into soil [one half gardening soil (Voila!®, S. Doubrias Inc.) and one half ProMix soil (Promix BXt, Premier Horticulture Ltee)] for four weeks in a Conviron-E15 growth chamber at 20°C ± 1°C with 12 hours daytime and 12 hours in dark with photon flux density minimum of 350 ± 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Humidity was not controlled in the growth chamber.

Kentucky bluegrass seed was collected from the field potted soil and held in the Conviron-E15 growth chamber as indicated above for 2 to 3 days before conducting the experiment.

5.3.2 Colorimetric assay of OxO activity

A colorimetric assay method with a modified procedure was used to test the OxO activity (Sugiyama et al., 1979). The assay buffer was made before the experiment following the procedure of Livingstone et al. (2005). Developing solution was prepared as follows: 57 μL of a 140 mg/mL horseradish peroxidase solution was added into 100 mL 0.1M sodium phosphate buffer, pH 6.0. 6 mg aminoantipyrene was dissolved in 30 μL N,N-dimethylaniline and added in to the sodium

phosphate buffer.

Leaf discs (5 mm diameter) of dandelion, broadleaf plantain, narrowleaf plantain, and Kentucky bluegrass were placed in 1.5 ml centrifuge tubes. Four hundred μL assay buffer were added into the tubes and incubated at 37°C for 20 minutes. Two hundred seventy μL developing buffer were added into tubes and incubated for 30 minutes at room temperature. The absorbance was measured at 550nm wavelength using Ultrospec 4300pro spectrophotometry.

Six repeats were for each group plants and were conducted on three consecutive days (two repeats per day). The experiment was repeated three times.

5.3.3 Data analysis

The SAS statistics package 9.4 was used to statistically analyze collected data. The dependent variable was OxO activities measurement of leaf discs by spectrophotometry with plant species as the fixed effect. Time was considered as a random effect since it may affect the absorbance of the developing solution. Measured discs were nested within time. Levene's test was used to test the homogeneity of variance ($P=0.05$). Parametric mixed model was used to analysis the OxO activities in the four plants. Bonferroni's test was used to compare the difference of OxO activities among the four species with $P=0.05$.

5.4 Results

The plant OxO values were confirmed by a parametric mixed model (SAS PROC GLIMMIX with DIST = N). The model was mixed due to the heterogeneity of variance, which was modeled using RANDOM statements (RANDOM _RESIDUAL_ / GROUP = plant; RANDOM time / sub = plant TYPE = VC). The standard structure of the variance-covariance matrix and the normal distribution specification were selected based on the fit statistics (Bayesian Information Criterion). The mixed model indicated that the plant levels of OxO were not equal ($F_{3,64} = 55.67$, $p < .0001$) (Table 5.1). Contrast to the hypothesis, dandelion possessed the highest OxO activity with 0.1481 while Kentucky bluegrass had the lowest value, 0.0022. The values of two plantain were in the middle: OxO activity in broadleaf plantain was nearly 0.05; the OxO in narrowleaf plantain was approximately half of in broadleaf plantain, with 0.0224. The result indicated that,

Table 5.1 Oxalic oxidase values in selected turfgrass inhabiting plants.

Plant Species	Mean of colormetric assay A_{550} with Std Error *
Dandelion	0.1481 ± 0.0161^a
Broadleaf plantain	0.0488 ± 0.0056^b
Narrowleaf plantain	0.0224 ± 0.0039^c
Kentucky bluegrass	0.0022 ± 0.0010^d

* The OxO value is the estimated values with standard errors. Estimate values sharring the same letters are not significantly different by Bonferri's test ($P=0.05$).

in the tested plants, the susceptibility variation is not due to the different OxO levels in healthy plant.

5.5 Discussion

The OxO values recorded was contrast to the estimated hypothesis where the grass was predicted to have the highest OxO activity and dandelion was estimated to have the lowest value. However, the opposite ranking may indicate the role of H₂O₂. Back to the evidence of our hypothesis: Reports shows that transferred OxO-generated gene enhance the resistance to *Sclerotinia spp* on different susceptible species (Donaldson et al., 2001; Dong et al., 2008; Livingstone et al., 2005). The transferred OxO-generated gene increases the level of the OxO activity on these plants and result in decreasing the damage to these susceptible plants, that is, enhancement to *S. minor*. However, in our experiment, grass is not a susceptible plant to *S. minor* and no damage on grass happens. This indicates that there may have other physical or chemical barriers that cause the infection failure on grass species so that grass does not have to waste resources to secret OxO for defending the pathogen when it is healthy. Vice versa, dandelion needs a high level of OxO to defend *S. minor* in healthy issue since they are susceptible to *S. minor* and do not own the “barriers” that grass has. This could also explain why broadleaf plantain and narrowleaf plantain has lower levels of OxO activities. It is known that *S. minor* has shown different infection ability to dandelion, broadleaf plantain and narrowleaf plantain. *S. minor* may face some barrier factors that are less strong than barriers in grass so that OxO is also secreted in a level to defend the infection. But it just needs to keep in a lower level in these two weeds than dandelion due to their own barriers.

The morphology of four plants will also affect the susceptibility. Dandelion rosette leaves are thin, glabrous. Broadleaf plantain leaves are thick, oval, glabrous or with short hair with several prominent veins from tip to the stalk. Narrowleaf plantain leaf has paralleled several veins with hairy surface. The leaf is also thick but is more likely to be a grass-like shape. It seems that thick hairy leaf is less susceptible. And also narrow leaf shape is also less susceptible.

In general, this experiment also indicates that the infection variation and interaction between *S. minor* and plant is not simply related with OA and OxO activities, a more complex mechanism may exist.

Chapter 6

Formulation improvements of *Sclerotinia minor* bioherbicide

6.1 Abstract

The commercial formulation of Sarritor[®] was 1.4-2.0 mm barley grits. However it is hard to produce for laboratory use due to lack of suitable grinding machine. Therefore, new grains are needed to be found to replace barley as a substrate for *S. minor* bioherbicide product. In this study, Japonica rice, Indica rice, millet, couscous were tested as new formulations. The hypothesis for this experiment is that at least one of the tested grains is hypothesized to be morphologically superior formulation than *S. minor* barley product for the *S. minor* bioherbicide product. The four grains were morphologically examined by shape examine, virulence tests, shelf life tests and field efficacy tests. Millet is the ideal substitute to barley among the tested grains for *S. minor*

bioherbicide product on virulence, shield life, field performance.

Keywords: *Sclerotinia minor* bioherbicide, formulation selection

6.2 Introduction

The current formulation of Sarritor[®] is 1.4 to 2.0 mm ground barley grits, which supports the growth of *Sclerotinia minor*, the active bioherbicide agent. The production process with barley grits is time-consuming to produce since no specific machine were found to produce standard barley grits. Therefore, alternative formulations were needed. When developping bioherbicide formulations, three criteria should be considered: 1) Formulation needs to have homogenous, firm form; 2) Formulation needs to maintain the efficacy of the active ingredient for relative long time period (shelf-life); 3) Components are easily accessible and economic.

Based on these requirements, several methods have been used to test the potential formulations. Measuring the diameter of granule and examine if clumping occurs in formulations. In the barley formulation, product clumping occasionally occurred. The presence of clumping mass may impede the water activity (a_w) stability, an important standard for shelf-life. Virulence of formulation were test on agar and detached leaf since the diameter of colony and leaf lesion are correlated with the virulence.

6.3 Methodology and materials

6.3.1 Grain substrate preparation

Barley seeds were ground and sorted through metal screens to produce 1.4 mm-2.0 mm barley grits. One hundred grams of the 1.4 to 2.0 mm ground barley grits and 50ml of water were combined in autoclaveable and breathable bags and autoclaved for 30 minutes at 103 kPa. In addition to barley, four other grains substrates; couscous, millet, Japonica rice and Indica rice were tested. Fifty milliliter water was mixed with 100 g millet, 100 g Japonica rice, 100 g Indica rice respectively in autoclavable and breathable bags before sterilizing. Pre-experiment shows that couscous with different water percentage shows sensitively different shape and efficacy. Therefore, three different couscous formulation combinations were made, with 40 ml water (couscous:40), 50 ml water (couscous:50) and 60 ml water (couscous:60), respectively. Bags were allowed to cool before the *S. minor* starter culture was added to the breathable bag.

6.3.2 Production of *Sclerotinia minor* inoculum

Sclerotinia minor (IMI 344141) was isolated and incubated using the same method as mentioned in Chapter 4. The isolate was collected from a diseased lettuce field in Sherrington, Quebec and the culture was stored as sclerotia at 4°C. Sclerotia are sterilized by a modified method of Shaheen et al. (2010). Sclerotia were submerged in 70% ethanol for 30 seconds, 9% hypochlorite for three minutes and dried on sterilized filter paper. Between each step, sterilized deionized water was

used to wash sclerotia twice. Dry sclerotia were placed on PDA plates in 9-cm petri dishes and incubated at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ in the dark. Five mm diameter mycelia discs were taken from the edge of five-day-old colonies and incubated for mycelia on other PDA plates in conditions as above.

The *S. minor* mycelia were used to produce *S. minor* inoculation bioherbicide based on the procedure of Abu-Dieyeh and Watson (2006): 5 agar plates from the margin of a colony were taken and incubated on 100ml MRS (Briere et al., 2000) in 250 ml Erlenmeyer flask on rotary shaker at 100rpm, 20°C in dark for 5 days.

The recipe of MRS is as followed: 10 g KNO_3 , 10 g sucrose, 5g KH_2PO_4 , 2.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.02 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ with 150 ml V-8 juice (Campbell Soup Company Inc.) per liter. Flask cultures were homogenized and mixed with sterilized moist grains in autoclavable bags. Bags were sealed and incubated at 20°C in the dark for four to five days. Bags were massaged every day in order to spread out fungus homogenously on grains. Five grains substrates were tested as *S. minor* formulation, couscous, millet and two types of rice (Japonica rice and Indica rice), with 1.4 mm to 2.0 mm barley grits as standard control. Formulas were then placed in a 0.5 mm mesh tray in sterile air cupboard and dried for 12 hours. The water activities of dried granules were tested by HygroLab-3 water activity system (Rotronic AG, Bassersdorf, Switzerland) (accuracy ± 0.03 at $23 \pm 2^{\circ}\text{C}$) to maintain at aw 0.4 to 0.6 (Abu-Dieyeh and Watson, 2006). Then formulations were sealed to 17.7 cm $\times$ 18.8 cm plastic zipper bags (Ziploc, Johnson and Son Ltee) and stored

at -20°C fridge.

6.3.3 Physical examination and water activity assay

The formulation shape was examined after drying to the proper water activity (0.4-0.6 a_w). Thirty granules of each formulation were randomly selected and their diameter was measured. The final water activity, stiffness extent and clumping phenomenon were recorded.

6.3.4 Virulence tests on agar plates

Potato dextrose agar (PDA) (DifcoTM, BD Medical Technology) in 9 cm petri dishes was used to test the virulence of the stored *S. minor* formulations. Ten granules from each formulation were randomly selected from each experimental formulation after 1, 3, and 5-months of storage at -20°C. Colony diameters of mycelium growth from granules were measured after 24 hours and 48 hours. The experiment was repeated once.

6.3.5 Virulence tests on detached leaves

Dandelion was grown from seed. Seeds were collected in spring 2016 in a field (McGill Macdonald Campus, St-Anne-de-Bellevue, Quebec). Seeds were stored at 4°C fridge after drying for one day. Stratification was used to dandelion before sowing in the 39 cm × 39 cm × 58 cm plastic pots to break the dormancy and synchronize germination. Seeds were submerged in deionized water in 25mL falcon tubes. The tubes were stirred for 4 to 5 hours before remove deionized water. Then seeds were placed in 4°C fridge for 5 days before sowing in soil. The plants were

transferred into 85 cm × 85 cm × 95 cm plastic pots after the third leaf emerges. Soil contains one half of gardening soil (Voila!®, S. Doubrias Inc.) and one half of ProMix soil (Promix BXt, Premier Horticulture Ltee). Plants were grown in a Conviron-E15 growth chambers at 20°C ± 1.5°C with 12 hours light with photon flux density minimum of 350 ± 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 12 hours dark. Plants were watered every two or three days according to the water condition in soil.

Three eight-week-old detached dandelion leaves were placed on 6 1/2 gauze faced disks (Rapid-Flo Milk Filters, Filter Fabrics Inc.) in a 15 cm petri dish containing 4ml deionized water. One granule of the *S. minor* formulants was placed on the leaf surface and plates were sealed with biofilm and placed in the dark at 20°C ± 1°C. Lesion diameters (lengthwise) were measured after 24h and 48h, respectively. The whole leaf lesion test was repeated once. Following Shaheen et al. (2010) the latter measurements were the parameter used to assess the virulence of the inoculum. Colony diameter after 48h over 3 cm indicates the high virulence of formulation; Colony diameter between 2 cm to 3 cm indicates moderate virulence; diameter between 1 cm to 2 cm indicates low virulence; below 1cm indicates hypovirulence. The experiment was repeated once.

6.3.6 Turfgrass system investigations

Field tests were carried out on a turfgrass area at McGill Macdonald campus in Saint-Anne-de-Bellevue, Quebec. Turfgrass was mowed every week except during a lengthy drought. No other maintenance was done in this area for the last three years. The grass sward was 90% Kentucky bluegrass and 10% creeping red fescue (*Festuca rubra*). The main weed species was dande-

lion with the intensity of 40 to 120 dandelion plants m^{-2} . Broadleaf plantain and white clover were less abundant than dandelion in this area but have high intensity partially during the experiment period. Other weeds species was sporadically distributed: ground ivy (*Nepeta glechoma* Benth.), common ragweed (*Ambrosia artemisiifolia* L.), birdsfoot trefoil (*Lotus corniculatus* L.), narrow leaf plantain, common vetch (*Vicia sativa* L.), mouseear chickweed (*Cerastium fontanum* Baumg.) and black medic (*Medicago lupulina* L.).

6.3.7 Experimental design and arrangement

A completed randomized block design was applied for testing new inoculation formulas with six treatments. Each plot was 25 cm $\times$ 25 cm with 25 cm width distance between all plots. The distance between each block was over 2m. Barley, couscous and millet inoculums were produced one week before the field test. Virulence of the four inoculums were tested on PDA plates and detached dandelion leaves before application. The six treatments were: untreated control, 10g m^{-2} millets inoculation, 10g m^{-2} couscous:40, 10 g m^{-2} couscous:50, 10 g m^{-2} couscous:60 and 10 g m^{-2} Sarritor[®]. Each treatment was evenly applied over the experiment plot. Plots were covered with two 30 cm $\times$ 35 cm jute burlap (TarraTex, Lenrod Industrial Ltd) sheets for the first three days in order to increase the efficacy of *S. minor* (Abu-Dieyeh and Watson, 2009). The experiment sites were watered for one hour for two days before the experiment due to the dry condition of the turfgrass in the summer (Figure 6.1). Also, the experimental plots were also watered for the next three consecutive days after bioherbicide application if there was no rainfall during those days. Weather condition and rainfall data during the experimental period

were recorded (See in Appendix). The whole experiment was conducted three times during the summer in 2016 (July 8, July 25 and August 25, respectively).

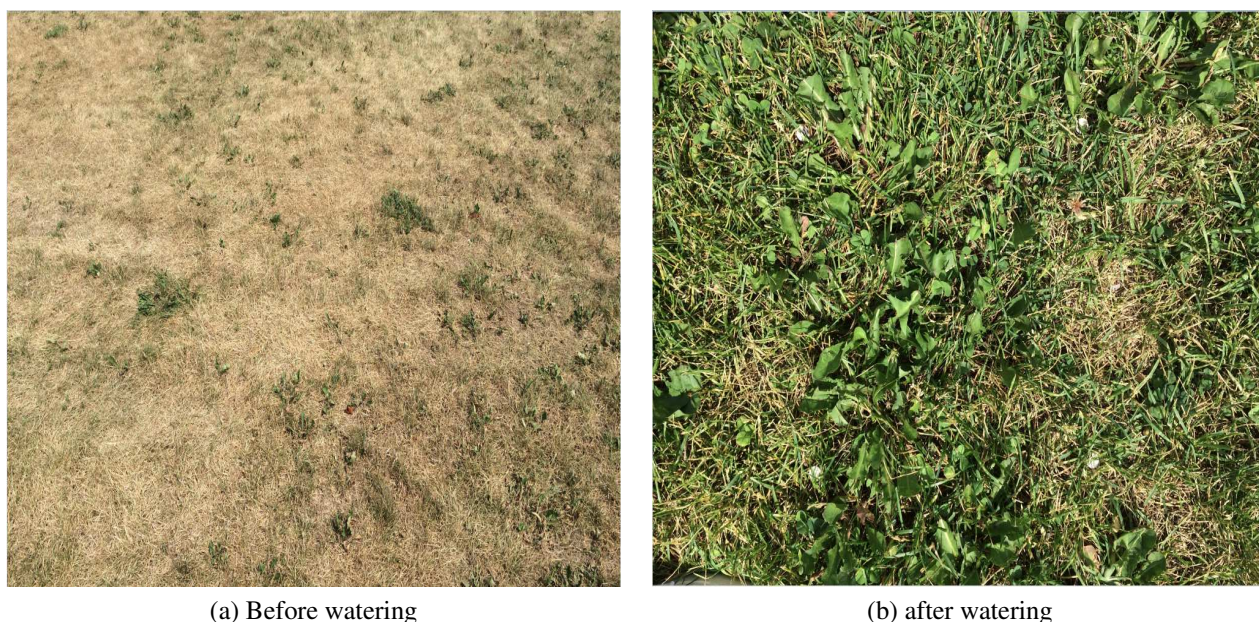


Fig. 6.1 Dry field condition prior to conducting *Sclerotinia minor* experiments and field condition after watering just before *S. minor* application.

6.3.8 Field test evaluation method

Weed numbers were counted one day before treatment, one-week after treatment, two-weeks after treatment and three-weeks after treatment. Visually assessed damage was also recorded each time for three weeks after application. Damage was assessed by 0-10 visual scale, where 0=no damage, or less than 10% damage in above-ground biomass compared within the same block. 1 = 11% to 20%, 2 = 21% to 30% damage... 10 = 100% damage on the plot compared to the control in the same block. Weather conditions and relative humidity was also recorded hourly during

the field test period based on data of Ste-Anne-de-Bellevue Station from Environmental Canada (Appendix A). Temperature and RH on the lawn were also recorded by a digital temperature and humidity indicator (H10C, Honeywell) on the day the bioherbicides were applied and 1-week, 2-weeks and 3-weeks after application.

6.3.9 Data Analysis

SAS statistics package 9.4 was used to statistically analyze collected data. For the shape examination, means and standard error of granule diameter were calculated. Tukey's test was used to do the multiple comparisons among groups at $P=0.05$. Homogeneity of variance was tested by Levene's test with $P=0.05$.

For the field test, the percentage of dandelion damage was calculated by formula:

$$DR = \frac{PO}{PRE} \quad (6.1)$$

Where DR means damage rate of dandelion plant; PO represents posttreatment number of dandelion; PRE represents the pretreatment number of dandelion in the same plot. The formulation overcame the differences of dandelion between plots. The data from field test of *S. minor* formulations were pooled since the experiment was repeated during a short period. The data were analyzed with one-way ANOVA for a randomized complete block design to determine the effect of treatment ($P=0.05$). The distribution was tested for data of DR. The model's distribution of

each experiment was selected according to Bayesian Information Criterion (BIC). SAS Qlim-mix procedure of repeated measurement was used to test the effect of time on the damage of dandelion. Tukey's test at $P=0.05$ was used to analyze the means with significant effects.

6.4 Results

6.4.1 Physical examination

All formulations were physically examined before conducting virulence and efficacy test. Couscous groups and millet grits were homogenous, firm and in spherical good shape after drying while both rice groups were fragile and irregular shape (Figure 6.2). Japonica rice formulation diameter was $5.11 \text{ mm} \pm 0.58 \text{ mm}$ and the Indica rice formulation diameter was $5.72 \text{ mm} \pm 0.77 \text{ mm}$. The range of Japonica rice formulations were from 2.00 mm to 19.80 mm and the range for Thailand rice was from 2.0 mm to 30.05 mm. This indicates the heterogeneity of rice formulations. The irregular shape was not suitable for field application therefore two rice groups were ruled out and further experiment was not taken on these two groups. The mean of millet diameter was $2.35 \text{ mm} \pm 0.18 \text{ mm}$ and the diameter range of couscous:40, couscous:50 and couscous:60 were $1.64 \text{ mm} \pm 0.16 \text{ mm}$, $1.63 \text{ mm} \pm 0.13 \text{ mm}$, $3.56 \text{ mm} \pm 1.37 \text{ mm}$, respectively. In the couscous groups, all three couscous groups showed localized clumping phenomenon (Figure 6.2): couscous:60 had higher percentage of clumping than couscous:50 while couscous:40 rarely occur clumping mass. Furthermore, couscous:60 had larger clumping than couscous:50 and couscous:40. Clumping mass over 5 mm were ruled out and couscous test continued. However, the formulations were

still evenly in general so that both three groups of couscous were kept and further test will be done on couscous groups. Millet was firm and good shape after drying and clumping mass rarely occurred in the millet formulation (less than 1% of total weight).

6.4.2 Virulence test on agar

The agar tests revealed the decrease in virulence of the four tested formulations with time (Figure 6.3). Besides, the virulence remaining ability of the four groups are different.

To be specific, in millet and couscous:60, results show that there was no significant difference between colony diameters in 1 month and 3 months while diameters in 5 months was significant less than in 3 months. In couscous:40, the colony diameter had a gradual decrease over 5 months although there was no significantly difference between 1 month and 3 months. In another couscous group (couscous:50), there were significant differences between each of two tested months. This result shows the virulence of couscous:40 and couscous:50 may not be very stable with the increase of time.

Millet had significant higher virulence among the tested formulations. Millet formulation had better performance over 5 months than the barley control group. Virulence of couscous:50 was highest after 1-month storage. However, couscou:60 remained the highest virulence among three couscous formulations after 3 months and 5 months. All three couscous formulations had inferior performances than the control and millet formulations.

