ALBUGO TRAGOPOGI (PERS)S.F.GRAY ON AMBROSIA ARTEMISIIFOLIA L.

by

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To my parents.

ABSTRACT

M.Sc. HARRY HARTMANN Plant Science

ALBUGO TRAGOPOGI (PERS)S.F.GRAY ON AMBROSIA ARTEMISIIFOLIA L.

A survey for potential biological control agents of Ambrosia artemisiifolia L. has been carried out in the area of Ste. Anne de Bellevue, Québec. Albugo tragopogi (Pers)S.F.Gray appeared destructive in the field and was selected for futher study. Zoospore release at temperatures of 5°C to 30°C in light and dark over 12 hours were tested. Optimum release occurred at 10°C and 15°C, both in light and dark.

Effects of infection on plant growth (biomass) and reproduction (flower heads, pollen, seeds) were studied. No significant effects were found except for plants showing systemic infection symptoms. In these plants, both vegetative and reproductive growth was suppressed by the disease.

The host range of A. tragopogi was tested under controlled conditions. Albugo tragopogi isolated from Ambrosia artemisiifolia infected Helianthus annus slightly but no other tested host. Cross inoculation studies with A. tragopogi isolates from Tragopogon pratensis and Helianthus annus (from South America) demonstrated that each of these isolates have restricted and exclusive host ranges.

A. tragopogi is not suitable for biocontrol of Ambrosia artemisiifolia because of the low virulence of the pathogen and the tremendous vigour of the target plant.

RESUME

M.Sc. HARRY HARTMANN Plant Science

ALBUGO TRAGOPOGI (PERS)S.F.GRAY SUR AMBROSIA ARTEMISIIFOLIA L.

Des recherches menées dans les environs de la ville de Ste-Anne de Bellevue, P.Q., afin de découvrir des organismes susceptibles de servir d'agents de contrôle biologique pour Ambrosia artemisiifolia L. ont permis d'établir qu'un champignon, Albugo tragopogi (Pers)S.F.Gray, paraissait efficace. Cet organisme fut donc choisi en vue d'une étude plus approfondie.

La libération des zoospores fut étudiée au cours d'une période de 12 heures, à des températures variant de 5°C à 30°C, à l'obscurité ainsi qu'à la lumière. Il fut déterminé que la libération des zoospores s'effectuait de façon optimale à 10°C ou à 15°C, quel que soit le régime lumineux employé.

Les effets de l'infection sur la croissance végétative (biomasse) et la reproduction (fleurs, pollen, graines) furent étudiés. Aucun effet significatif ne fut enrégistré, sauf chez les plantes montrant des signes d'infection généralisée. Chez ces dernières, toute croissance des parties végétatives et reproductrices était supprimée.

La variété et la spécificité des plantes-hôtes pour A. tragopogi firent l'objet d'une étude réalisée en milieu contrôlé. A. tragopogi, isolé chez A. artemisiifolia, a causé une légère infection chez Helianthus annus L. mais fut totalement inefficace chez toutes les autres espèces étudiées. Des inoculations croisées effectuées avec A. tragopogi isolé chez Tragopogon pratensis et H. annus (provenant d'Amérique du Sud) ont montré que chacun des isolats attaquait spécifiquement certaines espèces.

En conclusion, A. tragopogi ne constitue pas un agent efficace pour la lutte biologique contre A. artemisiifolia à cause de sa faible pathogénicité et de la très grande vigeur de la plante-hôte.

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CHAPTER I

LITERATURE REVIEW

Introduction

Ambrosia artemisiifolia L. is a major source of aeroallergenic pollen as well as an agricultural weed. Its control has proven difficult with herbicides, especially in urban areas because of its tremendous distribution and reproductive capabilities (Dickerson, 1968; Bassett and Crompton, 1975).

Harris and Piper (1970) suggested that Ambrosia artemisiifolia L. was suitable for biological control. They listed 217 taxa of insects parasitizing Ambrosia from which selection could begin for a suitable biocontrol organism. Insects, however, are not the only organisms with potential use as biological control agents. Daniel et al. (1973) have shown that an endemic, host specific pathogen (Colletotrichum gleosporioides), when cultured in the laboratory and applied as a mycoherbicide, controlled northern jointvetch (Aeschynomene virginica (L.) B.S.P.).

A survey of endemic pathogens of A. artemisiifolia L. in the Ste. Anne de Bellevue, Québec, area was undertaken for two successive seasons by this author. Two obligate parasites, Albugo tragopogi (Pers) S.F.Gray and Erysiphe cichoracearum Dc ex Mérat, were collected and isolated from infected A. artemisiifolia. A. tragopogi was selected for further study.