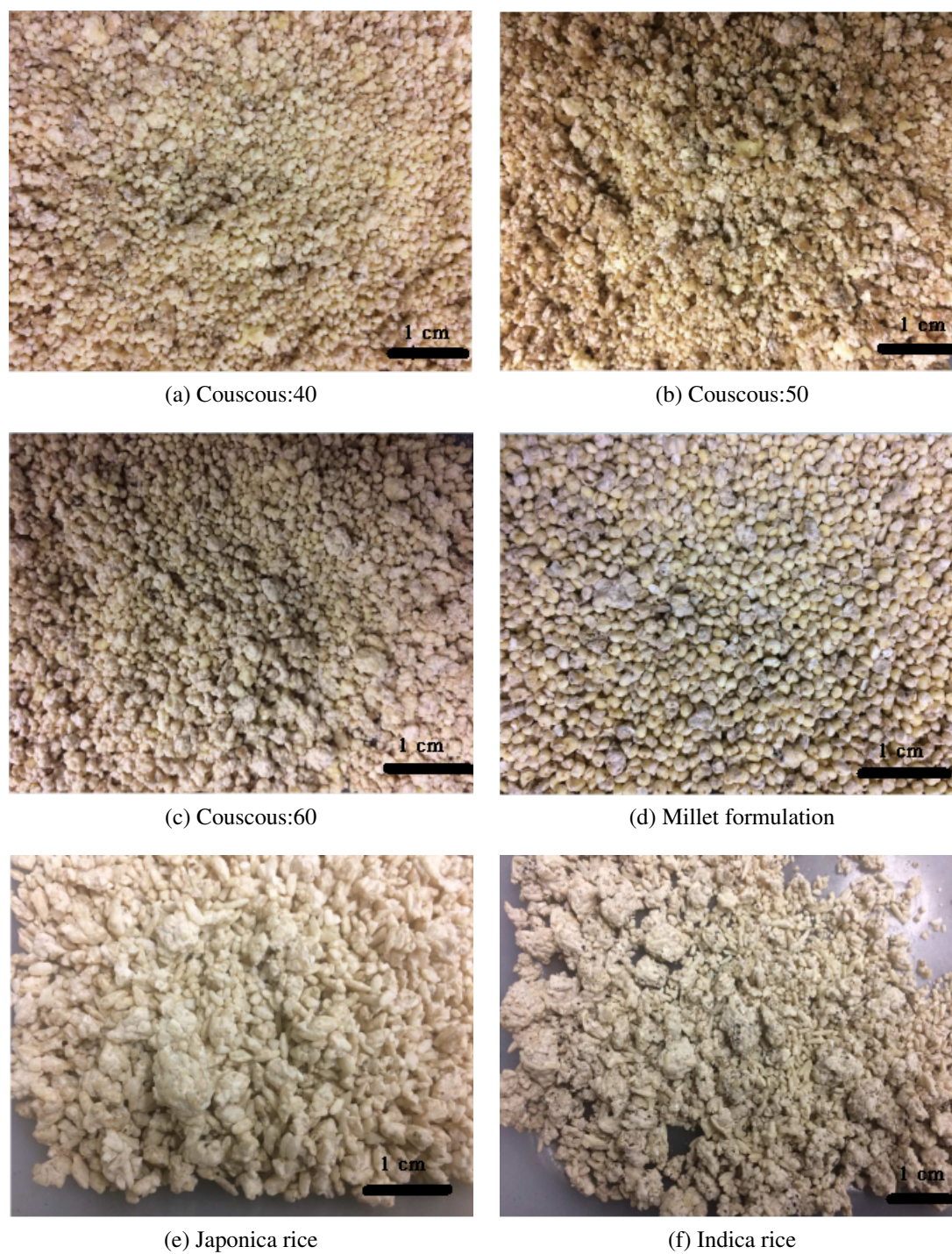


Fig. 6.2 Physical forms of four formulations of *Sclerotinia minor* after drying.

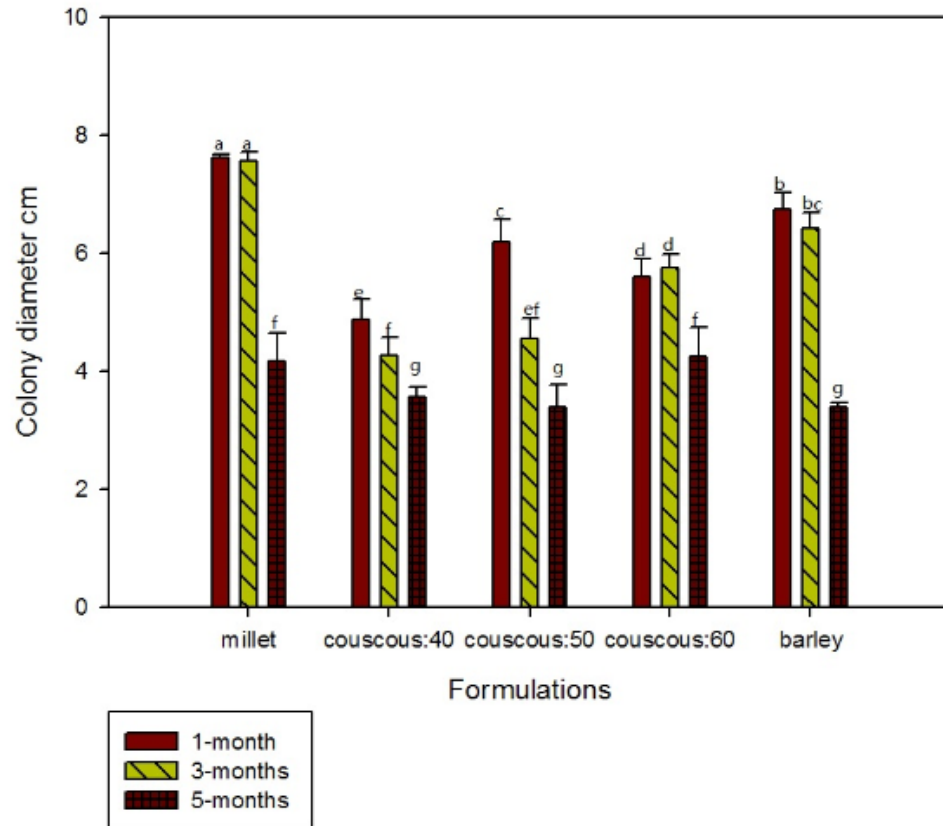


Fig. 6.3 Effect of storage and formulation on the virulence of *Sclerotinia minor* on potato dextrose agar.

It is interesting that, in couscous:60, the diameter after 3 months was slightly higher than 1 month. This indicates that couscous:60 had fluctuated and unstable status during the long period storage.

6.4.3 Virulence test on detached dandelion leaves

The data of two replicates were pooled due to the similarity of result. The virulence of the four formulations reduced with directly storage time become longer (Figure 6.3). Millet changed

most sharply among the tested formulations, from over 3 cm to less than 2 cm (Figure 6.4). Leaf lesion tests on couscous groups indicate the decay of virulence with month (Figures 6.5, 6.6, 6.7). Statistic analysis to the variance of virulence in each month shows that there was no differences in virulence among treatments in the first month ($F_{3,32}=2.69$, $P=0.0626$). While the virulence of three couscous groups drop from the third month, millet keeps the high virulence in the third month. Virulences of the tested formulation were different after 5 months: couscous:50 and couscous:60 had higher virulence than couscous:40 and millet group ($F_{3,32}=6.26$, $P=0.0018$).

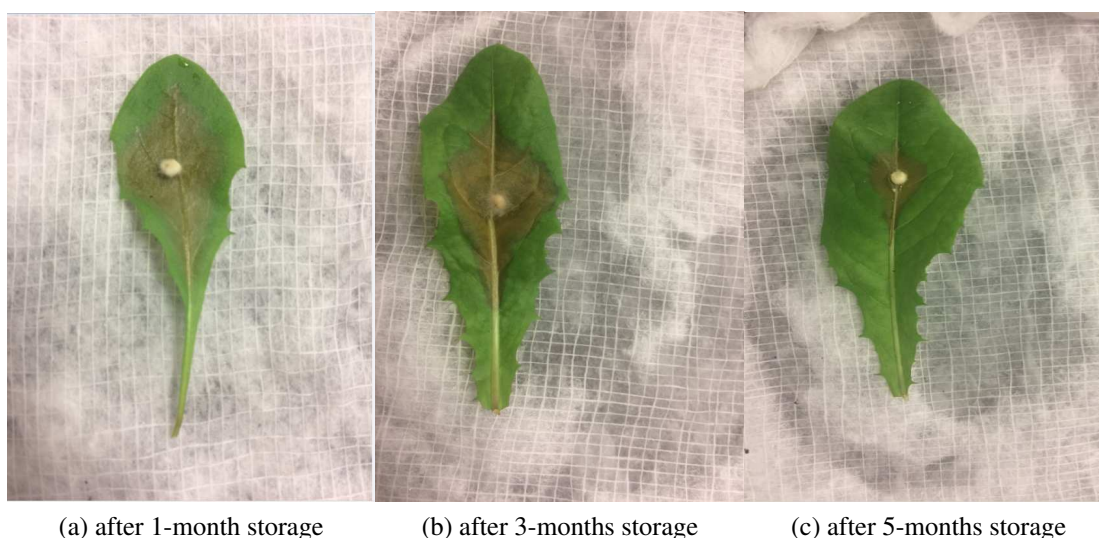


Fig. 6.4 Effect of storage length of millet on the colony diameter of *Sclerotinia minor* 48h after inoculation on dandelion leaves.

All the four tested formulations are in high virulence since the diameter of leaf lesion are over 3 cm. Three couscous groups become medium virulence in the third month while millet keeps the high virulence, with 3.90 ± 0.32 cm leaf lesions. The couscous:50 and couscous:60 remain at

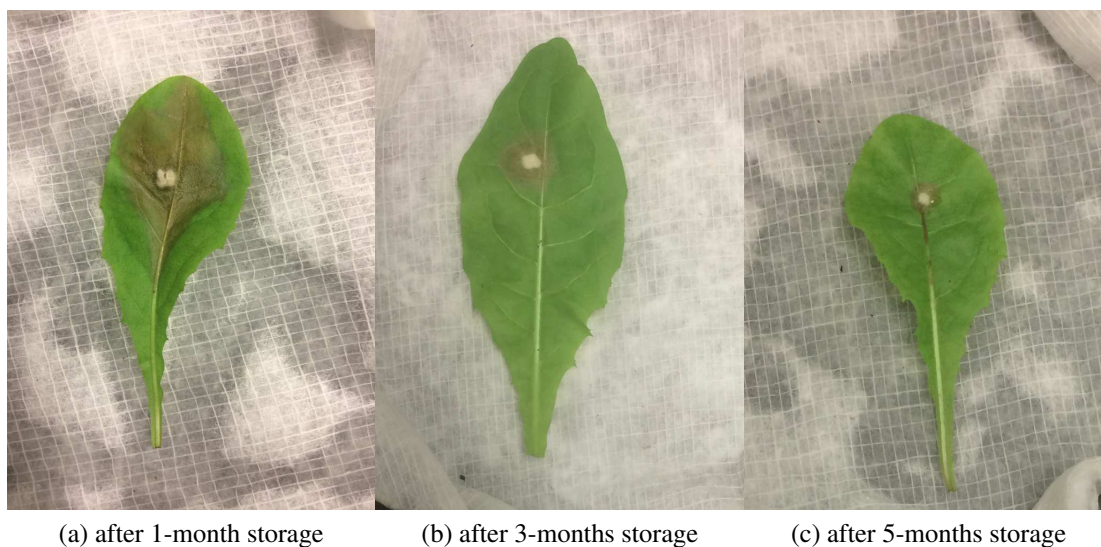


Fig. 6.5 Effect of storage length of couscous:40 on the colony diameter 48h after inoculation on dandelion leaf.

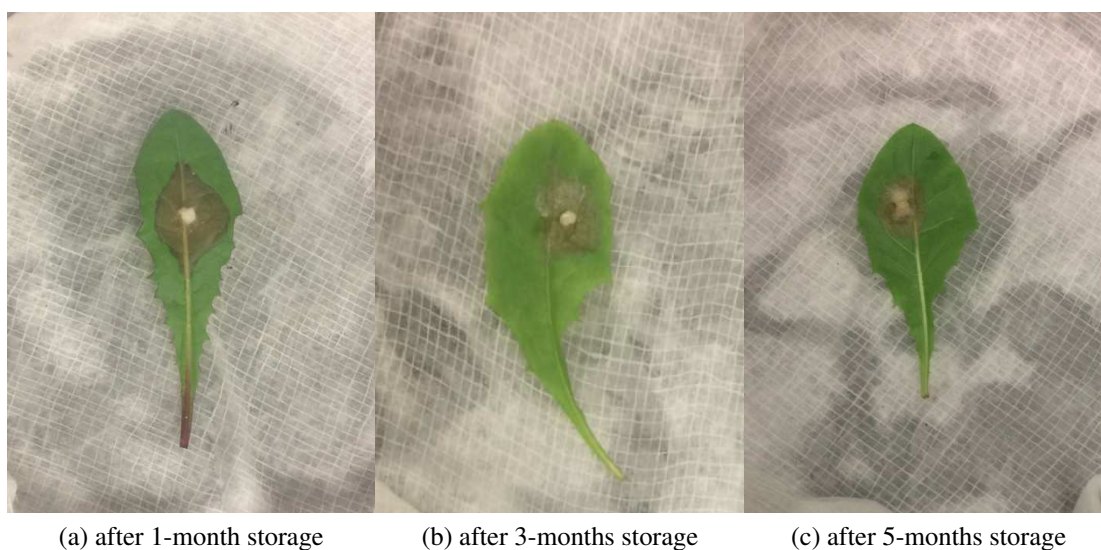


Fig. 6.6 Effect of storage length of couscous:50 on the colony diameter of *Sclerotinia minor* 48h after inoculation on dandelion leaves.

the medium virulence while the other two groups drop into low virulence.

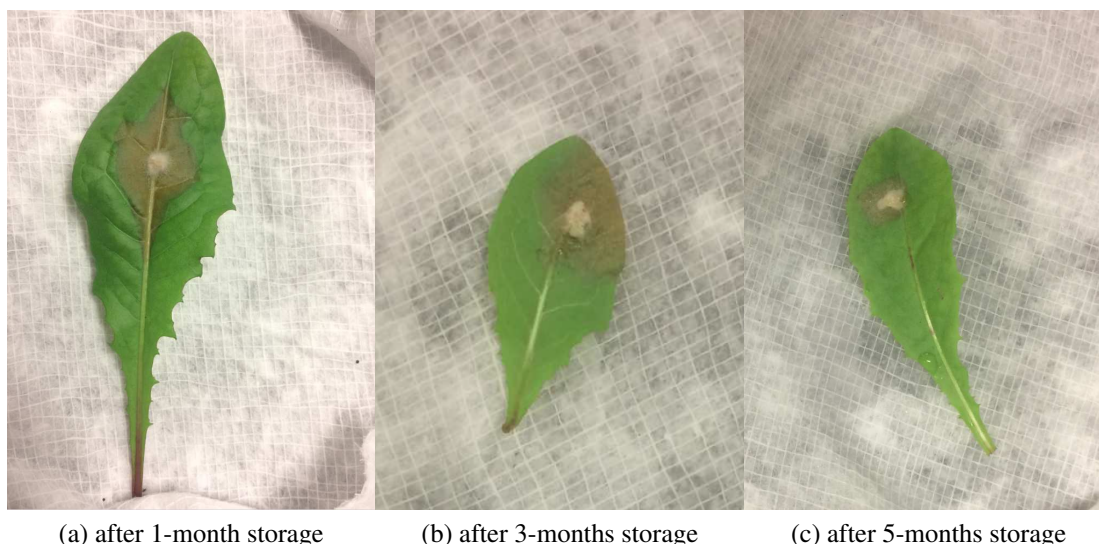


Fig. 6.7 Effect of storage length of couscous:60 on the colony diameter of *Sclerotinia minor* 48h after inoculation on dandelion leaves.

6.4.4 Field test to control dandelion

The first experiment was carried out at 5pm on July 8th, 2016 with 70% RH, at 22°C measured by digital indicator, the second replicates started on July 25th at 5pm with 25°C, 90% RH and the last replicate occurred on August 25th at 5pm with 25°C, 80% RH. (The weather data records from weather station are listed in Appendix A).

Dandelion controlling rate was mainly analyzed in the field test. The control of other target weeds on the lawn was recorded but not shown here as the other target weeds were not widely distributed in the experimental plots.

A generalized linear mixed model was used to test the dandelion damage percentage since it is a

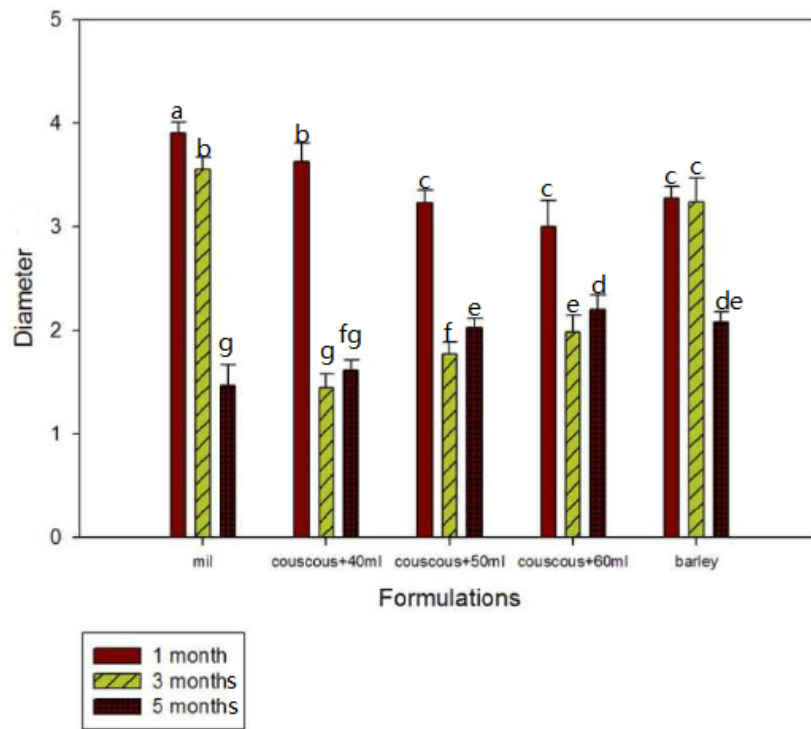


Fig. 6.8 Effect of storage and formulation on the virulence of the *Sclerotinia minor* bioherbicide formulation on detached dandelion leaves.

randomized complete block design with repeated measurement on three different weeks with the equal variance correlation on every week.

The field control of dandelion shows that *S. minor* inoculations germinated within 24 hours and killed dandelion rapidly (within 1 week) (Figures 6.9). Then some of the dandelion plants regrew in the consecutive week and this phenomenon happened in every treatment including positive control (Figure 6.10 to 6.14). The regrowth rate shows differences between replicates due to the different RH and rainfall during the experimental period.

The experimental data indicates that the percentage of damage to dandelion was not equal between different *S. minor* formulations ($F_{5,50}=40.24$, $P<0.001$). The multiple comparison result shows that there were statistically significant differences between every treatment and control ($P<0.001$). This indicates that couscous and millet formulations both have significant control on dandelion in field. The comparison between treatments and positive control shows that there was no significant difference on dandelion damage percentage between couscous formulation, millet formulations and standard control, which indicates that the new formulations could become substitutes of Sarritor[®] according to the field control performances. Figure 6.15 shows that millet treatment has slightly better control on dandelion compared with other treatments and positive control. This indicates that millet formulation is probably superior to the Sarritor[®] formulation and millet is a better choice as formulation for replacing barley grits in the future.

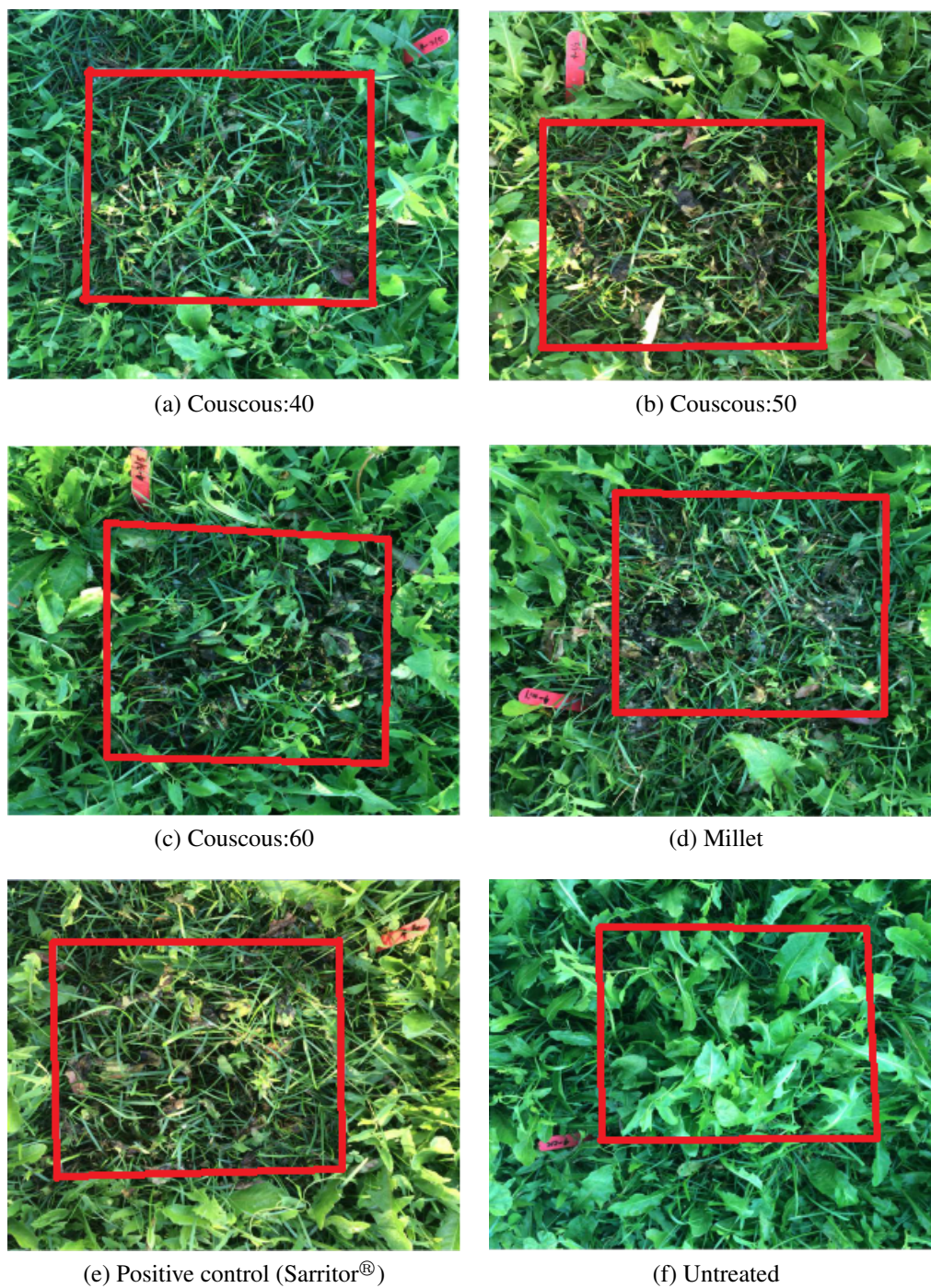


Fig. 6.9 Effect of formulation of *Sclerotinia minor* on dandelion control within field turf plots one week after application.

NOTE: Performance based on dandelion reduction and grass regrowth.



(a) Pretreatment at application



(b) One week after application



(c) Two weeks after application



(d) Three weeks after application

Fig. 6.10 Effect of the millet formulation of *Sclerotinia minor* bioherbicide on dandelion suppression within the field plots over time.

NOTE: Performance based on dandelion reduction and grass regrowth.

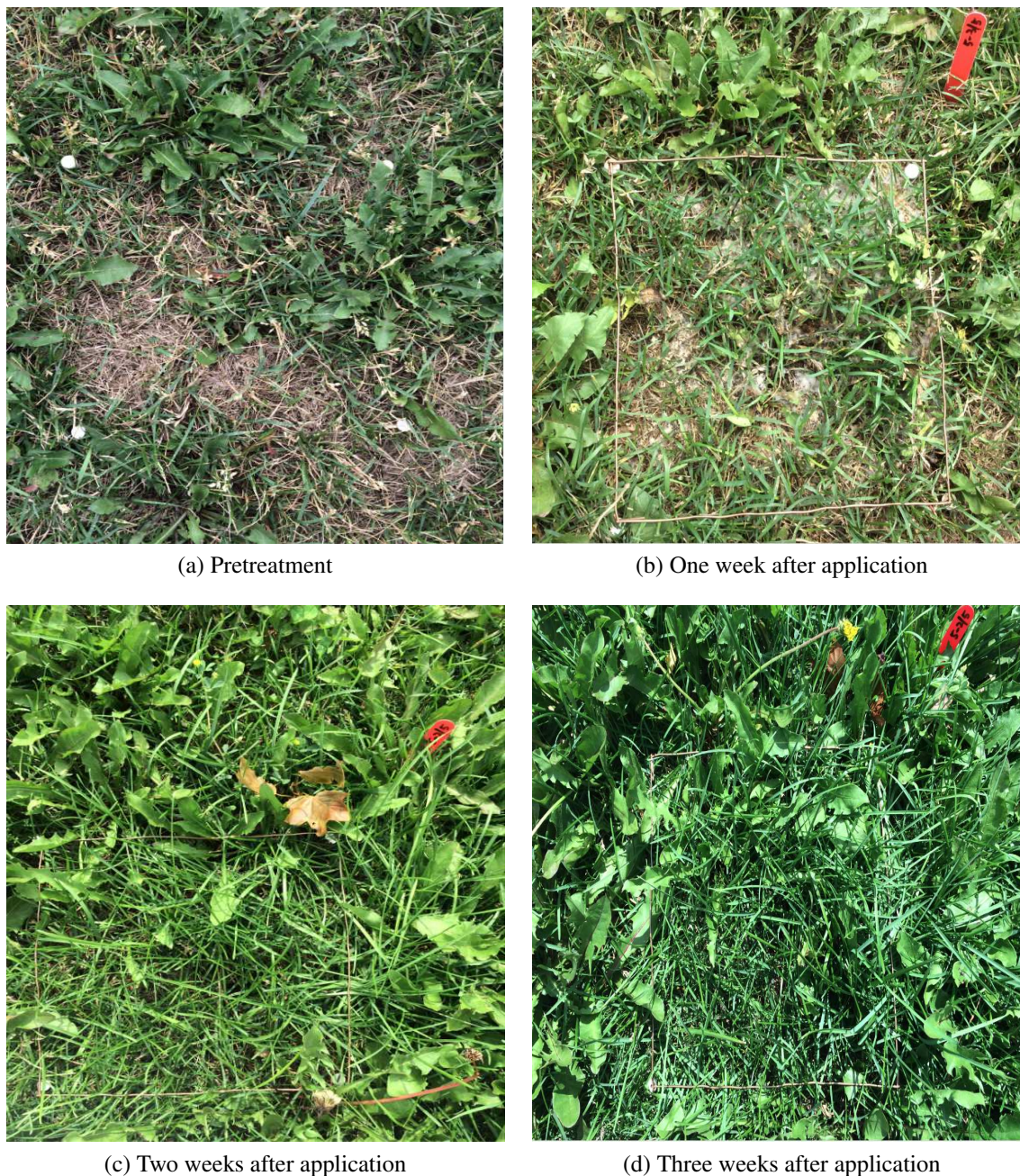


Fig. 6.11 Effect of the couscous:40 formulation of *Sclerotinia minor* bioherbicide on dandelion suppression within the field plots over time.

NOTE: Performance based on dandelion reduction and grass regrowth.

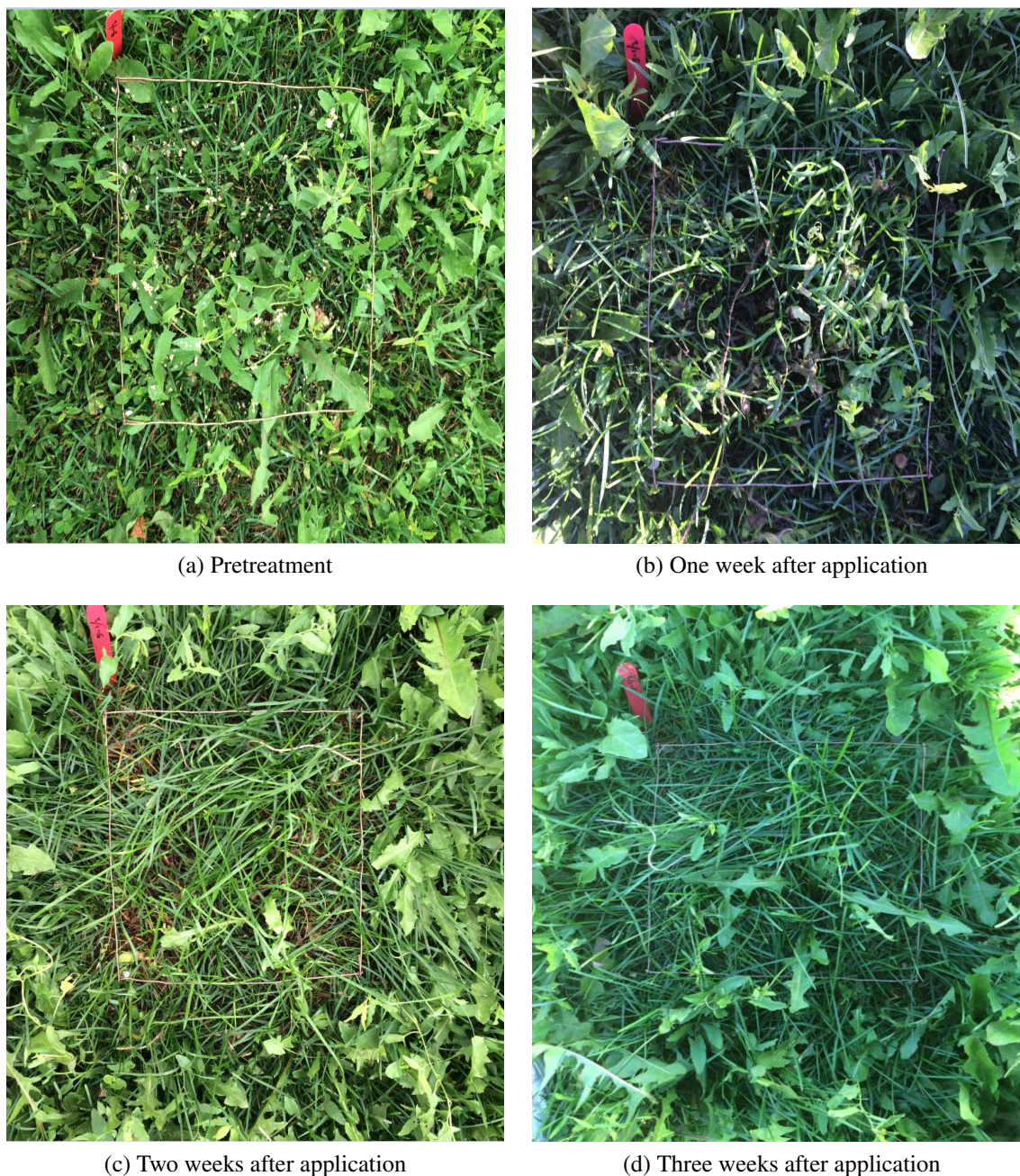


Fig. 6.12 Effect of the couscous:50 formulation of *Sclerotinia minor* bioherbicide on dandelion suppression within the field plots over time.

NOTE: Performance based on dandelion reduction and grass regrowth.

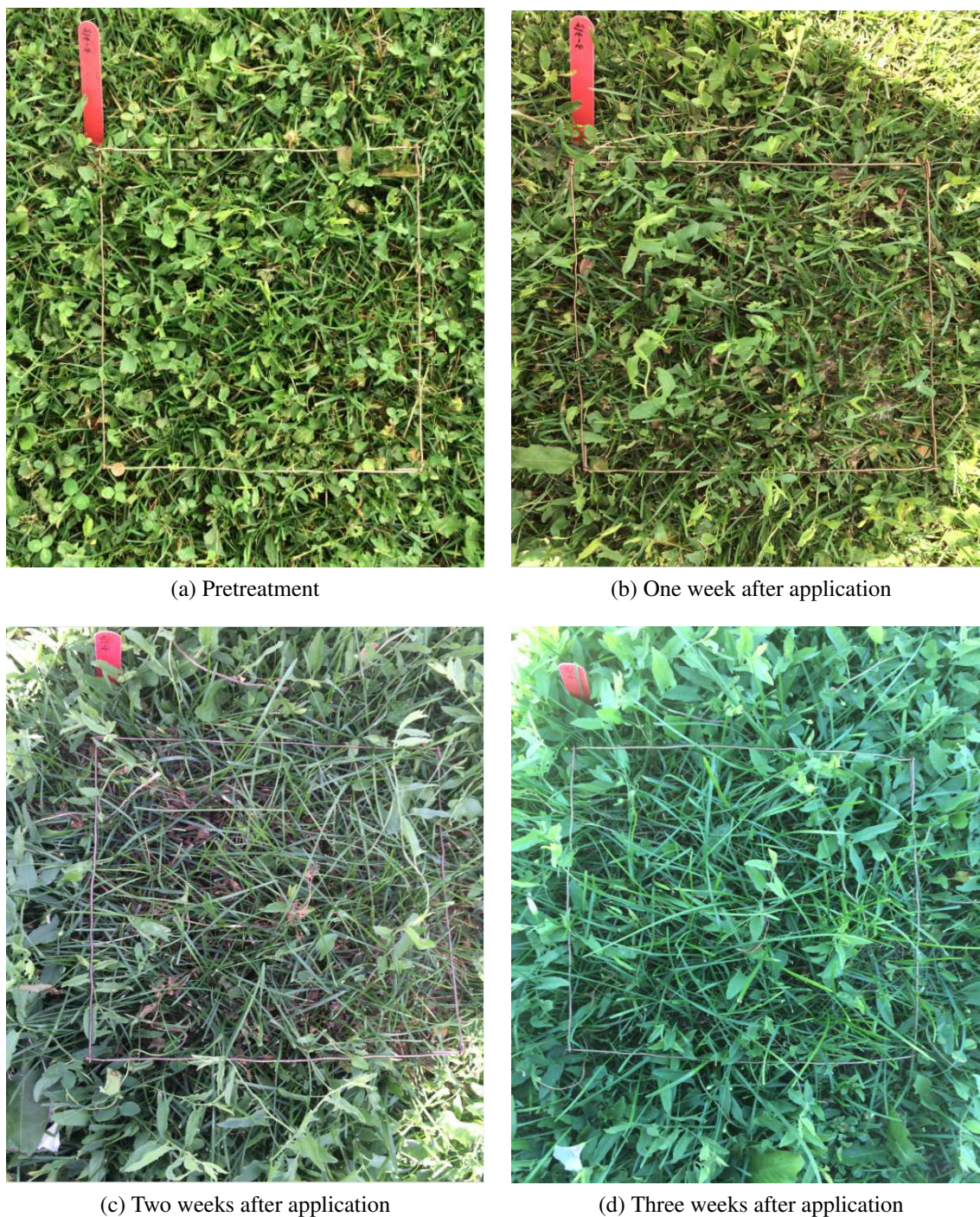


Fig. 6.13 Effect of the couscous:60 formulation of *Sclerotinia minor* bioherbicide on dandelion suppression within the field plots over time.

NOTE: Performance based on dandelion reduction and grass regrowth.

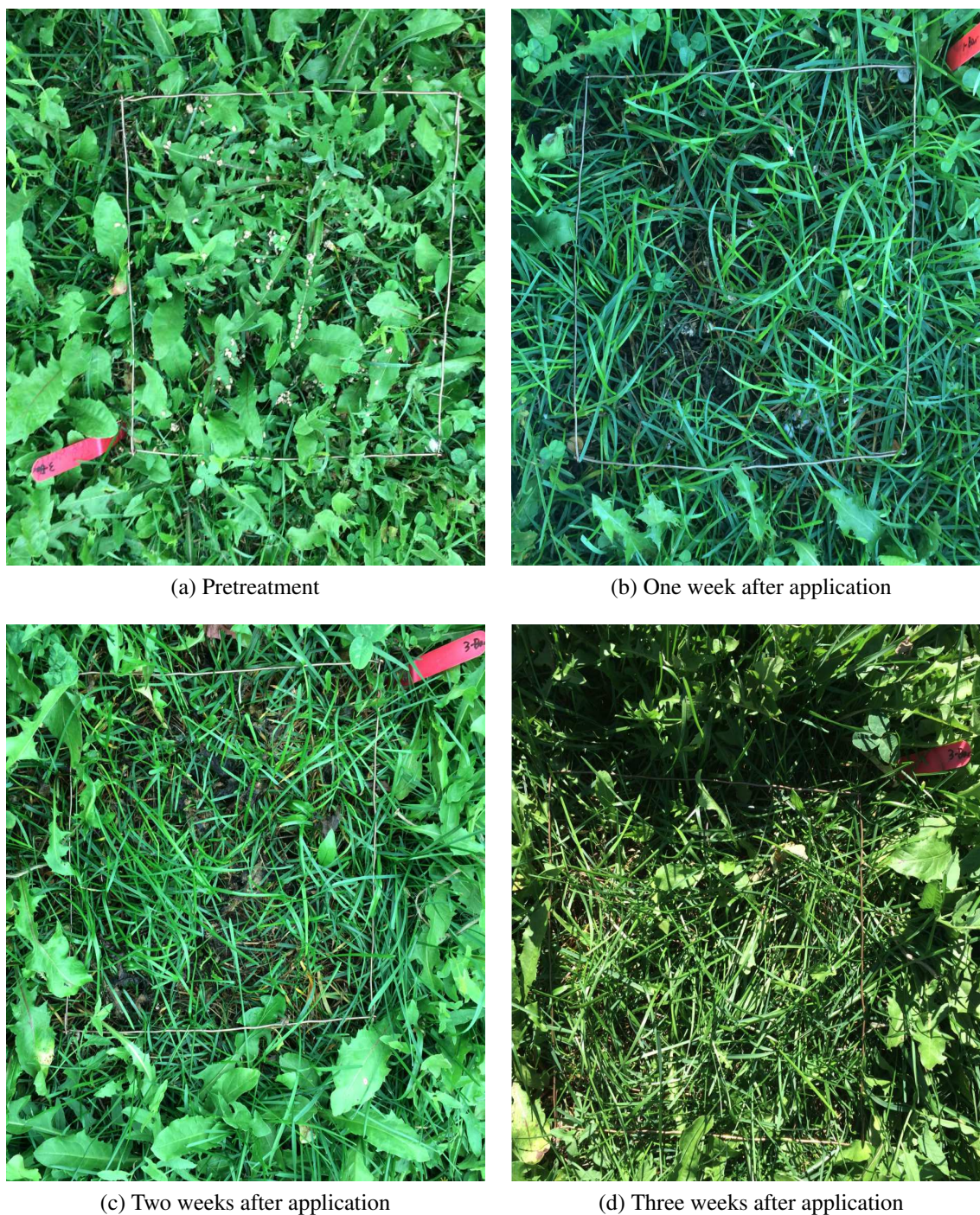


Fig. 6.14 Effect of Sarritor[®] (positive control) of *Sclerotinia minor* bioherbicide on dandelion suppression within the field plots over time.

NOTE: Performance based on dandelion reduction and grass regrowth.

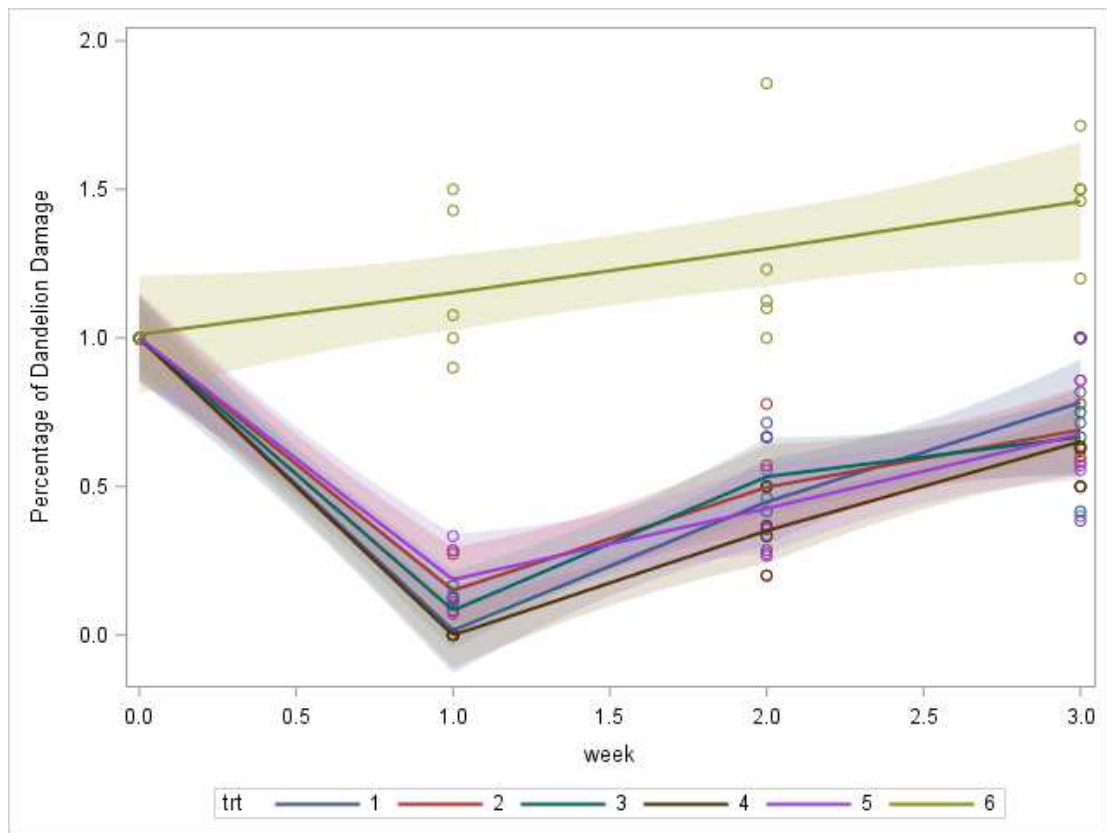


Fig. 6.15 Effect of *Sclerotinia minor* bioherbicide formulations to damage dandelion population over time.

trt 1 represents response of couscous:40 to time; trt 2 represents response of couscous:50 to time; trt 3 represents response of couscous:60 to time; trt 4 represents response of millet; trt 5 represents Sarritor[®] to time; trt 6 represents negative control to time.

6.5 Discussion

6.5.1 Superior formulation selection

The whole experiment of efficacy test shows no statistical differences among each group. However, millet group was superior to couscous groups and Sarritor[®] with the barley formulation on various aspects. First of all, millet is easy to acquire as ingredient and no further shape adjustment is needed in this process. Besides, millet shows less clumping mass (<1%) when *S. minor* is inoculated on. Agar test and detached leaf test also show that efficacy of this formulation keeps stable and at high level even after 5-months storage which is also a perfect trait as a herbicide product from the perspective of commercial uses. Field efficacy test also shows millets could rapidly kill dandelion with the help of jute burlap in summer (Abu-Dieyeh and Watson, 2009). *S. minor* is most favorable in moist dark environment, therefore it could be expected that, in autumn, millet inoculations would show effective and more persistent controlling on dandelion than summer application due to the high RH and dew point with lower sunlight.

Although all three couscous groups showed no obvious differences with the positive control, couscous is not the most suitable substitute to barley grits mainly because of the instability in formulation. As indicated in agar test result, clumping would break the homogeneity of formulations, which causes the uneven distribution of fungi. This idea is proved by the fluctuation in couscous:60 on agar test in 3 months and 5 months. Agar test in 3 months has more small for-

mulation couscous while test in 5 months has more clumping mass formulations that cause the “recover of virulence” in this group. Furthermore, field test shows some control failure scenarios in couscous group though the general damage control shows no statistical differences with other groups.

6.5.2 Dandelion regrowth and practical weed management in summer

Aged dandelion has long taproot that allows them to regrowth after losing the aboveground part of the plant although jute burlap helps prolong the survival time of *S. minor*. During the summer period, *S. minor* shows less effective and less consecutive control on plant regrowth since the fungi are exposed largely under sunlight, high temperature and less competition with the grass. Abu-Dieyeh and Watson (2007b) raised a long-term control strategy with combination of grass overseeding and *S. minor* application. Here, based on that strategy, we suggested a second-time bioherbicide should be applied two or three weeks after the first application when dandelion generates new above-ground tissue. This strategy would provide a better control on regrowth dandelion as well as provide time and space for the growth of grass to fill the gap after dandelions were killed. In that way, after the second applications, grass biomass will be increased, which will provide a healthier turfgrass environment for controlling dandelion. Dandelion control is usually recommended during autumn season. This experiment also provides a possibility of managing dandelion during summer time.

6.5.3 Drawback factors in the field

S. minor bioherbicide application in summer time has many drawback factors. As mentioned above, *S. minor* prefers moist, dark and cool environment while the weather conditions in summer in Quebec is mostly sunny, dry and high temperature this year. Another involved factor is ant nests. *S. minor* bioherbicide owns grain-based formulation which attracts insect around (mostly ants). If the inoculation cannot germinate soon ant colony will settle down around the application plots or target plants and consume the inoculations. Field test was carried out during summer time when it was not the perfect time for *S. minor* inoculation to develop because high temperature and strong sunlight have caused damage to *S. minor*. However, in this inferior scenario, the dandelion damage efficacy was over 70%, which indicates the potential use on lawn maintenance in summer with the help of jute burlap.

Chapter 7

Conclusions

According to the experimental result and analysis, it is concluded that:

1. Millet and couscous are capable to replace barley grits as the formulation substrate for the *S. minor* bioherbicide. Millet is the ideal substitute for barley grits since it has stable and even shape with superior efficacy on dandelion. Couscous is less stable than millet, mostly because the shape of couscous is sensitive to the volume of water mixed with and clumping mass happened sometimes that could affect the homogeneity and drying procedure.
2. Dandelion has the highest oxalic oxidase activity compared with broadleaf plantain, narrow-leaf plantain and common grass. A multiple mechanism regulates *S. minor* infection and host selections.
3. Sclerotia production of *S. minor* was related to the plant species, biomass of *S. minor* and the

location of *S. minor*. There are interactions among the three factors. Controlling the amount of *S. minor* in the field could impede sclerotia development and reduce danger to other susceptible plants.

Chapter 8

Additional work

8.1 Formulation improvement

Barley, millet and couscous are grains that could attract insects (e.g. ant) especially in summer. Ants formicaries were observed around the application plots in the field. The use of grains-based formulations could possibly lead to bioherbicide loss, control failure and off-target movement. Therefore, formulations need to be improved to avoid attracting insects or to further improve infection speed.

An alternative way to increase the efficacy of the bioherbicide is adding phytotoxins secreted by *S. minor*. Previously precursors of oxalic acid have been used to increase pathogenicity of *S. minor*. Enhancing the production of cellulose, polygalacturonase and pectinase enzymes through formulation and pH optimization should be studied further.

8.2 Investigation of *Sclerotinia minor*'s mode of action and interaction with susceptible plants

Recent studies indicate the important independent function of pH during the pathogenicity and sclerotia development in *S. sclerotiorum* (Xu et al., 2015). Testing the function of pH on *S. minor* and pH of exudates juice of different susceptible hosts that are infected in different stages may reveal the reasons for limited sclerotia development on dandelion.

Physical and chemical infection barriers in an array of susceptible plants could be investigated further to study the reason for reduced susceptibility in some weed species. The relationship between OxO and plant susceptibility could be further demonstrated by examining a list of weed host of *S. minor*. Physical barriers include various trichomes and hairs on the surface of leaves. Additionally, chemical barriers including pH or signal proteins may explain the tolerance of grass species.

8.3 Environmental safety investigation of the *Sclerotinia minor* bioherbicide in the United States

Sclerotinia minor IMI34414 has already been registered as a bioherbicide for turfgrass use in Canada (Health Canada, 2010) while the process of registration in the US is still on the way. Persistence of *S. minor* and sclerotia detection must be carried out to verify the safety of *S. minor*

bioherbicide in the field. Dandelion control with *S. minor* have been conducted in field trials in the United States and treated plot soil was collected for detection of *S. minor* residue. A molecular marker for *S. minor* IMI34414 strain was successfully developed enabling detection of *S. minor* sclerotia in the soil (Pan et al., 2010). However, the DNA extraction kits suggested by Pan et al. (2010) are not available and no alternative extraction method was found. Therefore, new soil DNA extraction methods are needed.

Appendix A

Data records of weather conditions from Ste-Anne-de-Bellevue station

This appendix shows the hourly temperature, relative humidity and dew point from July 8th to September 15th in 2016. Data in red color indicates the weather conditions when the experiments were carried out. The data is based on record of Environmental Canada.

Year	Month	Day	Time	Temp (°C)	Dew Point Temp (°C)	Rel Hum (%)
2016	7	8	0:00	19.3	16.6	85
2016	7	8	1:00	18.6	16.3	86
2016	7	8	2:00	17.6	15.6	88
2016	7	8	3:00	17.5	15	86
2016	7	8	4:00	16.8	14.5	86
2016	7	8	5:00	16.6	14.7	89
2016	7	8	6:00	17.7	15.1	85
2016	7	8	7:00	18.3	15.1	82
2016	7	8	8:00	19.2	14.9	76
2016	7	8	9:00	19.9	14.7	72
2016	7	8	10:00	20	15	73
2016	7	8	11:00	21.2	15	68
2016	7	8	12:00	21.5	15.1	67
2016	7	8	13:00	21.1	15.9	72
2016	7	8	14:00	21.7	15.7	69
2016	7	8	15:00	21.6	16.1	71

Year	Month	Day	Time	Temp (°C)	Dew Point Temp (°C)	Rel Hum (%)
2016	7	8	16:00	22.1	16	68
2016	7	8	17:00	21.7	16	70
2016	7	8	18:00	21.4	16	71
2016	7	8	19:00	20.9	15.9	73
2016	7	8	20:00	20.2	15.7	75
2016	7	8	21:00	20	15.6	76
2016	7	8	22:00	20	15.7	76
2016	7	8	23:00	19.5	15.8	79
2016	7	9	0:00	18.9	15.6	81
2016	7	9	1:00	18.5	15.7	84
2016	7	9	2:00	17.9	15.5	86
2016	7	9	3:00	17.8	15.4	86
2016	7	9	4:00	18	15.3	84
2016	7	9	5:00	16.6	15.8	95
2016	7	9	6:00	16.6	16.3	98
2016	7	9	7:00	18.4	18	98
2016	7	9	8:00	18.7	17.4	93
2016	7	9	9:00	18.8	17.2	91
2016	7	9	10:00	18.7	16.6	88
2016	7	9	11:00	17.9	17	94
2016	7	9	12:00	17.6	16.7	95
2016	7	9	13:00	17.5	16.6	95
2016	7	9	14:00	17.5	17	97
2016	7	9	15:00	17.7	17.1	96
2016	7	9	16:00	17.7	17.1	96
2016	7	9	17:00	18.2	17.2	94
2016	7	9	18:00	17.7	16.8	95
2016	7	9	19:00	17.6	16.6	94
2016	7	9	20:00	17.5	16.5	94
2016	7	9	21:00	17.4	16.4	94
2016	7	9	22:00	17.3	16.4	95
2016	7	9	23:00	17.1	16.1	94
2016	7	10	0:00	17.1	16.3	95
2016	7	10	1:00	17	16.4	96
2016	7	10	2:00	16.6	16.1	97
2016	7	10	3:00	15.6	15.3	98
2016	7	10	4:00	15.2	14.8	97
2016	7	10	5:00	15	14.6	98
2016	7	10	6:00	14.8	14.5	98
2016	7	10	7:00	15.1	14.5	96
2016	7	10	8:00	15.5	14.8	95

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	7	10	9:00	15.6	15	96
2016	7	10	10:00	16	15.1	94
2016	7	10	11:00	16.1	15.3	95
2016	7	10	12:00	17.2	15.3	89
2016	7	10	13:00	17.4	15.2	87
2016	7	10	14:00	18	15	83
2016	7	10	15:00	18.4	15.2	82
2016	7	10	16:00	19	15.8	81
2016	7	10	17:00	18.8	14.8	77
2016	7	10	18:00	18.4	14.4	77
2016	7	10	19:00	18	14.6	81
2016	7	10	20:00	15.9	14.2	90
2016	7	10	21:00	14.1	13.6	97
2016	7	10	22:00	13	12.7	99
2016	7	10	23:00	12.5	12.4	100
2016	7	11	0:00	12.2	12.1	100
2016	7	11	1:00	12	12	100
2016	7	11	2:00	12.2	12.2	100
2016	7	11	3:00	12.2	12.2	100
2016	7	11	4:00	14	14	100
2016	7	11	5:00	14	14	100
2016	7	11	6:00	14.4	14.4	100
2016	7	11	7:00	15.4	15.4	100
2016	7	11	8:00	17.7	16.2	91
2016	7	11	9:00	19.8	17.3	86
2016	7	11	10:00	21.4	16.9	75
2016	7	11	11:00	22.9	17.8	73
2016	7	11	12:00	24.9	18.1	66
2016	7	11	13:00	25.8	16.1	55
2016	7	11	14:00	26.8	14.4	47
2016	7	11	15:00	26.9	14.6	47
2016	7	11	16:00	26.9	15.1	48
2016	7	11	17:00	26.5	15	49
2016	7	11	18:00	25.8	13.6	47
2016	7	11	19:00	24.6	13	49
2016	7	11	20:00	22.4	13.2	56
2016	7	11	21:00	20.8	13.5	63
2016	7	11	22:00	20	14.4	70
2016	7	11	23:00	20.5	13.9	66
2016	7	12	0:00	19.9	14.9	73
2016	7	12	1:00	19.6	15.2	76

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	7	12	2:00	19.1	15	77
2016	7	12	3:00	18.2	14.9	81
2016	7	12	4:00	18.4	15.2	81
2016	7	12	5:00	18	15.3	84
2016	7	12	6:00	18.3	15.5	84
2016	7	12	7:00	19.9	15.5	76
2016	7	12	8:00	21	15.8	72
2016	7	12	9:00	22.9	14.1	58
2016	7	12	10:00	24.8	15.3	56
2016	7	12	11:00	26.1	15.6	52
2016	7	12	12:00	26.7	13.2	43
2016	7	12	13:00	27.2	15.3	48
2016	7	12	14:00	27.8	16.5	50
2016	7	12	15:00	28.4	17.8	53
2016	7	12	16:00	29.2	15.4	43
2016	7	12	17:00	28	16.5	50
2016	7	12	18:00	27.2	16.7	53
2016	7	12	19:00	26.2	17.3	58
2016	7	12	20:00	24.4	18.2	68
2016	7	12	21:00	23.9	17.2	66
2016	7	12	22:00	21.7	16.8	74
2016	7	12	23:00	20.3	16.8	80
2016	7	13	0:00	20.4	15.8	75
2016	7	13	1:00	18.6	16	85
2016	7	13	2:00	17.2	16.2	94
2016	7	13	3:00	17.1	16.2	95
2016	7	13	4:00	17.3	16.6	96
2016	7	13	5:00	20.7	16.8	78
2016	7	13	6:00	21.4	17.6	79
2016	7	13	7:00	23.1	17.8	72
2016	7	13	8:00	24.9	19	70
2016	7	13	9:00	27.6	20.2	64
2016	7	13	10:00	29.1	20.7	61
2016	7	13	11:00	30.1	21	58
2016	7	13	12:00	32.3	20.7	51
2016	7	13	13:00	33.1	19.7	45
2016	7	13	14:00	32.5	20.6	50
2016	7	13	15:00	32	21.5	54
2016	7	13	16:00	32	20.6	51
2016	7	13	17:00	31.8	19.9	49
2016	7	13	18:00	32	19.7	48

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	7	13	19:00	30.3	21	58
2016	7	13	20:00	28.7	21.1	64
2016	7	13	21:00	28.1	20.6	64
2016	7	13	22:00	26.9	20.8	70
2016	7	13	23:00	26.8	21.4	72
2016	7	14	0:00	25.9	21.5	77
2016	7	14	1:00	25.2	21.2	79
2016	7	14	2:00	24.3	20.8	81
2016	7	14	3:00	24	19.7	77
2016	7	14	4:00	23.1	19.8	82
2016	7	14	5:00	22.7	19.9	84
2016	7	14	6:00	24	20.5	81
2016	7	14	7:00	25.5	21.2	77
2016	7	14	8:00	27	21.5	72
2016	7	14	9:00	26.9	21.8	74
2016	7	14	10:00	26.1	22	79
2016	7	14	11:00	25.1	21.2	79
2016	7	14	12:00	23.4	22.3	94
2016	7	14	13:00	25.3	23.2	88
2016	7	14	14:00	25.3	21.4	79
2016	7	14	15:00	27.1	21.8	73
2016	7	14	16:00	27.3	21	69
2016	7	14	17:00	27	20.5	68
2016	7	14	18:00	27.1	20.4	67
2016	7	14	19:00	26	20.7	73
2016	7	14	20:00	25.7	20.6	74
2016	7	14	21:00	25	20.6	77
2016	7	14	22:00	24.4	20.9	81
2016	7	14	23:00	18.9	18	94
2016	7	15	0:00	19.7	18.9	95
2016	7	15	1:00	20.2	18.4	90
2016	7	15	2:00	20.5	18.8	90
2016	7	15	3:00	20.7	18.8	89
2016	7	15	4:00	20.4	18.4	88
2016	7	15	5:00	20.3	18.5	89
2016	7	15	6:00	20.7	18.7	88
2016	7	15	7:00	21.6	18.6	83
2016	7	15	8:00	22.1	19.2	84
2016	7	15	9:00	22.8	18.7	78
2016	7	15	10:00	24.1	19.3	75
2016	7	15	11:00	25.4	18.4	65

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	7	15	12:00	26.5	18.4	61
2016	7	15	13:00	27.2	18.8	60
2016	7	15	14:00	25.7	18.4	64
2016	7	15	15:00	26.7	18.6	61
2016	7	15	16:00	28	18.7	57
2016	7	15	17:00	26.8	18.5	61
2016	7	15	18:00	19.7	17.4	87
2016	7	15	19:00	19.6	18	90
2016	7	15	20:00	19.3	18.5	95
2016	7	15	21:00	19.4	18.8	97
2016	7	15	22:00	19.7	19	96
2016	7	15	23:00	19.4	18.7	96
2016	7	16	0:00	19.2	18.6	96
2016	7	16	1:00	19.2	18.5	96
2016	7	16	2:00	18.6	17.6	94
2016	7	16	3:00	17.6	15.7	89
2016	7	16	4:00	16.4	15	92
2016	7	16	5:00	15.6	14.8	95
2016	7	16	6:00	15.8	13.5	86
2016	7	16	7:00	17.1	13.9	81
2016	7	16	8:00	17.4	13.9	80
2016	7	16	9:00	18.9	15.5	81
2016	7	16	10:00	19.7	14	70
2016	7	16	11:00	20.7	14.6	68
2016	7	16	12:00	20.8	14.6	68
2016	7	16	13:00	21.7	15.6	68
2016	7	16	14:00	22.7	16	66
2016	7	16	15:00	22.6	14.7	61
2016	7	16	16:00	22.4	15.9	67
2016	7	16	17:00	23.2	16.3	65
2016	7	16	18:00	21.9	16.4	71
2016	7	16	19:00	21.3	16.6	75
2016	7	16	20:00	19.4	17.2	87
2016	7	16	21:00	19.5	18	91
2016	7	16	22:00	19.6	18	91
2016	7	16	23:00	19.6	17.7	89
2016	7	17	0:00	19.2	18.2	94
2016	7	17	1:00	18.9	17.8	93
2016	7	17	2:00	18.8	17.1	90
2016	7	17	3:00	19.3	16.8	86
2016	7	17	4:00	18.5	17	91

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	7	17	5:00	18.2	17.4	95
2016	7	17	6:00	18.7	17.8	94
2016	7	17	7:00	20	16.7	81
2016	7	17	8:00	21.5	16.4	73
2016	7	17	9:00	22.1	16	68
2016	7	17	10:00	22.8	15.5	63
2016	7	17	11:00	23.8	15.9	61
2016	7	17	12:00	24.2	15.9	60
2016	7	17	13:00	25	17	62
2016	7	17	14:00	25.7	15.2	52
2016	7	17	15:00	25.9	14.6	50
2016	7	17	16:00	26.1	13.9	47
2016	7	17	17:00	25.2	13	47
2016	7	17	18:00	24.8	13.9	51
2016	7	17	19:00	23.9	13.5	52
2016	7	17	20:00	20.6	15.7	74
2016	7	17	21:00	19	15.7	81
2016	7	17	22:00	21.6	16.6	73
2016	7	17	23:00	21.2	15.8	71
2016	7	18	0:00	21.1	15	68
2016	7	18	1:00	20.8	15.2	70
2016	7	18	2:00	21.4	14.8	66
2016	7	18	3:00	18.2	17.3	95
2016	7	18	4:00	19.4	17.2	87
2016	7	18	5:00	18.9	17.4	91
2016	7	18	6:00	21.5	17.6	78
2016	7	18	7:00	23.2	18.9	76
2016	7	18	8:00	25.3	19.9	72
2016	7	18	9:00	25.1	20.2	74
2016	7	18	10:00	24.9	21.4	81
2016	7	18	11:00	27.1	21.6	72
2016	7	18	12:00	24.6	18.5	69
2016	7	18	13:00	21.9	19.7	87
2016	7	18	14:00	26.2	21	73
2016	7	18	15:00	27.9	17.8	54
2016	7	18	16:00	27.7	16.1	49
2016	7	18	17:00	27.4	15	47
2016	7	18	18:00	26.3	15.2	50
2016	7	18	19:00	23.7	14.3	56
2016	7	18	20:00	21.6	14.1	62
2016	7	18	21:00	20.7	13.2	62

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	7	18	22:00	19.1	12.9	67
2016	7	18	23:00	19.5	13.2	67
2016	7	19	0:00	19.2	13.1	68
2016	7	19	1:00	17.9	13.2	74
2016	7	19	2:00	16.5	12.6	78
2016	7	19	3:00	15.6	12.9	84
2016	7	19	4:00	14.2	11.9	86
2016	7	19	5:00	14.4	11.5	83
2016	7	19	6:00	16.1	12	77
2016	7	19	7:00	17.4	12.7	74
2016	7	19	8:00	18.1	12.4	69
2016	7	19	9:00	19.3	12.2	64
2016	7	19	10:00	20.4	11.3	56
2016	7	19	11:00	19.1	11.5	61
2016	7	19	12:00	20.9	12.4	58
2016	7	19	13:00	21	11.1	53
2016	7	19	14:00	22.2	11.9	52
2016	7	19	15:00	21.8	9.8	46
2016	7	19	16:00	22.2	9.6	45
2016	7	19	17:00	22.1	8.8	43
2016	7	19	18:00	20.7	9.2	48
2016	7	19	19:00	20.1	8.9	48
2016	7	19	20:00	17.9	10.1	60
2016	7	19	21:00	15.1	11.2	78
2016	7	19	22:00	16.8	12.1	74
2016	7	19	23:00	16.6	12.6	77
2016	7	20	0:00	15.9	11.8	76
2016	7	20	1:00	15.3	11.8	80
2016	7	20	2:00	14.8	12	83
2016	7	20	3:00	14.3	11.9	86
2016	7	20	4:00	14.4	11.9	85
2016	7	20	5:00	14.7	12.3	85
2016	7	20	6:00	15.5	12.1	80
2016	7	20	7:00	16.7	12.4	76
2016	7	20	8:00	18.6	12.8	69
2016	7	20	9:00	21	13.1	61
2016	7	20	10:00	22.7	12.8	54
2016	7	20	11:00	23.7	12.2	49
2016	7	20	12:00	24.4	12.8	48
2016	7	20	13:00	25.1	11.6	43
2016	7	20	14:00	25.6	10.7	39

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	7	20	15:00	25.7	10.9	40
2016	7	20	16:00	26	11.8	41
2016	7	20	17:00	25.7	11.8	42
2016	7	20	18:00	25	12.2	45
2016	7	20	19:00	23.8	12.8	50
2016	7	20	20:00	22.2	14	60
2016	7	20	21:00	21.4	14.4	64
2016	7	20	22:00	20.4	14.7	70
2016	7	20	23:00	20.5	14.5	68
2016	7	21	0:00	19.5	13.9	70
2016	7	21	1:00	18.6	14.3	76
2016	7	21	2:00	18.6	14.4	76
2016	7	21	3:00	18.1	13.8	76
2016	7	21	4:00	17.7	13.3	76
2016	7	21	5:00	17.4	13.5	78
2016	7	21	6:00	17.7	13.3	75
2016	7	21	7:00	18.9	13.6	71
2016	7	21	8:00	20.3	14.7	70
2016	7	21	9:00	22.1	15.2	65
2016	7	21	10:00	24.2	15.9	60
2016	7	21	11:00	25.9	15.6	53
2016	7	21	12:00	27.7	15.1	46
2016	7	21	13:00	28.6	16.9	49
2016	7	21	14:00	29.2	17.1	48
2016	7	21	15:00	30	15.9	43
2016	7	21	16:00	29.7	16.7	45
2016	7	21	17:00	29.6	17.2	47
2016	7	21	18:00	28.3	16.8	50
2016	7	21	19:00	27.9	16.3	49
2016	7	21	20:00	27.1	15.5	49
2016	7	21	21:00	26.6	15.2	50
2016	7	21	22:00	26.4	15.5	51
2016	7	21	23:00	26.2	15.2	51
2016	7	22	0:00	26.3	14.8	49
2016	7	22	1:00	20.7	18.6	88
2016	7	22	2:00	21.9	19.5	87
2016	7	22	3:00	22.1	19	83
2016	7	22	4:00	23.1	19.2	79
2016	7	22	5:00	22.9	19.2	80
2016	7	22	6:00	23.5	19.3	77
2016	7	22	7:00	23.6	20.1	81

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	7	22	8:00	25.1	21.4	80
2016	7	22	9:00	25.9	21.5	77
2016	7	22	10:00	25.9	21.4	76
2016	7	22	11:00	27	21.4	71
2016	7	22	12:00	28.8	20.3	60
2016	7	22	13:00	29.8	19.8	55
2016	7	22	14:00	30.8	17.3	45
2016	7	22	15:00	31.3	16.8	42
2016	7	22	16:00	31	16.5	42
2016	7	22	17:00	29.7	17.7	49
2016	7	22	18:00	27.8	18.2	56
2016	7	22	19:00	26.5	18.6	62
2016	7	22	20:00	24.1	19.2	74
2016	7	22	21:00	24.6	19.8	75
2016	7	22	22:00	25.4	17.4	61
2016	7	22	23:00	23.9	17.9	70
2016	7	23	0:00	22.4	18.7	80
2016	7	23	1:00	21.7	18.9	85
2016	7	23	2:00	21.6	18.8	84
2016	7	23	3:00	20.7	18.1	85
2016	7	23	4:00	20.3	18.1	87
2016	7	23	5:00	20.2	18.2	89
2016	7	23	6:00	20.7	18.3	86
2016	7	23	7:00	21.3	18.7	85
2016	7	23	8:00	21.4	18.7	85
2016	7	23	9:00	22.9	19.2	80
2016	7	23	10:00	25.2	20	73
2016	7	23	11:00	27.4	20.1	64
2016	7	23	12:00	26.3	18.7	63
2016	7	23	13:00	19.5	17.9	90
2016	7	23	14:00	18.3	17.7	97
2016	7	23	15:00	19.3	18.8	97
2016	7	23	16:00	20.4	19.1	92
2016	7	23	17:00	21.4	19.5	89
2016	7	23	18:00	22.1	19.5	85
2016	7	23	19:00	21.4	19.7	91
2016	7	23	20:00	20.4	19.5	95
2016	7	23	21:00	20.6	19.5	94
2016	7	23	22:00	20.1	18.8	92
2016	7	23	23:00	18.4	17	92
2016	7	24	0:00	18.7	15.2	80

Year	Month	Day	Time	Temp (°C)	Dew Point Temp (°C)	Rel Hum (%)
2016	7	24	1:00	17.6	13.5	77
2016	7	24	2:00	16.5	12.2	76
2016	7	24	3:00	13.6	12.4	92
2016	7	24	4:00	12.6	12.1	97
2016	7	24	5:00	12.1	11.9	99
2016	7	24	6:00	15.7	14.4	92
2016	7	24	7:00	18.2	13.9	76
2016	7	24	8:00	18.8	13.6	72
2016	7	24	9:00	20	12.8	63
2016	7	24	10:00	21.3	14.6	65
2016	7	24	11:00	22	14.6	63
2016	7	24	12:00	22.5	14.9	62
2016	7	24	13:00	23.6	14.9	58
2016	7	24	14:00	23.5	13.8	55
2016	7	24	15:00	24.2	12.8	49
2016	7	24	16:00	24.4	13.3	50
2016	7	24	17:00	24.7	14.5	53
2016	7	24	18:00	23.5	17.3	68
2016	7	24	19:00	21.3	16.7	75
2016	7	24	20:00	20.5	17.6	83
2016	7	24	21:00	19.7	17.4	86
2016	7	24	22:00	20.3	17.7	86
2016	7	24	23:00	21.1	15.8	72
2016	7	25	0:00	21	16	73
2016	7	25	1:00	20.3	15.9	76
2016	7	25	2:00	19.8	16.4	81
2016	7	25	3:00	18.8	16.2	85
2016	7	25	4:00	16.5	15.3	93
2016	7	25	5:00	16.2	15.6	96
2016	7	25	6:00	18.4	17.5	95
2016	7	25	7:00	20.3	17	81
2016	7	25	8:00	20.6	17.5	83
2016	7	25	9:00	20	17.7	87
2016	7	25	10:00	20.5	18.6	89
2016	7	25	11:00	21.9	19.2	84
2016	7	25	12:00	22.4	19.6	84
2016	7	25	13:00	23.5	20	80
2016	7	25	14:00	23.6	20.4	82
2016	7	25	15:00	25.6	21.3	77
2016	7	25	16:00	23.4	22.1	93
2016	7	25	17:00	24.5	22.2	87

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	7	25	18:00	24.7	22	85
2016	7	25	19:00	24	21.8	87
2016	7	25	20:00	23.9	21.6	87
2016	7	25	21:00	22.4	20	86
2016	7	25	22:00	21.1	19.5	91
2016	7	25	23:00	20.4	19.7	96
2016	7	26	0:00	21	20.4	96
2016	7	26	1:00	21.3	20	93
2016	7	26	2:00	20.9	19.3	91
2016	7	26	3:00	20.5	18.5	88
2016	7	26	4:00	19.7	17.6	88
2016	7	26	5:00	19.2	17.3	89
2016	7	26	6:00	19.7	17.7	88
2016	7	26	7:00	20.7	18.3	86
2016	7	26	8:00	22.3	19.1	82
2016	7	26	9:00	22.3	19.1	82
2016	7	26	10:00	22.7	18.8	78
2016	7	26	11:00	24.8	19.6	73
2016	7	26	12:00	24.5	18.3	68
2016	7	26	13:00	24.7	17.9	66
2016	7	26	14:00	25.4	18.8	67
2016	7	26	15:00	25.9	16.9	58
2016	7	26	16:00	27	17.4	56
2016	7	26	17:00	26.7	17.8	58
2016	7	26	18:00	26.3	17.9	60
2016	7	26	19:00	25	18.1	65
2016	7	26	20:00	23	17.8	72
2016	7	26	21:00	20.5	18	86
2016	7	26	22:00	19.7	18.1	91
2016	7	26	23:00	19	17.5	91
2016	7	27	0:00	19.1	18.5	96
2016	7	27	1:00	18.7	17.5	93
2016	7	27	2:00	19.9	18.1	90
2016	7	27	3:00	19.2	17.6	90
2016	7	27	4:00	19.6	18	90
2016	7	27	5:00	19.2	18.1	93
2016	7	27	6:00	20.2	18.3	89
2016	7	27	7:00	21.2	18.5	85
2016	7	27	8:00	22.7	18.5	77
2016	7	27	9:00	24.2	19.6	76
2016	7	27	10:00	25.6	19.7	70

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	7	27	11:00	26.7	18.6	61
2016	7	27	12:00	28	17.3	52
2016	7	27	13:00	28.7	16.5	48
2016	7	27	14:00	29.1	17.7	50
2016	7	27	15:00	29.1	17.5	50
2016	7	27	16:00	29.3	17.4	49
2016	7	27	17:00	29.2	18.4	52
2016	7	27	18:00	28.6	17.7	52
2016	7	27	19:00	27.3	18.3	58
2016	7	27	20:00	26	18.3	63
2016	7	27	21:00	25.5	18.6	66
2016	7	27	22:00	24.8	18	66
2016	7	27	23:00	23.3	18.3	74
2016	7	28	0:00	21.6	19.1	86
2016	7	28	1:00	20.9	19.1	89
2016	7	28	2:00	20.6	19.1	91
2016	7	28	3:00	19.7	18.8	95
2016	7	28	4:00	21	18.3	85
2016	7	28	5:00	20.3	17.2	82
2016	7	28	6:00	21	17.5	80
2016	7	28	7:00	21.3	17.1	77
2016	7	28	8:00	22.4	17.4	73
2016	7	28	9:00	23	17.6	72
2016	7	28	10:00	23.3	17.6	70
2016	7	28	11:00	23.2	17.8	72
2016	7	28	12:00	24	18.7	72
2016	7	28	13:00	25.2	18.7	67
2016	7	28	14:00	25.9	15.5	53
2016	7	28	15:00	26.7	17.1	56
2016	7	28	16:00	27.3	16.5	52
2016	7	28	17:00	26.7	16	52
2016	7	28	18:00	25.5	17.5	61
2016	7	28	19:00	24.9	18.9	69
2016	7	28	20:00	21.6	18.2	81
2016	7	28	21:00	20.3	16.5	78
2016	7	28	22:00	18.4	16.1	86
2016	7	28	23:00	17.4	15.9	91
2016	7	29	0:00	16.2	15	92
2016	7	29	1:00	16.8	14.8	88
2016	7	29	2:00	17.9	14	78
2016	7	29	3:00	17.2	13.6	79

Year	Month	Day	Time	Temp (°C)	Dew Point Temp (°C)	Rel Hum (%)
2016	7	29	4:00	15.5	13.4	88
2016	7	29	5:00	15.8	13.4	86
2016	7	29	6:00	17.9	14	78
2016	7	29	7:00	19.1	14.2	73
2016	7	29	8:00	20.8	13.8	64
2016	7	29	9:00	21.9	13.5	59
2016	7	29	10:00	22.7	13.8	57
2016	7	29	11:00	23.8	14	54
2016	7	29	12:00	24.3	12	46
2016	7	29	13:00	24.7	14.3	52
2016	7	29	14:00	24.6	13.8	51
2016	7	29	15:00	25.7	13.4	47
2016	7	29	16:00	26	13.9	47
2016	7	29	17:00	25.3	13.8	49
2016	7	29	18:00	24.5	15.1	56
2016	7	29	19:00	23.7	13.9	54
2016	7	29	20:00	22.3	12.9	55
2016	7	29	21:00	20.5	13.2	63
2016	7	29	22:00	20.5	12.8	61
2016	7	29	23:00	20	12.8	63
2016	7	30	0:00	18.3	13.8	75
2016	7	30	1:00	17	13.9	82
2016	7	30	2:00	16.4	14	86
2016	7	30	3:00	15.8	14.2	90
2016	7	30	4:00	16.1	14.3	89
2016	7	30	5:00	16.3	13.8	85
2016	7	30	6:00	18	13.9	77
2016	7	30	7:00	19.3	13.1	67
2016	7	30	8:00	20.2	12.6	61
2016	7	30	9:00	20.9	12.3	58
2016	7	30	10:00	22	11.5	51
2016	7	30	11:00	23.2	11	46
2016	7	30	12:00	23.8	12.9	50
2016	7	30	13:00	24.1	12.3	48
2016	7	30	14:00	24.9	12.5	46
2016	7	30	15:00	25.5	12.8	45
2016	7	30	16:00	25.3	12.3	44
2016	7	30	17:00	24.6	11.2	43
2016	7	30	18:00	24.4	10.9	43
2016	7	30	19:00	23.2	12.7	52
2016	7	30	20:00	18.8	14.7	77

Year	Month	Day	Time	Temp (°C)	Dew Point Temp (°C)	Rel Hum (%)
2016	7	30	21:00	18.4	14.5	78
2016	7	30	22:00	17.7	14.3	81
2016	7	30	23:00	16.5	14.3	87
2016	7	31	0:00	15.1	14	93
2016	7	31	1:00	14.5	13.7	95
2016	7	31	2:00	14.4	13.7	96
2016	7	31	3:00	14.2	13.7	97
2016	7	31	4:00	14.4	14.1	98
2016	7	31	5:00	14.1	13.9	99
2016	7	31	6:00	15.8	15.6	99
2016	7	31	7:00	20	16.3	79
2016	7	31	8:00	22.3	17.2	73
2016	7	31	9:00	23.9	15.1	58
2016	7	31	10:00	24.8	12.4	46
2016	7	31	11:00	25.8	12.5	44
2016	7	31	12:00	26.7	11.8	40
2016	7	31	13:00	27.2	11.7	38
2016	7	31	14:00	27.6	13.2	41
2016	7	31	15:00	27.5	11.9	38
2016	7	31	16:00	27.8	12.5	39
2016	7	31	17:00	27.5	13.3	42
2016	7	31	18:00	26.6	13.4	44
2016	7	31	19:00	25.4	13.4	47
2016	7	31	20:00	19.6	15.3	76
2016	7	31	21:00	18.8	14.7	77
2016	7	31	22:00	17.9	14.9	83
2016	7	31	23:00	17.7	14.4	81
2016	8	1	0:00	17.9	15.3	85
2016	8	1	1:00	16.5	14.8	90
2016	8	1	2:00	17	14.9	87
2016	8	1	3:00	16.8	14.5	86
2016	8	1	4:00	17.3	15.3	88
2016	8	1	5:00	18.7	15.6	82
2016	8	1	6:00	18.7	15.5	82
2016	8	1	7:00	19.3	15.6	79
2016	8	1	8:00	19.8	15.8	78
2016	8	1	9:00	20.7	16.2	76
2016	8	1	10:00	23	16	65
2016	8	1	11:00	24.8	16.4	60
2016	8	1	12:00	25.6	16.6	58
2016	8	1	13:00	25.7	16.6	57

Year	Month	Day	Time	Temp (°C)	Dew Point Temp (°C)	Rel Hum (%)
2016	8	1	14:00	26.2	17.5	59
2016	8	1	15:00	26.3	16.5	55
2016	8	1	16:00	26	16.5	56
2016	8	1	17:00	25.3	15.8	56
2016	8	1	18:00	24.1	15.6	59
2016	8	1	19:00	23	16	65
2016	8	1	20:00	22.1	16.5	71
2016	8	1	21:00	21.5	17.3	77
2016	8	1	22:00	18	16.2	89
2016	8	1	23:00	17.4	16.4	94
2016	8	2	0:00	16.3	15.9	97
2016	8	2	1:00	16.5	16.2	98
2016	8	2	2:00	16.5	16.1	98
2016	8	2	3:00	15.5	15.1	97
2016	8	2	4:00	15.4	15.2	99
2016	8	2	5:00	15.2	14.8	98
2016	8	2	6:00	17.9	16.8	94
2016	8	2	7:00	19.9	17.5	86
2016	8	2	8:00	21.4	17.4	78
2016	8	2	9:00	23	17.3	70
2016	8	2	10:00	24.5	16.1	60
2016	8	2	11:00	25.9	14.9	50
2016	8	2	12:00	26.6	13.6	45
2016	8	2	13:00	27.3	13.8	44
2016	8	2	14:00	28.1	14.3	43
2016	8	2	15:00	28.4	14.3	42
2016	8	2	16:00	28	11.3	35
2016	8	2	17:00	28.2	11.9	37
2016	8	2	18:00	27	13.2	43
2016	8	2	19:00	25.8	14.4	49
2016	8	2	20:00	20.5	15.8	75
2016	8	2	21:00	19.1	15.6	81
2016	8	2	22:00	17.9	15.3	85
2016	8	2	23:00	18	15.9	88
2016	8	3	0:00	16.7	15.6	93
2016	8	3	1:00	16.3	15.5	95
2016	8	3	2:00	15.6	15.2	97
2016	8	3	3:00	15.6	15.4	98
2016	8	3	4:00	15.6	15.4	99
2016	8	3	5:00	14.9	14.7	99
2016	8	3	6:00	16.6	16.2	97

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	8	3	7:00	21.4	17.4	78
2016	8	3	8:00	23.2	16.7	67
2016	8	3	9:00	24.8	16.4	59
2016	8	3	10:00	26.2	16.2	54
2016	8	3	11:00	26.7	16.4	53
2016	8	3	12:00	27.8	15.4	47
2016	8	3	13:00	28.3	15.3	45
2016	8	3	14:00	29.2	15.6	44
2016	8	3	15:00	29.6	13.4	37
2016	8	3	16:00	29.8	14.3	39
2016	8	3	17:00	29.7	12.8	36
2016	8	3	18:00	28.1	15.5	46
2016	8	3	19:00	26.9	16	51
2016	8	3	20:00	25.4	17	60
2016	8	3	21:00	24.9	17.6	64
2016	8	3	22:00	25	17.7	64
2016	8	3	23:00	24.6	16.9	62
2016	8	4	0:00	24	16.5	63
2016	8	4	1:00	23.4	16.2	64
2016	8	4	2:00	22.7	16.7	69
2016	8	4	3:00	22.2	16.7	71
2016	8	4	4:00	20.4	16.6	79
2016	8	4	5:00	21.1	16.5	75
2016	8	4	6:00	21.7	17.2	76
2016	8	4	7:00	21.9	17.5	76
2016	8	4	8:00	23.5	17.2	68
2016	8	4	9:00	25	18.4	67
2016	8	4	10:00	26.6	18.4	61
2016	8	4	11:00	27.9	18.3	56
2016	8	4	12:00	29.4	18.4	52
2016	8	4	13:00	30.3	18.5	49
2016	8	4	14:00	31	16.6	42
2016	8	4	15:00	31.2	16.7	42
2016	8	4	16:00	32	16.5	39
2016	8	4	17:00	31.4	16.1	40
2016	8	4	18:00	30.7	16	41
2016	8	4	19:00	28.9	18.1	52
2016	8	4	20:00	26.6	19.7	66
2016	8	4	21:00	25.4	19.9	72
2016	8	4	22:00	24.4	20	77
2016	8	4	23:00	24	18.8	73

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	8	5	0:00	24.3	18.2	69
2016	8	5	1:00	23.8	18.5	72
2016	8	5	2:00	22.7	18.2	76
2016	8	5	3:00	21.4	18.9	86
2016	8	5	4:00	19.7	18.4	92
2016	8	5	5:00	18.4	17.9	97
2016	8	5	6:00	21.8	19.9	89
2016	8	5	7:00	23.8	19.2	76
2016	8	5	8:00	25.6	20.1	72
2016	8	5	9:00	27.2	18.8	60
2016	8	5	10:00	28	18.9	58
2016	8	5	11:00	28.8	19.4	57
2016	8	5	12:00	30.5	18.6	49
2016	8	5	13:00	31.8	18.7	46
2016	8	5	14:00	32.2	16.9	40
2016	8	5	15:00	32.3	14.9	35
2016	8	5	16:00	32.6	16.2	37
2016	8	5	17:00	32.1	15.5	37
2016	8	5	18:00	30.7	17.3	45
2016	8	5	19:00	30	18	49
2016	8	5	20:00	29.3	18.5	52
2016	8	5	21:00	28	19.2	59
2016	8	5	22:00	26.6	19.1	64
2016	8	5	23:00	24.2	19.9	77
2016	8	6	0:00	23.3	20.1	82
2016	8	6	1:00	22.8	20.2	85
2016	8	6	2:00	22.4	20.4	89
2016	8	6	3:00	22	20.4	91
2016	8	6	4:00	21.6	20.4	93
2016	8	6	5:00	21.4	20.3	93
2016	8	6	6:00	21.7	20.5	93
2016	8	6	7:00	22.1	19.8	87
2016	8	6	8:00	23.3	19.2	78
2016	8	6	9:00	24.4	18.7	71
2016	8	6	10:00	24.8	17.1	62
2016	8	6	11:00	25.7	15.3	53
2016	8	6	12:00	27.4	14.7	46
2016	8	6	13:00	27.5	15.1	47
2016	8	6	14:00	27.7	13.1	41
2016	8	6	15:00	27.5	12.3	39
2016	8	6	16:00	27	9.8	34

Year	Month	Day	Time	Temp (°C)	Dew Point Temp (°C)	Rel Hum (%)
2016	8	6	17:00	25.2	11.8	43
2016	8	6	18:00	25.1	12.2	44
2016	8	6	19:00	22.8	13.2	55
2016	8	6	20:00	21.5	13.1	59
2016	8	6	21:00	21.3	13.2	60
2016	8	6	22:00	21.1	12.8	59
2016	8	6	23:00	21.5	13	58
2016	8	7	0:00	20.3	13.2	64
2016	8	7	1:00	20.2	13.5	65
2016	8	7	2:00	18.6	14	75
2016	8	7	3:00	18.4	15	81
2016	8	7	4:00	18.9	14.1	74
2016	8	7	5:00	18.6	14.3	76
2016	8	7	6:00	19.1	15.2	78
2016	8	7	7:00	19.7	15.2	75
2016	8	7	8:00	21.5	15.5	69
2016	8	7	9:00	22.1	15.2	65
2016	8	7	10:00	22.8	14.3	59
2016	8	7	11:00	24.4	13.9	52
2016	8	7	12:00	25.4	13.3	47
2016	8	7	13:00	23.6	15	58
2016	8	7	14:00	24.5	13	49
2016	8	7	15:00	24.4	13.2	50
2016	8	7	16:00	24.8	13	48
2016	8	7	17:00	24.3	12.9	49
2016	8	7	18:00	23.5	10.7	44
2016	8	7	19:00	21.7	11.1	51
2016	8	7	20:00	20.8	11.8	57
2016	8	7	21:00	18.9	12.2	65
2016	8	7	22:00	17.7	12.6	72
2016	8	7	23:00	17.9	12.3	70
2016	8	8	0:00	17.2	13.1	76
2016	8	8	1:00	18	13.7	76
2016	8	8	2:00	17.3	13.1	76
2016	8	8	3:00	17.6	14	79
2016	8	8	4:00	16.4	13.8	85
2016	8	8	5:00	16	12.9	82
2016	8	8	6:00	16.5	12.9	79
2016	8	8	7:00	18.9	13.5	71
2016	8	8	8:00	20.5	13.5	64
2016	8	8	9:00	22.7	13.8	57

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	8	8	10:00	24.3	12.9	49
2016	8	8	11:00	26.2	12.5	43
2016	8	8	12:00	26.6	10.7	37
2016	8	8	13:00	28	12.8	39
2016	8	8	14:00	27.6	11.5	37
2016	8	8	15:00	28	9.6	32
2016	8	8	16:00	27.8	10.1	33
2016	8	8	17:00	27.2	12.2	39
2016	8	8	18:00	26.1	12.7	43
2016	8	8	19:00	23.9	13.3	51
2016	8	8	20:00	21.7	13.2	59
2016	8	8	21:00	20.8	12.5	59
2016	8	8	22:00	16.8	12.7	77
2016	8	8	23:00	15.4	12.7	84
2016	8	9	0:00	14.7	12.6	87
2016	8	9	1:00	13.9	11.7	87
2016	8	9	2:00	13.6	12.1	91
2016	8	9	3:00	12.5	11.6	94
2016	8	9	4:00	11.3	10.4	94
2016	8	9	5:00	11.2	10.8	97
2016	8	9	6:00	13.4	12.9	97
2016	8	9	7:00	17.7	13.5	76
2016	8	9	8:00	21.7	9.8	47
2016	8	9	9:00	22.7	8.1	39
2016	8	9	10:00	23.3	9.1	40
2016	8	9	11:00	24.5	9	37
2016	8	9	12:00	26.2	10.5	37
2016	8	9	13:00	27	11.3	37
2016	8	9	14:00	27.2	11.3	37
2016	8	9	15:00	28.7	11.9	35
2016	8	9	16:00	28.1	12.2	37
2016	8	9	17:00	28.1	13.5	41
2016	8	9	18:00	27.1	14.3	46
2016	8	9	19:00	25.7	15.2	52
2016	8	9	20:00	25	14.5	52
2016	8	9	21:00	24.2	15	57
2016	8	9	22:00	23.7	15.7	61
2016	8	9	23:00	20.9	15	69
2016	8	10	0:00	17.5	14.9	85
2016	8	10	1:00	17.5	14.9	85
2016	8	10	2:00	17.4	14.8	85

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	8	10	3:00	17.5	15.4	88
2016	8	10	4:00	18.3	16.2	88
2016	8	10	5:00	18.8	16.5	86
2016	8	10	6:00	21.3	16.7	75
2016	8	10	7:00	22.1	17.6	76
2016	8	10	8:00	23.3	17.8	71
2016	8	10	9:00	26.8	19	63
2016	8	10	10:00	28.1	19.9	61
2016	8	10	11:00	28.9	19.6	57
2016	8	10	12:00	29	20.2	59
2016	8	10	13:00	31.1	20.5	53
2016	8	10	14:00	31.5	18.8	47
2016	8	10	15:00	32.1	19.7	48
2016	8	10	16:00	31.8	19.8	49
2016	8	10	17:00	30.8	20	52
2016	8	10	18:00	30.4	19.9	53
2016	8	10	19:00	29	20.1	59
2016	8	10	20:00	27.8	20.1	63
2016	8	10	21:00	27.3	20.5	67
2016	8	10	22:00	27.1	21.4	71
2016	8	10	23:00	26.2	21.2	74
2016	8	11	0:00	25.4	20.3	73
2016	8	11	1:00	24.8	20	75
2016	8	11	2:00	24	20	78
2016	8	11	3:00	23.3	19.7	80
2016	8	11	4:00	22.7	19.3	81
2016	8	11	5:00	22	19.2	84
2016	8	11	6:00	22.1	19.5	85
2016	8	11	7:00	23.3	19.8	81
2016	8	11	8:00	25.1	20.1	74
2016	8	11	9:00	26.6	20.2	68
2016	8	11	10:00	26.8	20.1	67
2016	8	11	11:00	28.5	18.7	55
2016	8	11	12:00	29.2	19.2	55
2016	8	11	13:00	29.2	19.7	57
2016	8	11	14:00	30.2	19.6	53
2016	8	11	15:00	30.9	20	52
2016	8	11	16:00	31.3	19.4	49
2016	8	11	17:00	31.6	19.3	48
2016	8	11	18:00	30.5	19.6	52
2016	8	11	19:00	27.6	20.5	65

Year	Month	Day	Time	Temp (°C)	Dew Point Temp (°C)	Rel Hum (%)
2016	8	11	20:00	25.4	20.5	74
2016	8	11	21:00	27.5	21.3	69
2016	8	11	22:00	26.5	21.8	76
2016	8	11	23:00	26.9	21.7	73
2016	8	12	0:00	26.6	20.9	71
2016	8	12	1:00	26.1	19.2	66
2016	8	12	2:00	25.5	19.4	69
2016	8	12	3:00	25.1	19.1	70
2016	8	12	4:00	24.7	19.1	71
2016	8	12	5:00	23	20.4	85
2016	8	12	6:00	22.3	20.3	89
2016	8	12	7:00	21.6	20	91
2016	8	12	8:00	20.3	19.7	96
2016	8	12	9:00	19.5	18.5	94
2016	8	12	10:00	20.4	18.3	88
2016	8	12	11:00	20.4	17.8	85
2016	8	12	12:00	20.1	17.3	84
2016	8	12	13:00	19.8	17.2	85
2016	8	12	14:00	19.5	17.2	87
2016	8	12	15:00	18.5	17.4	94
2016	8	12	16:00	18.5	17.5	94
2016	8	12	17:00	18.8	17.4	92
2016	8	12	18:00	18.2	17.1	93
2016	8	12	19:00	18.4	16.4	89
2016	8	12	20:00	18.6	16.4	87
2016	8	12	21:00	18.7	16.8	89
2016	8	12	22:00	19	16.9	88
2016	8	12	23:00	19.2	16.9	87
2016	8	13	0:00	18.9	17.5	91
2016	8	13	1:00	19	17.1	89
2016	8	13	2:00	19.2	16.6	85
2016	8	13	3:00	18.3	16.4	89
2016	8	13	4:00	17.5	16.5	94
2016	8	13	5:00	16.7	15.8	94
2016	8	13	6:00	16.3	15.4	94
2016	8	13	7:00	16.7	15.7	94
2016	8	13	8:00	17.1	15.7	91
2016	8	13	9:00	17.6	15.9	90
2016	8	13	10:00	17.7	16.7	94
2016	8	13	11:00	17.9	17.4	97
2016	8	13	12:00	18.5	18	97

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	8	13	13:00	18.9	18.3	96
2016	8	13	14:00	19.4	18.7	96
2016	8	13	15:00	19.8	19	95
2016	8	13	16:00	20	19.4	96
2016	8	13	17:00	19.9	19.4	97
2016	8	13	18:00	20	19.6	97
2016	8	13	19:00	19.8	19.4	98
2016	8	13	20:00	19.6	19.2	97
2016	8	13	21:00	19.2	18.9	98
2016	8	13	22:00	19	18.8	99
2016	8	13	23:00	18.8	18.6	99
2016	8	14	0:00	18.4	18.3	99
2016	8	14	1:00	18.2	18.1	99
2016	8	14	2:00	17.7	17.6	99
2016	8	14	3:00	17.5	17.5	100
2016	8	14	4:00	17.4	17.3	100
2016	8	14	5:00	17.3	17.1	99
2016	8	14	6:00	17.2	17.1	99
2016	8	14	7:00	17.6	17.4	99
2016	8	14	8:00	18.3	17.9	98
2016	8	14	9:00	19.7	19.3	97
2016	8	14	10:00	21.6	20.9	96
2016	8	14	11:00	24	21.8	87
2016	8	14	12:00	24.4	21.1	82
2016	8	14	13:00	23.8	20.2	80
2016	8	14	14:00	24.7	19.8	74
2016	8	14	15:00	25.2	19.9	73
2016	8	14	16:00	24.1	18.3	70
2016	8	14	17:00	23.4	18.2	73
2016	8	14	18:00	22.2	17.9	76
2016	8	14	19:00	21.3	17.8	80
2016	8	14	20:00	21	17.5	80
2016	8	14	21:00	20.6	17.4	82
2016	8	14	22:00	19.4	16.9	85
2016	8	14	23:00	19.6	17.4	87
2016	8	15	0:00	19.2	17	87
2016	8	15	1:00	19.4	17	86
2016	8	15	2:00	18.6	17.2	92
2016	8	15	3:00	17.5	16.8	95
2016	8	15	4:00	17	16.6	97
2016	8	15	5:00	16.9	16.6	98

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	8	15	6:00	18.3	17.4	95
2016	8	15	7:00	19.3	17.8	91
2016	8	15	8:00	20.5	17.3	82
2016	8	15	9:00	21.5	17.4	77
2016	8	15	10:00	22.8	17.1	70
2016	8	15	11:00	24	16	61
2016	8	15	12:00	25	16.8	61
2016	8	15	13:00	25.7	16.6	57
2016	8	15	14:00	25.5	16.2	57
2016	8	15	15:00	26.5	17.1	56
2016	8	15	16:00	26.2	16.1	54
2016	8	15	17:00	25.8	16.4	56
2016	8	15	18:00	24.7	15.6	57
2016	8	15	19:00	23.4	15.9	63
2016	8	15	20:00	21.7	16.5	72
2016	8	15	21:00	19.1	16.9	87
2016	8	15	22:00	18.5	16.7	89
2016	8	15	23:00	18.7	16.8	88
2016	8	16	0:00	18.6	16.1	86
2016	8	16	1:00	18.5	16.6	89
2016	8	16	2:00	19.3	17.9	92
2016	8	16	3:00	17.4	16.7	96
2016	8	16	4:00	17.5	17.1	98
2016	8	16	5:00	16	15.7	99
2016	8	16	6:00	17.3	17.1	99
2016	8	16	7:00	20.2	18.6	91
2016	8	16	8:00	21.1	18.3	84
2016	8	16	9:00	21.7	18.2	81
2016	8	16	10:00	22.8	18.4	76
2016	8	16	11:00	23.9	18.6	72
2016	8	16	12:00	23.4	19.6	79
2016	8	16	13:00	21.5	20.4	94
2016	8	16	14:00	19.8	17.1	84
2016	8	16	15:00	19.6	17.9	90
2016	8	16	16:00	20.2	19.6	96
2016	8	16	17:00	19.8	19.5	98
2016	8	16	18:00	19.6	19.4	99
2016	8	16	19:00	18.8	18.7	99
2016	8	16	20:00	17.8	17.7	100
2016	8	16	21:00	18	17.9	100
2016	8	16	22:00	18.2	18.1	100

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	8	16	23:00	18	17.9	100
2016	8	17	0:00	17	16.7	98
2016	8	17	1:00	16.9	16.5	97
2016	8	17	2:00	16.7	16.2	97
2016	8	17	3:00	17	15.9	94
2016	8	17	4:00	17.3	16.1	93
2016	8	17	5:00	17.6	16.1	91
2016	8	17	6:00	17.9	16.3	90
2016	8	17	7:00	18.6	17.2	92
2016	8	17	8:00	19.9	17.1	84
2016	8	17	9:00	21.4	17.4	78
2016	8	17	10:00	22.6	18.7	79
2016	8	17	11:00	23.4	18.1	72
2016	8	17	12:00	24.2	16.8	63
2016	8	17	13:00	25.3	17.2	61
2016	8	17	14:00	26	18.2	62
2016	8	17	15:00	25.8	17.1	58
2016	8	17	16:00	25.7	17.8	61
2016	8	17	17:00	25.4	17.4	61
2016	8	17	18:00	24.7	16.7	61
2016	8	17	19:00	23.2	17.8	71
2016	8	17	20:00	22.5	17.8	75
2016	8	17	21:00	22.2	17.7	76
2016	8	17	22:00	22.4	17.1	72
2016	8	17	23:00	22	18.4	80
2016	8	18	0:00	21.9	18.3	80
2016	8	18	1:00	21.6	17.2	76
2016	8	18	2:00	20.9	16.7	77
2016	8	18	3:00	19.7	17.4	86
2016	8	18	4:00	19.2	17.7	91
2016	8	18	5:00	19.4	18.1	92
2016	8	18	6:00	19.9	17.9	88
2016	8	18	7:00	20.8	18.1	85
2016	8	18	8:00	21.9	19.1	84
2016	8	18	9:00	22.6	19.4	82
2016	8	18	10:00	23.3	19.2	78
2016	8	18	11:00	23.9	19.6	77
2016	8	18	12:00	24.1	19.4	75
2016	8	18	13:00	25	19.5	71
2016	8	18	14:00	26	18.4	63
2016	8	18	15:00	26.4	19.1	64

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	8	18	16:00	25.2	19.5	71
2016	8	18	17:00	26	18.8	64
2016	8	18	18:00	25.4	18.7	67
2016	8	18	19:00	22.1	17.1	74
2016	8	18	20:00	20.5	16	75
2016	8	18	21:00	20.1	15.1	73
2016	8	18	22:00	18.1	14.9	81
2016	8	18	23:00	16.3	14.9	92
2016	8	19	0:00	15.9	15	94
2016	8	19	1:00	14.9	14.4	97
2016	8	19	2:00	14.6	14.3	98
2016	8	19	3:00	14.5	14.3	99
2016	8	19	4:00	14	13.9	99
2016	8	19	5:00	13.8	13.7	100
2016	8	19	6:00	15.5	15.4	99
2016	8	19	7:00	19.2	15.8	81
2016	8	19	8:00	21	14.6	67
2016	8	19	9:00	22.2	15.8	67
2016	8	19	10:00	23.6	15.8	62
2016	8	19	11:00	24.7	16.2	59
2016	8	19	12:00	25.3	14.8	52
2016	8	19	13:00	25.4	12.9	46
2016	8	19	14:00	25.9	14.5	49
2016	8	19	15:00	26.3	15	50
2016	8	19	16:00	26.2	15.4	51
2016	8	19	17:00	26.2	16.4	55
2016	8	19	18:00	25.6	16	55
2016	8	19	19:00	23.8	17	66
2016	8	19	20:00	20.4	17.6	84
2016	8	19	21:00	23	16.5	67
2016	8	19	22:00	21.4	16.8	75
2016	8	19	23:00	21.3	16.2	73
2016	8	20	0:00	21.1	15.3	69
2016	8	20	1:00	19.4	15.4	78
2016	8	20	2:00	19	15.6	81
2016	8	20	3:00	20.2	15	72
2016	8	20	4:00	19.2	15.1	77
2016	8	20	5:00	19.3	15.5	79
2016	8	20	6:00	17.2	16.1	93
2016	8	20	7:00	20.1	17	83
2016	8	20	8:00	21.4	17	76

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	8	20	9:00	23	18.1	74
2016	8	20	10:00	24.5	17.8	66
2016	8	20	11:00	25.2	18.9	68
2016	8	20	12:00	26.8	19.9	66
2016	8	20	13:00	27.6	18.1	57
2016	8	20	14:00	28	17.5	53
2016	8	20	15:00	27.5	17.4	54
2016	8	20	16:00	27.6	17.2	53
2016	8	20	17:00	27.4	19.2	61
2016	8	20	18:00	26.9	18.1	59
2016	8	20	19:00	26.1	18.7	64
2016	8	20	20:00	25.3	18.8	67
2016	8	20	21:00	24.7	18.6	69
2016	8	20	22:00	24.5	18.4	69
2016	8	20	23:00	24.1	17.6	67
2016	8	21	0:00	24.1	17	65
2016	8	21	1:00	24	16.3	62
2016	8	21	2:00	23.5	16.7	66
2016	8	21	3:00	23.5	17.7	70
2016	8	21	4:00	23.2	18.6	75
2016	8	21	5:00	22.8	18.9	79
2016	8	21	6:00	22.8	19.1	80
2016	8	21	7:00	23.1	19.3	79
2016	8	21	8:00	23.9	19.5	77
2016	8	21	9:00	25.3	19.9	72
2016	8	21	10:00	26.1	20.5	71
2016	8	21	11:00	25.3	21.5	80
2016	8	21	12:00	22	20.2	89
2016	8	21	13:00	22.4	21.2	93
2016	8	21	14:00	21.9	21.2	96
2016	8	21	15:00	21.8	21.3	97
2016	8	21	16:00	21.7	21.2	97
2016	8	21	17:00	21.2	19.7	91
2016	8	21	18:00	20.4	17.9	86
2016	8	21	19:00	20	17.8	87
2016	8	21	20:00	20	18.1	89
2016	8	21	21:00	20.3	18.4	89
2016	8	21	22:00	20.6	18	85
2016	8	21	23:00	20.3	17.9	87
2016	8	22	0:00	19	16.7	87
2016	8	22	1:00	18	14.4	79

Year	Month	Day	Time	Temp (°C)	Dew Point Temp (°C)	Rel Hum (%)
2016	8	22	2:00	16.8	13.8	83
2016	8	22	3:00	15.9	12.3	79
2016	8	22	4:00	14.2	11.9	86
2016	8	22	5:00	13.9	9.9	77
2016	8	22	6:00	13.6	9.4	76
2016	8	22	7:00	14.4	9.4	72
2016	8	22	8:00	15.4	10.5	72
2016	8	22	9:00	16.7	11.5	71
2016	8	22	10:00	17	10.7	66
2016	8	22	11:00	18.6	11.7	64
2016	8	22	12:00	19.2	10.5	57
2016	8	22	13:00	19.1	9.6	54
2016	8	22	14:00	19.8	10.7	56
2016	8	22	15:00	19.9	10.6	55
2016	8	22	16:00	20.7	11	54
2016	8	22	17:00	20.6	10.4	52
2016	8	22	18:00	19.6	11	58
2016	8	22	19:00	18.3	11.2	63
2016	8	22	20:00	17.1	11.5	70
2016	8	22	21:00	15.9	11.7	76
2016	8	22	22:00	16.1	11.2	73
2016	8	22	23:00	15.4	11.4	77
2016	8	23	0:00	15.2	12.2	83
2016	8	23	1:00	14.8	12.7	87
2016	8	23	2:00	14.4	12.5	88
2016	8	23	3:00	14.6	13	91
2016	8	23	4:00	13.7	12.4	92
2016	8	23	5:00	13.6	12.2	91
2016	8	23	6:00	14.1	12	87
2016	8	23	7:00	15.9	12.3	79
2016	8	23	8:00	17.5	13.5	77
2016	8	23	9:00	19.1	13.7	71
2016	8	23	10:00	20.8	14	65
2016	8	23	11:00	22.1	14	60
2016	8	23	12:00	22.8	14.2	58
2016	8	23	13:00	23.7	13.1	51
2016	8	23	14:00	24.5	14.8	55
2016	8	23	15:00	25	15.4	55
2016	8	23	16:00	25.3	15.2	54
2016	8	23	17:00	25	15.8	56
2016	8	23	18:00	24.3	15.3	57

Year	Month	Day	Time	Temp (°C)	Dew Point Temp (°C)	Rel Hum (%)
2016	8	23	19:00	23.2	15.3	61
2016	8	23	20:00	22.6	15.4	64
2016	8	23	21:00	22	15.5	67
2016	8	23	22:00	21.6	15.7	69
2016	8	23	23:00	21	15.6	71
2016	8	24	0:00	20.1	15.8	76
2016	8	24	1:00	19.6	15.9	79
2016	8	24	2:00	19.4	16	81
2016	8	24	3:00	19.6	16.9	85
2016	8	24	4:00	19.4	16.4	83
2016	8	24	5:00	18.6	16	85
2016	8	24	6:00	18.4	16.2	87
2016	8	24	7:00	19.5	16.3	82
2016	8	24	8:00	21.3	17.5	79
2016	8	24	9:00	22.8	17.5	72
2016	8	24	10:00	24.1	18.3	70
2016	8	24	11:00	24.9	18.7	69
2016	8	24	12:00	25.7	16.9	58
2016	8	24	13:00	26.6	17.4	57
2016	8	24	14:00	27.4	16.9	53
2016	8	24	15:00	28	17.7	53
2016	8	24	16:00	28	18.3	56
2016	8	24	17:00	27.4	18.4	58
2016	8	24	18:00	25	21	79
2016	8	24	19:00	23.8	19.2	75
2016	8	24	20:00	22.9	18.8	78
2016	8	24	21:00	23.1	18.8	77
2016	8	24	22:00	22.8	18.5	77
2016	8	24	23:00	22.8	18.2	75
2016	8	25	0:00	22.5	17.8	75
2016	8	25	1:00	22.5	17.4	73
2016	8	25	2:00	22.3	17	72
2016	8	25	3:00	22	17.3	75
2016	8	25	4:00	22.5	17.5	73
2016	8	25	5:00	22.5	17.8	75
2016	8	25	6:00	22	18.9	83
2016	8	25	7:00	23.2	18.7	76
2016	8	25	8:00	23.7	19.1	76
2016	8	25	9:00	24.5	19.8	75
2016	8	25	10:00	24.6	19.9	75
2016	8	25	11:00	22.9	20.8	88

Year	Month	Day	Time	Temp (°C)	Dew Point Temp (°C)	Rel Hum (%)
2016	8	25	12:00	21.9	21.2	96
2016	8	25	13:00	22.6	21.8	95
2016	8	25	14:00	23.1	22	93
2016	8	25	15:00	25.5	22.7	85
2016	8	25	16:00	26	22.5	81
2016	8	25	17:00	26.3	22.4	79
2016	8	25	18:00	26	22.4	81
2016	8	25	19:00	25.6	22.1	81
2016	8	25	20:00	25.1	22	83
2016	8	25	21:00	24.7	21.7	84
2016	8	25	22:00	24.7	21.8	84
2016	8	25	23:00	23.8	21.7	88
2016	8	26	0:00	23.4	21.5	89
2016	8	26	1:00	23.5	21.4	88
2016	8	26	2:00	23	21.5	91
2016	8	26	3:00	22.8	21.3	91
2016	8	26	4:00	22.5	21.2	93
2016	8	26	5:00	21.8	20.9	94
2016	8	26	6:00	21.6	20.7	95
2016	8	26	7:00	22.2	20.2	89
2016	8	26	8:00	23.2	20	82
2016	8	26	9:00	23.6	20.1	81
2016	8	26	10:00	24.7	20.5	78
2016	8	26	11:00	25.4	17.8	63
2016	8	26	12:00	26	17.7	60
2016	8	26	13:00	26.5	17.1	56
2016	8	26	14:00	26.9	15.6	50
2016	8	26	15:00	26.9	16.7	54
2016	8	26	16:00	26.6	15.7	51
2016	8	26	17:00	26.3	16.5	55
2016	8	26	18:00	24.8	16.4	60
2016	8	26	19:00	22.6	16.5	68
2016	8	26	20:00	19.3	17.1	87
2016	8	26	21:00	18.9	17.6	92
2016	8	26	22:00	18.3	17.2	94
2016	8	26	23:00	17.6	16.3	92
2016	8	27	0:00	18.1	16.9	93
2016	8	27	1:00	18.4	17.5	95
2016	8	27	2:00	18.7	16.8	89
2016	8	27	3:00	19.1	16	82
2016	8	27	4:00	18.7	15.1	80

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	8	27	5:00	16	14.4	90
2016	8	27	6:00	16.8	15.4	92
2016	8	27	7:00	19.2	14.5	74
2016	8	27	8:00	20	14.2	69
2016	8	27	9:00	21.1	14.2	65
2016	8	27	10:00	22.3	14.7	62
2016	8	27	11:00	23.3	14.1	56
2016	8	27	12:00	23.9	15.4	59
2016	8	27	13:00	24.2	14.8	56
2016	8	27	14:00	25	15	54
2016	8	27	15:00	25.2	15.5	55
2016	8	27	16:00	25.7	15.5	53
2016	8	27	17:00	24.7	13.5	50
2016	8	27	18:00	23.4	15.2	60
2016	8	27	19:00	23	14.8	60
2016	8	27	20:00	22.3	15.5	65
2016	8	27	21:00	21.6	15.6	69
2016	8	27	22:00	18.5	16.1	86
2016	8	27	23:00	18.2	16.7	91
2016	8	28	0:00	20	16.7	81
2016	8	28	1:00	20.6	16.1	75
2016	8	28	2:00	19.9	16	78
2016	8	28	3:00	20	15.7	77
2016	8	28	4:00	18.6	17	90
2016	8	28	5:00	18.3	16.9	92
2016	8	28	6:00	18.8	16.7	88
2016	8	28	7:00	19.1	16.8	87
2016	8	28	8:00	19.8	17.6	88
2016	8	28	9:00	22	18.1	78
2016	8	28	10:00	22.8	17.5	72
2016	8	28	11:00	22.9	18.5	76
2016	8	28	12:00	24.8	19.4	72
2016	8	28	13:00	26.6	20.4	69
2016	8	28	14:00	26.7	20.6	69
2016	8	28	15:00	27.3	21.2	69
2016	8	28	16:00	25.9	22.5	81
2016	8	28	17:00	25.8	21.3	76
2016	8	28	18:00	25.4	22.8	86
2016	8	28	19:00	24.4	22.7	91
2016	8	28	20:00	24	20.7	82
2016	8	28	21:00	23.2	20.7	86

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	8	28	22:00	22.3	20.9	91
2016	8	28	23:00	22.4	21	92
2016	8	29	0:00	22.3	21.1	93
2016	8	29	1:00	21.5	19.7	90
2016	8	29	2:00	20.8	18.4	86
2016	8	29	3:00	20.6	18	85
2016	8	29	4:00	19.4	16.6	84
2016	8	29	5:00	18.7	15.7	83
2016	8	29	6:00	18.7	16.5	87
2016	8	29	7:00	18.9	15.8	82
2016	8	29	8:00	19.5	15.2	76
2016	8	29	9:00	20.2	14.8	71
2016	8	29	10:00	21.4	14.6	65
2016	8	29	11:00	21.4	14.3	64
2016	8	29	12:00	22.3	13.7	58
2016	8	29	13:00	23	13.8	56
2016	8	29	14:00	23.4	13.9	55
2016	8	29	15:00	22.9	14.2	58
2016	8	29	16:00	23.6	14.1	55
2016	8	29	17:00	23.6	15	58
2016	8	29	18:00	22.4	15	63
2016	8	29	19:00	20.7	14.4	67
2016	8	29	20:00	19.5	14.9	75
2016	8	29	21:00	19.3	15.7	80
2016	8	29	22:00	17.9	14.8	82
2016	8	29	23:00	16.6	15.1	91
2016	8	30	0:00	17.5	15.8	90
2016	8	30	1:00	17.1	14.9	87
2016	8	30	2:00	16.8	15	89
2016	8	30	3:00	16.6	14.6	88
2016	8	30	4:00	16.5	14.8	90
2016	8	30	5:00	16.2	14.5	90
2016	8	30	6:00	16	14.4	90
2016	8	30	7:00	17.4	15.2	87
2016	8	30	8:00	18.5	15.5	83
2016	8	30	9:00	18.8	15.9	83
2016	8	30	10:00	20.6	16.5	77
2016	8	30	11:00	22.7	17.7	74
2016	8	30	12:00	23.9	16.3	62
2016	8	30	13:00	23.6	15.9	62
2016	8	30	14:00	24.9	16	58

Year	Month	Day	Time	Temp (°C)	Dew Point Temp (°C)	Rel Hum (%)
2016	8	30	15:00	24.5	16.3	61
2016	8	30	16:00	24.1	16.6	63
2016	8	30	17:00	24	17.5	67
2016	8	30	18:00	23.6	17.8	70
2016	8	30	19:00	23.1	17.5	71
2016	8	30	20:00	22.4	17.8	75
2016	8	30	21:00	22.4	16.9	71
2016	8	30	22:00	22.1	16.5	71
2016	8	30	23:00	21.8	16.4	72
2016	8	31	0:00	21.5	16.3	72
2016	8	31	1:00	20.7	16.3	76
2016	8	31	2:00	20.8	16.8	78
2016	8	31	3:00	19	18.1	94
2016	8	31	4:00	19	18.2	95
2016	8	31	5:00	19.1	18	94
2016	8	31	6:00	19.1	18.1	94
2016	8	31	7:00	19.2	18.2	94
2016	8	31	8:00	20.3	19.1	93
2016	8	31	9:00	21.7	19.7	88
2016	8	31	10:00	21.8	19.3	86
2016	8	31	11:00	22.7	20	85
2016	8	31	12:00	22.2	19.8	87
2016	8	31	13:00	23.6	20.1	81
2016	8	31	14:00	24.7	21	80
2016	8	31	15:00	25	20.4	76
2016	8	31	16:00	22.1	20	88
2016	8	31	17:00	20.7	18.2	86
2016	8	31	18:00	20	18.8	93
2016	8	31	19:00	19.9	18.4	91
2016	8	31	20:00	19.8	19.2	96
2016	8	31	21:00	19.3	17.9	91
2016	8	31	22:00	19.2	17.1	87
2016	8	31	23:00	18	15.5	86
2016	9	1	0:00	17.6	14.9	85
2016	9	1	1:00	16.4	14.4	88
2016	9	1	2:00	15	14	94
2016	9	1	3:00	13.6	13.1	96
2016	9	1	4:00	14.1	13.3	95
2016	9	1	5:00	14.4	13.1	92
2016	9	1	6:00	14	12.9	93
2016	9	1	7:00	16.6	14	85

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	9	1	8:00	17.7	13.7	77
2016	9	1	9:00	18.7	14	74
2016	9	1	10:00	19.5	14.1	71
2016	9	1	11:00	19.9	14.2	70
2016	9	1	12:00	20.1	14	68
2016	9	1	13:00	20.7	14	66
2016	9	1	14:00	20.8	15.5	72
2016	9	1	15:00	21.1	14.4	66
2016	9	1	16:00	21.8	14.4	63
2016	9	1	17:00	20.8	15.3	71
2016	9	1	18:00	20.7	14.6	68
2016	9	1	19:00	19.6	15.1	75
2016	9	1	20:00	18.9	15.1	78
2016	9	1	21:00	17.8	15.5	87
2016	9	1	22:00	16.8	15.6	92
2016	9	1	23:00	17.2	14.4	84
2016	9	2	0:00	18.5	12.9	70
2016	9	2	1:00	17.5	12.4	72
2016	9	2	2:00	16.5	11.6	73
2016	9	2	3:00	16	11	72
2016	9	2	4:00	15.3	10.6	74
2016	9	2	5:00	14.5	10.1	75
2016	9	2	6:00	13.9	10.3	79
2016	9	2	7:00	Missing	Missing	Missing
2016	9	2	8:00	Missing	Missing	Missing
2016	9	2	9:00	Missing	Missing	Missing
2016	9	2	10:00	Missing	Missing	Missing
2016	9	2	11:00	Missing	Missing	Missing
2016	9	2	12:00	Missing	Missing	Missing
2016	9	2	13:00	20.4	12	59
2016	9	2	14:00	20.6	11.6	56
2016	9	2	15:00	20.8	10.9	53
2016	9	2	16:00	20.3	10.1	52
2016	9	2	17:00	20.3	11.5	57
2016	9	2	18:00	18.8	11.1	61
2016	9	2	19:00	17.6	11.6	68
2016	9	2	20:00	16.6	11.7	73
2016	9	2	21:00	16.6	12.4	76
2016	9	2	22:00	14.8	11.8	82
2016	9	2	23:00	13.1	11.4	89
2016	9	3	0:00	13.7	11.1	84

Year	Month	Day	Time	Temp (°C)	Dew Point Temp (°C)	Rel Hum (%)
2016	9	3	1:00	12.2	10.4	89
2016	9	3	2:00	11.7	10.8	94
2016	9	3	3:00	11.2	10.6	96
2016	9	3	4:00	9.9	9.6	98
2016	9	3	5:00	9.4	9.3	99
2016	9	3	6:00	9.6	9.5	99
2016	9	3	7:00	13.3	11.8	91
2016	9	3	8:00	15.1	11.6	79
2016	9	3	9:00	17.1	11.7	70
2016	9	3	10:00	19	11.5	62
2016	9	3	11:00	19.5	11.7	61
2016	9	3	12:00	20.4	12.6	61
2016	9	3	13:00	21.1	12.3	57
2016	9	3	14:00	21.4	12.4	57
2016	9	3	15:00	21.6	11.9	54
2016	9	3	16:00	21.9	12.5	55
2016	9	3	17:00	21.7	12.2	55
2016	9	3	18:00	19.4	14.4	73
2016	9	3	19:00	15.6	13.9	89
2016	9	3	20:00	14.6	13.6	94
2016	9	3	21:00	13.9	13.1	95
2016	9	3	22:00	13.6	13.1	97
2016	9	3	23:00	12.7	12.4	98
2016	9	4	0:00	12.5	12.4	99
2016	9	4	1:00	12.1	12	99
2016	9	4	2:00	11.5	11.5	100
2016	9	4	3:00	11.9	11.9	100
2016	9	4	4:00	10.9	10.9	100
2016	9	4	5:00	11.4	11.4	100
2016	9	4	6:00	11.3	11.3	100
2016	9	4	7:00	14.6	14.6	100
2016	9	4	8:00	16.9	15.1	89
2016	9	4	9:00	19.5	15.5	78
2016	9	4	10:00	21.3	15.4	69
2016	9	4	11:00	22.2	12.5	54
2016	9	4	12:00	22.8	12.5	52
2016	9	4	13:00	23.4	14.9	59
2016	9	4	14:00	23.8	13.1	51
2016	9	4	15:00	24.2	13.1	50
2016	9	4	16:00	24.4	13	49
2016	9	4	17:00	24.4	12.8	48

Year	Month	Day	Time	Temp (°C)	Dew Point Temp (°C)	Rel Hum (%)
2016	9	4	18:00	21.2	16.5	75
2016	9	4	19:00	16.7	15	90
2016	9	4	20:00	15.8	14.7	93
2016	9	4	21:00	15.2	14.4	95
2016	9	4	22:00	14.6	13.9	96
2016	9	4	23:00	13.9	13.4	97
2016	9	5	0:00	13.1	12.9	99
2016	9	5	1:00	12.7	12.7	100
2016	9	5	2:00	13.1	13.1	100
2016	9	5	3:00	12.3	12.3	100
2016	9	5	4:00	11.4	11.4	100
2016	9	5	5:00	11.2	11.2	100
2016	9	5	6:00	12.1	12.1	100
2016	9	5	7:00	15	15	100
2016	9	5	8:00	18	16.4	91
2016	9	5	9:00	21.1	18	83
2016	9	5	10:00	22.7	18.3	76
2016	9	5	11:00	23.7	17.9	70
2016	9	5	12:00	24.9	16.1	58
2016	9	5	13:00	25.5	16.3	57
2016	9	5	14:00	26.1	16.3	55
2016	9	5	15:00	26.5	13.8	46
2016	9	5	16:00	25.8	14.7	50
2016	9	5	17:00	25.5	15.4	54
2016	9	5	18:00	22.5	17.7	75
2016	9	5	19:00	19.2	17.2	88
2016	9	5	20:00	17	15.6	92
2016	9	5	21:00	17.1	16.5	96
2016	9	5	22:00	16.4	15.8	96
2016	9	5	23:00	14.8	14.4	98
2016	9	6	0:00	14.9	14.8	100
2016	9	6	1:00	14.6	14.6	100
2016	9	6	2:00	13.6	13.6	100
2016	9	6	3:00	13.9	13.9	100
2016	9	6	4:00	13.3	13.3	100
2016	9	6	5:00	12.7	12.7	100
2016	9	6	6:00	12.4	12.4	100
2016	9	6	7:00	17	17	100
2016	9	6	8:00	18.8	18.1	96
2016	9	6	9:00	22.1	18.3	79
2016	9	6	10:00	23.5	19.6	79

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	9	6	11:00	24.6	19.6	74
2016	9	6	12:00	26.1	16.6	56
2016	9	6	13:00	26.4	15.8	52
2016	9	6	14:00	27.2	17.1	54
2016	9	6	15:00	27.3	16	50
2016	9	6	16:00	27.2	15.4	48
2016	9	6	17:00	26.4	16.5	54
2016	9	6	18:00	22.9	17.9	74
2016	9	6	19:00	19.1	17.1	88
2016	9	6	20:00	17.9	16.2	90
2016	9	6	21:00	17.6	16	91
2016	9	6	22:00	18.6	17.7	94
2016	9	6	23:00	18.5	16.5	88
2016	9	7	0:00	16.5	14.9	91
2016	9	7	1:00	15.6	15.2	97
2016	9	7	2:00	14.9	14.5	98
2016	9	7	3:00	14.2	13.8	98
2016	9	7	4:00	15	14.9	99
2016	9	7	5:00	16.6	15.8	95
2016	9	7	6:00	18.1	17.2	95
2016	9	7	7:00	19.8	17.9	89
2016	9	7	8:00	21.9	18.7	82
2016	9	7	9:00	23	18.4	75
2016	9	7	10:00	24.7	19.1	71
2016	9	7	11:00	26.6	20	67
2016	9	7	12:00	27.4	19.5	62
2016	9	7	13:00	28.6	18.3	54
2016	9	7	14:00	28.8	18.8	55
2016	9	7	15:00	28.5	18.9	56
2016	9	7	16:00	28.8	17.7	51
2016	9	7	17:00	26.8	22	75
2016	9	7	18:00	25.9	21.5	77
2016	9	7	19:00	23.8	21.5	87
2016	9	7	20:00	23.1	20.6	86
2016	9	7	21:00	22.6	20.9	90
2016	9	7	22:00	22.8	21	89
2016	9	7	23:00	22.4	21.1	92
2016	9	8	0:00	22.6	21.2	92
2016	9	8	1:00	21.8	21.3	97
2016	9	8	2:00	21.8	21.2	96
2016	9	8	3:00	21.5	21.2	98

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	9	8	4:00	21.5	21.2	99
2016	9	8	5:00	21.2	21	99
2016	9	8	6:00	21.8	21.6	99
2016	9	8	7:00	21.4	20.9	97
2016	9	8	8:00	22.2	21.2	94
2016	9	8	9:00	23.5	21.7	90
2016	9	8	10:00	23.2	21.4	89
2016	9	8	11:00	24.2	21.4	85
2016	9	8	12:00	24	21.8	88
2016	9	8	13:00	24.4	21.8	86
2016	9	8	14:00	23.8	22.7	94
2016	9	8	15:00	23.8	22.7	94
2016	9	8	16:00	23.3	22.5	96
2016	9	8	17:00	23.9	23	95
2016	9	8	18:00	23.5	22.9	96
2016	9	8	19:00	23.3	23	98
2016	9	8	20:00	24	23.6	98
2016	9	8	21:00	24.1	23.8	98
2016	9	8	22:00	24.1	23.2	95
2016	9	8	23:00	23.6	22.7	95
2016	9	9	0:00	23	21.9	94
2016	9	9	1:00	22.7	21.4	92
2016	9	9	2:00	22.2	21	93
2016	9	9	3:00	21.8	20.8	94
2016	9	9	4:00	21.7	20.6	94
2016	9	9	5:00	21	19.5	91
2016	9	9	6:00	20.7	19.1	91
2016	9	9	7:00	21.2	19.3	89
2016	9	9	8:00	22.1	19.5	85
2016	9	9	9:00	23	19.3	80
2016	9	9	10:00	23.2	17.9	73
2016	9	9	11:00	23.8	18.6	73
2016	9	9	12:00	24.9	18.3	67
2016	9	9	13:00	25.9	18.6	64
2016	9	9	14:00	25.9	17.4	59
2016	9	9	15:00	26.2	17.5	59
2016	9	9	16:00	25.9	16.9	57
2016	9	9	17:00	25.2	16	57
2016	9	9	18:00	23.1	16.1	65
2016	9	9	19:00	20	17	83
2016	9	9	20:00	18.7	17.9	95

Year	Month	Day	Time	Temp (°C)	Dew Point Temp (°C)	Rel Hum (%)
2016	9	9	21:00	18.6	17.4	93
2016	9	9	22:00	19.6	17.6	88
2016	9	9	23:00	18.6	17.6	94
2016	9	10	0:00	18.8	17.9	95
2016	9	10	1:00	17.9	17.1	96
2016	9	10	2:00	16.5	16.3	99
2016	9	10	3:00	16.7	16.6	99
2016	9	10	4:00	15.9	15.8	100
2016	9	10	5:00	15.6	15.6	100
2016	9	10	6:00	15.6	15.6	100
2016	9	10	7:00	18.3	17.3	94
2016	9	10	8:00	21.4	17.1	77
2016	9	10	9:00	22.5	17.6	74
2016	9	10	10:00	24	19.2	74
2016	9	10	11:00	25.7	20.1	71
2016	9	10	12:00	26.4	19.7	66
2016	9	10	13:00	26.3	19.2	65
2016	9	10	14:00	25.7	19.1	67
2016	9	10	15:00	25.9	19	66
2016	9	10	16:00	24.5	20.8	80
2016	9	10	17:00	25	20.4	76
2016	9	10	18:00	24.5	20	76
2016	9	10	19:00	24	20	78
2016	9	10	20:00	24.1	20.2	79
2016	9	10	21:00	24.2	20.4	79
2016	9	10	22:00	23.7	20.6	83
2016	9	10	23:00	22.9	20.8	88
2016	9	11	0:00	22.3	20.4	89
2016	9	11	1:00	22.3	20.7	91
2016	9	11	2:00	22.5	20.9	91
2016	9	11	3:00	22.7	20.9	90
2016	9	11	4:00	20.6	18.6	88
2016	9	11	5:00	19.1	15.8	81
2016	9	11	6:00	19	16.5	85
2016	9	11	7:00	18.7	16.3	86
2016	9	11	8:00	17.1	11.8	71
2016	9	11	9:00	16.8	10.6	67
2016	9	11	10:00	16.3	9.7	65
2016	9	11	11:00	16.8	9.9	64
2016	9	11	12:00	17	8.8	59
2016	9	11	13:00	17.5	8.8	57

Year	Month	Day	Time	Temp (°C)	Dew Point Temp (°C)	Rel Hum (%)
2016	9	11	14:00	17.3	7.8	54
2016	9	11	15:00	17.8	8.3	54
2016	9	11	16:00	18	8.5	54
2016	9	11	17:00	17.8	8.7	55
2016	9	11	18:00	16.6	8.8	60
2016	9	11	19:00	15.3	9	66
2016	9	11	20:00	14.7	8.6	67
2016	9	11	21:00	14	8.9	71
2016	9	11	22:00	12.5	9	80
2016	9	11	23:00	12.6	9.3	81
2016	9	12	0:00	11.5	9	85
2016	9	12	1:00	9.6	8.8	95
2016	9	12	2:00	11.7	9	84
2016	9	12	3:00	11.7	8.3	80
2016	9	12	4:00	11.2	9.1	87
2016	9	12	5:00	11.8	9.3	85
2016	9	12	6:00	11.8	9.5	86
2016	9	12	7:00	12.8	10.1	84
2016	9	12	8:00	14.4	11.1	80
2016	9	12	9:00	17	12.7	76
2016	9	12	10:00	18.8	12.4	66
2016	9	12	11:00	20.4	13.2	63
2016	9	12	12:00	20.9	13.2	61
2016	9	12	13:00	21.7	12.4	56
2016	9	12	14:00	22.4	11.5	50
2016	9	12	15:00	22.5	10.9	48
2016	9	12	16:00	22.4	10.7	48
2016	9	12	17:00	21.8	10	47
2016	9	12	18:00	20.3	9.9	51
2016	9	12	19:00	19.1	10.8	59
2016	9	12	20:00	18.9	10.6	59
2016	9	12	21:00	18.8	9.1	53
2016	9	12	22:00	17.6	9.9	60
2016	9	12	23:00	16.5	11.6	73
2016	9	13	0:00	15.6	11.9	79
2016	9	13	1:00	15.2	12.4	83
2016	9	13	2:00	14.9	11.1	78
2016	9	13	3:00	13.6	11	84
2016	9	13	4:00	13.4	10.8	84
2016	9	13	5:00	12.9	11	88
2016	9	13	6:00	12.5	11.1	91

Year	Month	Day	Time	Temp (°C)	Dew Point Temp (°C)	Rel Hum (%)
2016	9	13	7:00	14.9	12.5	86
2016	9	13	8:00	16.9	12.9	78
2016	9	13	9:00	19.1	14	73
2016	9	13	10:00	21.8	15.6	68
2016	9	13	11:00	22.9	14.4	59
2016	9	13	12:00	24.6	13.5	50
2016	9	13	13:00	25.2	12.8	46
2016	9	13	14:00	25.7	13.3	46
2016	9	13	15:00	25.6	13.2	46
2016	9	13	16:00	25.8	13.7	47
2016	9	13	17:00	24.4	14.1	53
2016	9	13	18:00	23.4	13.2	53
2016	9	13	19:00	22.6	13	54
2016	9	13	20:00	22	14.1	61
2016	9	13	21:00	21.6	14.7	65
2016	9	13	22:00	21.6	14.7	65
2016	9	13	23:00	21	14.7	67
2016	9	14	0:00	20.7	15.3	71
2016	9	14	1:00	22	15.3	66
2016	9	14	2:00	20.8	15.5	72
2016	9	14	3:00	20.6	15.8	74
2016	9	14	4:00	20.8	16.6	77
2016	9	14	5:00	19.6	17.8	89
2016	9	14	6:00	19.3	18.1	93
2016	9	14	7:00	19.8	18.4	91
2016	9	14	8:00	20	18	89
2016	9	14	9:00	18.7	14.8	78
2016	9	14	10:00	17	13.9	82
2016	9	14	11:00	18.8	13	69
2016	9	14	12:00	18.6	11.4	63
2016	9	14	13:00	17.8	9.4	58
2016	9	14	14:00	18.1	9.4	57
2016	9	14	15:00	18	8.9	56
2016	9	14	16:00	18.1	8.7	54
2016	9	14	17:00	17.3	8.8	58
2016	9	14	18:00	16.4	8.7	60
2016	9	14	19:00	14.6	9.4	71
2016	9	14	20:00	14.7	8.3	66
2016	9	14	21:00	14.1	8.1	67
2016	9	14	22:00	12.2	8.2	77
2016	9	14	23:00	11.8	8	78

Year	Month	Day	Time	Temp (° C)	Dew Point Temp (° C)	Rel Hum (%)
2016	9	15	0:00	9.5	7.1	85
2016	9	15	1:00	8.2	7.2	93
2016	9	15	2:00	8.8	6.7	87
2016	9	15	3:00	9.4	6.4	82
2016	9	15	4:00	8.4	6	85
2016	9	15	5:00	7.3	5.8	90
2016	9	15	6:00	6.2	5.7	97
2016	9	15	7:00	9.9	7.4	84
2016	9	15	8:00	11.6	7.2	75
2016	9	15	9:00	13	6.5	65
2016	9	15	10:00	13.6	7.1	65
2016	9	15	11:00	15.3	7.2	59
2016	9	15	12:00	15.6	7.1	57
2016	9	15	13:00	16.4	7.2	55
2016	9	15	14:00	16.7	6.1	50
2016	9	15	15:00	17.1	7.5	53
2016	9	15	16:00	16.6	7.4	55
2016	9	15	17:00	16.5	7.2	54
2016	9	15	18:00	13.5	8.7	73
2016	9	15	19:00	10.4	8.9	91
2016	9	15	20:00	9.4	8.3	93
2016	9	15	21:00	9.3	8.6	95
2016	9	15	22:00	9.1	8.5	96
2016	9	15	23:00	8.6	8.2	97

References

- Abu-Dieyeh, M. H. 2006. Population dynamics of dandelion (*Taraxacum officinal*) in turfgrass as influenced by a biological control agent, *Sclerotinia minor*. PhD thesis, McGill University, Montreal, Canada.
- Abu-Dieyeh, M. H. and Watson, A. K. 2006. Effect of turfgrass mowing height on biocontrol of dandelion with *Sclerotinia minor*. *Biocontrol Science and Technology*, 16(5):509–524.
- Abu-Dieyeh, M. H. and Watson, A. K. 2007a. Efficacy of *Sclerotinia minor* for dandelion control: effect of dandelion accession, age and grass competition. *Weed Research*, 47(1):63–72.
- Abu-Dieyeh, M. H. and Watson, A. K. 2007b. Grass overseeding and a fungus combine to control *Taraxacum officinale*. *Journal of Applied Ecology*, 44(1):115–124.
- Abu-Dieyeh, M. H. and Watson, A. K. 2007c. Population dynamics of broadleaf weeds in turfgrass as influenced by chemical and biological control methods. *Weed Science*, 55(4):371–380.
- Abu-Dieyeh, M. H. and Watson, A. K. 2009. Increasing the efficacy and extending the effective application period of a granular turf bioherbicide by covering with jute fabric. *Weed Technology*, 23(4):524–530.
- Adams, P. 1987. Effects of soil temperature, moisture, and depth on survival and activity of

- Sclerotinia minor*, *Sclerotium cepivorum*, and *Sporidesmium sclerotivorum*. *Plant Disease*, 71(2):170–174.
- Adams, P. 1989. Comparison of antagonists of *Sclerotinia* species. *Phytopathology*, 79(12):1345–1347.
- Adams, P. and Ayers, W. 1979. Ecology of *Sclerotinia* species. *Phytopathology*, 69(8):896–899.
- Alexander, B. and Stewart, A. 1994. Survival of sclerotia of *Sclerotinia* and *Sclerotium* spp in New Zealand horticultural soil. *Soil Biology and Biochemistry*, 26(10):1323–1329.
- Auld, B. 2002. Bioherbicidal formulations. *Australian Provisional Patent Application*, 2002952094.
- Auld, B. A. 1993. Vegetable oil suspension emulsions reduce dew dependence of a mycoherbicide. *Crop Protection*, 12(6):477–479.
- Auld, B. A., Hetherington, S. D., and Smith, H. E. 2003. Advances in bioherbicide formulation. *Weed Biology and Management*, 3(2):61–67.
- Bailey, K. 2010. Canadian innovations in microbial biopesticides. *Canadian Journal of Plant Pathology*, 32(2):113–121.
- Bailey, K. 2014. The bioherbicide approach to weed control using plant pathogens. *Integrated Pest Management: Current Concepts and Ecological Perspective*, pages 245–266.
- Bennett, A. and Hindal, D. 1989. Mycelial growth and oxalate production by five strains of *Cryphonectria parasitica* in selected liquid culture media. *Mycologia*, 81(4):554–560.

- Berna, A. and Bernier, F. 1997. Regulated expression of a wheat germin gene in tobacco: oxalate oxidase activity and apoplastic localization of the heterologous protein. *Plant Molecular Biology*, 33(3):417–429.
- Bothast, R. J., Schisler, D. A., Jackson, M. A., VanCauwenberge, J. E., and Slininger, P. J. 1993. Use of pregelatinized starch and other polysaccharides for improved storage and efficacy of biocontrol agents. In *Pesticide Formulations and Application Systems: 13th Volume*. ASTM International.
- Bourdoot, G., Harvey, I., Hurrell, G., and Saville, D. 1995. Demographic and biomass production consequences of inundative treatment of *Cirsium arvense* with *Sclerotinia sclerotiorum*. *Biocontrol Science and Technology*, 5(1):11–26.
- Boyette, C. D. 1994. Unrefined corn oil improves the mycoherbicidal activity of *Colletotrichum truncatum* for hemp sesbania (*Sesbania exaltata*) control. *Weed Technology*, 8:526–529.
- Boyette, C. D., Quimby Jr, P. C., Connick Jr, W. J., Daigle, D. J., and Fulgham, F. E. 1991. Progress in the production, formulation, and application of mycoherbicides. In *Microbial Control of Weeds*, pages 209–222. Springer.
- Briere, S. C., Watson, A. K., and Hallett, S. G. 2000. Oxalic acid production and mycelial biomass yield of *Sclerotinia minor* for the formulation enhancement of a granular turf bioherbicide. *Biocontrol Science and Technology*, 10(3):281–289.
- Brosten, B. S. and Sands, D. C. 1986. Field trials of *Sclerotinia sclerotiorum* to control Canada thistle (*Cirsium arvense*). *Weed Science*, pages 377–380.
- Cessna, S. G., Sears, V. E., Dickman, M. B., and Low, P. S. 2000. Oxalic acid, a pathogenicity

- factor for *Sclerotinia sclerotiorum*, suppresses the oxidative burst of the host plant. *The Plant Cell*, 12(11):2191–2199.
- Chen, C. and Dickman, M. B. 2005. cAMP blocks MAPK activation and sclerotial development via Rap-1 in a PKA-independent manner in *Sclerotinia sclerotiorum*. *Molecular Microbiology*, 55(1):299–311.
- Chen, C., Harel, A., Gorovoits, R., Yarden, O., and Dickman, M. B. 2004. MAPK regulation of sclerotial development in *Sclerotinia sclerotiorum* is linked with pH and cAMP sensing. *Molecular Plant-Microbe Interactions*, 17(4):404–413.
- Chet, I. and Henis, Y. 1968. The control mechanism of sclerotial formation in *Sclerotium rolfsii* Sacc. *Microbiology*, 54(2):231–236.
- Chet, I. and Henis, Y. 1975. Sclerotial morphogenesis in fungi. *Annual Review of Phytopathology*, 13(1):169–192.
- Chiriboga, J. 1966. Purification and properties of oxalic acid oxidase. *Archives of Biochemistry and Biophysics*, 116:516–523.
- Chivers, A. 1929. A comparative study of *Sclerotinia minor* Jagger and *Sclerotinia intermedia* Ramsey in culture. *Phytopathology*, 19:301–309.
- Coley-Smith, J. and Cooke, R. 1971. Survival and germination of fungal sclerotia. *Annual Review of Phytopathology*, 9(1):65–92.
- Connick, W. J., Boyette, C. D., and Mcalpine, J. R. 1991. Formulation of mycoherbicides using a pasta-like process. *Biological Control*, 1(4):281–287.
- Dillard, H. and Grogan, R. 1985. Relationship between sclerotial spatial pattern and density of *Sclerotinia minor* and the incidence of lettuce drop. *Phytopathology*, 75(1):90–94.

- Donaldson, P. A., Anderson, T., Lane, B. G., Davidson, A. L., and Simmonds, D. H. 2001. Soybean plants expressing an active oligomeric oxalate oxidase from the wheat gf-2.8 (germin) gene are resistant to the oxalate-secreting pathogen *Sclerotinia sclerotiorum*. *Physiological and Molecular Plant Pathology*, 59(6):297–307.
- Dong, X., Ji, R., Guo, X., Foster, S. J., Chen, H., Dong, C., Liu, Y., Hu, Q., and Liu, S. 2008. Expressing a gene encoding wheat oxalate oxidase enhances resistance to *Sclerotinia sclerotiorum* in oilseed rape (*Brassica napus*). *Planta*, 228(2):331–340.
- Duan, Y., Ge, C., Liu, S., Wang, J., and Zhou, M. 2013. A two-component histidine kinase Shk1 controls stress response, sclerotial formation and fungicide resistance in *Sclerotinia sclerotiorum*. *Molecular Plant Pathology*, 14(7):708–718.
- Dumas, B., Freyssinet, G., and Pallett, K. E. 1995. Tissue-specific expression of germin-like oxalate oxidase during development and fungal infection of barley seedlings. *Plant Physiology*, 107(4):1091–1096.
- Fan, H., Yu, G., Liu, Y., Zhang, X., Liu, J., Zhang, Y., Rollins, J. A., Sun, F., and Pan, H. 2016. An atypical forkhead-containing transcription factor SsFKH1 is involved in sclerotial formation and is essential for pathogenicity in *Sclerotinia sclerotiorum*. *Molecular Plant Pathology*. doi:10.1111/mpp.12453.
- Fravel, D. R. and Lewis, J. A. 1992. Production, formulation and delivery of beneficial microbes for biocontrol of plant pathogens. In *Pesticide Formulations and Application Systems: Eleventh Volume*. ASTM International.
- Georgiou, C. D., Patsoukis, N., Papapostolou, I., and Zervoudakis, G. 2006. Sclerotial metamorphosis in filamentous fungi is induced by oxidative stress. *Integrative and Comparative Biology*, 46(6):691–712.

- Georgiou, C. D. and Petropoulou, K. P. 2002. The role of ascorbic acid role in the differentiation of sclerotia in *Sclerotinia minor*. *Mycopathologia*, 154(2):71–77.
- Georgiou, C. D., Zervoudakis, G., Tairis, N., and Kornaros, M. 2001. β -Carotene production and its role in sclerotial differentiation of *Sclerotium rolfsii*. *Fungal Genetics and Biology*, 34(1):11–20.
- Greaves, M. P. and MacQueen, M. D. 1990. The use of mycoherbicides in the field. *Aspects of Applied Biology*, 24:163–168.
- Green, S., Stewart-Wade, S. M., Boland, G. J., Teshler, M. P., and Liu, S. H. 1998. Formulating microorganisms for biological control of weeds. *Plant-Microbe Interactions and Biological Control*, Marcel Dekker, New York, pages 249–281.
- Guimaraes, R. L. and Stotz, H. U. 2004. Oxalate production by *Sclerotinia sclerotiorum* deregulates guard cells during infection. *Plant Physiology*, 136(3):3703–3711.
- Hao, J. and Subbarao, K. 2005. Comparative analyses of lettuce drop epidemics caused by *Sclerotinia minor* and *S. sclerotiorum*. *Plant Disease*, 89(7):717–725.
- Harel, A., Gorovits, R., and Yarden, O. 2005. Changes in protein kinase A activity accompany sclerotial development in *Sclerotinia sclerotiorum*. *Phytopathology*, 95(4):397–404.
- Havir, E. A. and Anagnostakis, S. L. 1985. Oxaloacetate acetylhydrolase activity in virulent and hypovirulent strains of *Endothia (Cryphonectria) parasitica*. *Physiological plant Pathology*, 26(1):1–9.
- Health Canada 2010. *Sclerotinia minor* strain IMI 344141. Registration decision RD 2010-08. 22 September 2010. *Pest Management Regulatory Agency*. Health Canada HC Pub: 100362. IBBN: 978-1-100-16450-2 (978-1-100-16451-9). 6p.

- Heller, A. and Witt-Geiges, T. 2013. Oxalic acid has an additional, detoxifying function in *Sclerotinia sclerotiorum* pathogenesis. *PloS one*, 8(8):e72292.
- Hu, X., Bidney, D. L., Yalpani, N., Duvick, J. P., Crasta, O., Folkerts, O., and Lu, G. 2003. Over-expression of a gene encoding hydrogen peroxide-generating oxalate oxidase evokes defense responses in sunflower. *Plant Physiology*, 133(1):170–181.
- Ikotun, T. 1984. Production of oxalic acid by *Penicillium oxalicum* in culture and in infected yam tissue and interaction with macerating enzyme. *Mycopathologia*, 88(1):9–14.
- Imolehin, E., Grogan, R., and Duniway, J. 1980. Effect of temperature and moisture tension on growth, sclerotial production, germination, and infection by *Sclerotinia minor* [lettuce]. *Phytopathology*, 70:1153–1157.
- Jagger, I. C. 1920. *Sclerotinia minor*, n. sp., the cause of a decay of lettuce, celery, and other crops. *Journal of Agricultural Research*, 20:331–334.
- Jarvis, W. R. 1985. *Sclerotinia minor* as the cause of lettuce drop in southwestern Ontario. *Canadian Plant Disease Survey*, 65(1):7.
- Jones, E. and Stewart, A. 1997. Biological control of *Sclerotinia minor* in lettuce using trichoderma species. In *Proceedings of the New Zealand Plant Protection Conference*, pages 154–158. New Zealand Plant Protection Society Inc.
- Jurick II, W. M., Dickman, M. B., and Rollins, J. A. 2004. Characterization and functional analysis of a cAMP-dependent protein kinase catalytic subunit gene (*pka1*) in *Sclerotinia sclerotiorum*. *Physiological and Molecular Plant Pathology*, 64(3):155–163.
- Klein, T., Auld, B., and Fang, W. 1995. Evaluation of oil suspension emulsions of *Colletotrichum orbiculare* as a mycoherbicide in field trials. *Crop Protection*, 14(3):193–197.

- Lane, B., Dunwell, J. M., Ray, J., Schmitt, M., and Cuming, A. 1993. Germin, a protein marker of early plant development, is an oxalate oxidase. *Journal of Biological Chemistry*, 268(17):12239–12242.
- Lane, B. G., Cuming, A. C., Frégeau, J., Carpita, N. C., Hurkman, W. J., Bernier, F., Dratewka-kos, E., and Kennedy, T. D. 1992. Germin isoforms are discrete temporal markers of wheat development. *European Journal of Biochemistry*, 209(3):961–969.
- Leggett, M. and Gleddie, S. 1995. 4 developing biofertilizer and biocontrol agents that meet farmers' expectations. *Advances in Plant Pathology*, 11:59–74.
- Liang, H., Maynard, C. A., Allen, R. D., and Powell, W. A. 2001. Increased *Septoria musiva* resistance in transgenic hybrid poplar leaves expressing a wheat oxalate oxidase gene. *Plant Molecular Biology*, 45(6):619–629.
- Livingstone, D. M., Hampton, J. L., Phipps, P. M., and Grabau, E. A. 2005. Enhancing resistance to *Sclerotinia minor* in peanut by expressing a barley oxalate oxidase gene. *Plant Physiology*, 137(4):1354–1362.
- Loomis, W. 1938. The control of dandelions in lawns. *Journal of Agricultural Research*, 56:855–68.
- Lumsden, R. 1979. Histology and physiology of pathogenesis in plant diseases caused by *Sclerotinia* species. *Phytopathology*, 69(8):890–895.
- Lyu, X., Shen, C., Fu, Y., Xie, J., Jiang, D., Li, G., and Cheng, J. 2016. A small secreted virulence-related protein is essential for the necrotrophic interactions of *Sclerotinia sclerotiorum* with its host plants. *PLoS Pathog*, 12(2):e1005435.

- Melzer, M. and Boland, G. 1994. Epidemiology of lettuce drop caused by *Sclerotinia minor*. *Canadian Journal of Plant Pathology*, 16(3):170–176.
- Melzer, M., Smith, E., and Boland, G. 1997. Index of plant hosts of *Sclerotinia minor*. *Canadian Journal of Plant Pathology*, 19(3):272–280.
- Pan, L., Ash, G., Ahn, B., and Watson, A. 2010. Development of strain specific molecular markers for the *Sclerotinia minor* bioherbicide strain IMI 344141. *Biocontrol Science and Technology*, 20(9):939–959.
- Papapostolou, I. and Georgiou, C. D. 2010. Superoxide radical induces sclerotial differentiation in filamentous phytopathogenic fungi: a superoxide dismutase mimetics study. *Microbiology*, 156(3):960–966.
- Patterson, C. L. and Grogan, R. G. 1985. Differences in epidemiology and control of lettuce drop caused by *Sclerotinia minor* and *Sclerotinia sclerotiorum*. *Plant Disease*, 69(9):766–770.
- Phipps, P. M. and Porter, D. M. 1982. *Sclerotinia* blight of soybean caused by *Sclerotinia minor* and *Sclerotinia sclerotiorum*. *Plant Disease*, 66(2):163–165.
- Porter, D. M. and Beute, M. K. 1974. *Sclerotinia* blight of peanuts. *Phytopathology*, 64(2):263–264.
- Quimby, J. P., Zidack, N. K., Boyette, C. D., and Grey, W. E. 1999. A simple method for stabilizing and granulating fungi. *Biocontrol Science and Technology*, 9(1):5–8.
- Reeleder, R. and Charbonneau, F. 1987. Incidence and severity of diseases caused by *Botrytis cinerea*, *Pythium tracheiphilum*, and *Sclerotinia* spp. on lettuce in Quebec, 1985–1986. *Canadian Plant Disease Survey*, 67(2):45–46.

- Ridgway, H., Rabeendran, N., Eade, K., and Stewart, A. 2001. Application timing of *Coniothyrium minitans* A69 influences biocontrol of *Sclerotinia minor* in lettuce. In *Proceedings of the New Zealand Plant Protection Conference*, pages 89–92. New Zealand Plant Protection Society; 1998.
- Ritschkoff, A.-C., Rättö, M., Buchert, J., and Viikari, L. 1995. Effect of carbon source on the production of oxalic acid and hydrogen peroxide by brown-rot fungus *Poria placenta*. *Journal of Biotechnology*, 40(3):179–186.
- Rollins, J. A. and Dickman, M. B. 1998. Increase in endogenous and exogenous cyclic AMP levels inhibits sclerotial development in *Sclerotinia sclerotiorum*. *Applied and Environmental Microbiology*, 64(7):2539–2544.
- Rollins, J. A. and Dickman, M. B. 2001. pH signaling in *Sclerotinia sclerotiorum*: identification of a pacC/RIM1 homolog. *Applied and Environmental Microbiology*, 67(1):75–81.
- Schnick, P. J., Stewart-Wade, S. M., and Boland, G. J. 2002. 2, 4-D and *S. minor* to control common dandelion. *Weed Science*, 50(2):173–178.
- Sedun, F. and Brown, J. 1989. Comparison of three methods to assess resistance in sunflower to basal stem rot caused by *Sclerotinia sclerotiorum* and *S. minor*. *Plant Disease*, 73(1):52–55.
- Shaheen, I. Y., Abu-Dieyeh, M. H., Ash, G. J., and Watson, A. K. 2010. Physiological characterization of the dandelion bioherbicide, *Sclerotinia minor* IMI 344141. *Biocontrol Science and Technology*, 20(1):57–76.
- Stewart-Wade, S., Neumann, S., Collins, L., and Boland, G. 2002. The biology of Canadian weeds. 117. *Taraxacum officinale* GH Weber ex Wiggers. *Canadian Journal of Plant Science*, 82(4):825–853.

- Subbarao, K. V. 1998. Progress toward integrated management of lettuce drop. *Plant Disease*, 82(10):1068–1078.
- Sugiura, M., Yamamura, H., Harano, K., Sasaki, M., Morikawa, M., and Tsuboi, M. 1979. Purification and properties of oxalate oxidase from barley seedlings. *Chemical and Pharmaceutical Bulletin*, 27(9):2003–2007.
- Toms, H. 1964. Plant diseases of southern British Columbia. *Canadian Plant Disease Survey*, 44:143–225.
- Veluchamy, S., Williams, B., Kim, K., and Dickman, M. B. 2012. The CuZn superoxide dismutase from *Sclerotinia sclerotiorum* is involved with oxidative stress tolerance, virulence, and oxalate production. *Physiological and Molecular Plant Pathology*, 78:14–23.
- Walker, H. L. and Connick Jr, W. J. 1983. Sodium alginate for production and formulation of mycoherbicides. *Weed Science*, 31(3):333–338.
- Wang, S.-Y. C. and le Tourneau, D. 1971. Carbon sources, growth, sclerotium formation and carbohydrate composition of *Sclerotinia sclerotiorum*. *Archiv für Mikrobiologie*, 80(3):219–233.
- Wheeler, B. and Sharan, N. 1965. The production of sclerotia by *Sclerotium rolfsii*: I. effects of varying the supply of nutrients in an agar medium. *Transactions of the British Mycological Society*, 48(2):291–301.
- Willetts, H. and Bullock, S. 1992. Developmental biology of sclerotia. *Mycological Research*, 96(10):801–816.
- Willetts, H. and Wong, J. 1980. The biology of *Sclerotinia sclerotiorum*, *S. trifoliorum*, and *S. minor* with emphasis on specific nomenclature. *The Botanical Review*, 46(2):101–165.

- Woodard, K. and Newman, J. 1993. First report of *Sclerotinia minor* on Texas Bluebonnet. *Plant Disease*, 77:318.
- Xu, L., Xiang, M., White, D., and Chen, W. 2015. pH dependency of sclerotial development and pathogenicity revealed by using genetically defined oxalate-minus mutants of *Sclerotinia sclerotiorum*. *Environmental Microbiology*, 17(8):2896–2909.
- Yu, Y., Jiang, D., Xie, J., Cheng, J., Li, G., Yi, X., and Fu, Y. 2012. Ss-Sl2, a novel cell wall protein with PAN modules, is essential for sclerotial development and cellular integrity of *Sclerotinia sclerotiorum*. *PloS one*, 7(4):e34962.
- Yu, Y., Xiao, J., Zhu, W., Yang, Y., Mei, J., Bi, C., Qian, W., Qing, L., and Tan, W. 2016. Ss-Rhs1, a secretory Rhs repeat-containing protein, is required for the virulence of *Sclerotinia sclerotiorum*. *Molecular Plant Pathology*. doi:10.1111/mpp.12459.
- Zhang, W. and Watson, A. K. 1997. Efficacy of *Exserohilum monoceras* for the control of *Echinochloa* species in rice (*Oryza sativa*). *Weed Science*, pages 144–150.
- Zhu, W., Wei, W., Fu, Y., Cheng, J., Xie, J., Li, G., Yi, X., Kang, Z., Dickman, M. B., and Jiang, D. 2013. A secretory protein of necrotrophic fungus *Sclerotinia sclerotiorum* that suppresses host resistance. *PLoS One*, 8(1):e53901.