Williams (1892) observed in 1891 an area of South Dakota in which A. artemisiifolia was severely damaged by A. tragopogi and most plants did not set seed. A. tragopogi, or white rust, belongs to the Oomycetes. There were sufficient indications in the literature to show the existance of various forma specialis within the species (Pfister, 1927; Biga, 1955; Novotel'nova and Minasyan, 1970).

The present study was carried out to investigate the possibility of using this endemic pathogen (A. tragopogi) as biological control agent of A. artemisiifolia. The biology of the pathogen was studied to develop a technique for maintaining the pathogen under laboratory conditions and to define its ecological requirements. The host range of A. tragopogi was studied to determine its specificity and the damage to its host was evaluated under controlled environmental conditions.

1. Ambrosia artemisiifolia L (Common Ragweed)

(a) Description (after Gleason & Cronquist, 1963; Bassett & Crompton, 1975)

Annual herb, 5-200 cm high with a tap root. Stems branching at least above, variously hairy or in part subglabrous. Leaves opposite below, alternate above, petiolate, once or twice pinnatifided narrowly to broadly ovate or elliptic in outline, 4-10 cm. Staminate heads 10-200 flowered, in spikes terminating the stems and branchlets. Pistillate heads one-flowered, sessile, borne below the staminate ones, in the axiles of the upper leaves; male and female flowers on different parts of the same plant. Achenes obovate, 2.5 mm broad by 3.5 mm long with a terminal beak up to 2 mm long surrounded by a ring of spines 1 mm long; fruiting involucre woody. Pappus lacking. Flowering in Canada from August to October.

(b) Taxonomy

Synonyms of A. artemisiifolia L. include A. artemisiifolia elatior (L) Ses, A. diversifolia (Piper) Ryd., A. elatior L., A. glandulosa Ryd., A. maritima L. and A. media Ryd. (Payne, 1962a). It occurs in the Heliantheae subtribe of the Heleniae tribe of the Compositae family (Gleason and Cronquist, 1963). The genus Ambrosia consists of 41 species (Fernald, 1950; Payne, 1964). The unusual and highly modified morphology of Ambrosia has led to taxonomic confusion at all levels, from subspecies to tribe (Dickerson, 1968).

Payne (1964) stated that, since Ambrosia and Franseria are not sufficiently different morphologically and cytologically, they should be classified as congeneric under Ambrosia. He also proposed subdividing the

new taxon into four subgenera: Franseria, Xanthidium, Cercomeris, and Ambrosia. Furthermore, he suggested placing Ambrosia in a separate tribe of Compositae - Ambrosieae.

A. artemisiifolia is very variable morphologically (Gebben, 1965). Payne (1962) has identified four major forms: northeastern coarse lobed, southeastern lance lobed, southcentral dissected leaved and western prominent veined. A. artemisiifolia is dominated by specimens which are intermediate between the above-listed forms (Payne, 1962a,b).

(c) Distribution

A. artemisiifolia is abundant in cereal crops and cultivated row crops, common in fields in initial stages of abandonment, and conspicuous in roadside habitats and eroded soil surfaces (Dickerson, 1968; Fernald, 1950; Gebben, 1965; Lawalrée, 1947; Thompson, 1943, Simmons, 1928).

A. artemisiifolia is distributed throughout Canada but is rare in British Columbia, Alberta and Saskatchewan (Moss, 1956). Southern Ontario and Québec, especially along the St. Lawrence and Ottawa rivers, have very dense populations, while in the Atlantic provinces (Nova Scotia, Prince Edward Island and Newfoundland), the species is less common (Bassett and Frankton, 1971).

The genus Ambrosia is American, with half of the species found in North America. Many species of Ambrosia are found in and about the Sonora desert which is considered to be the centre of origin (Dickerson, 1968). The plant can be found in most of the U.S.A. except Arizona, New Mexico, Nevada, Utah and Colorado (Payne, 1962). A. artemisiifolia is also known to occur as an introduced species in Asia, Europe, South America and possibly in Africa (Vasilev, 1958; Payne, 1962; Numata et al., 1964).

(d) Economic Importance

(i) Beneficial

Allen and Pearson (1945) noted the value of Ambrosia species as wild avian food. Roedel and Thornton (1942) studied the oil contents of A. artemisiifolia and reported that it had about the same oil content as

soybeans but with better drying properties. Utilization in paint or varnish was suggested.

(ii) Detrimental

Durham (1931) estimated that about 90 percent of the late summer pollen was produced by A. artemisiifolia and A. trifida. A. artemisiifolia is the most important cause of hayfever in eastern North America (Bassett and Frankton, 1971). Estimates of the percentage of the U.S. population suffering allergy due to Ambrosia pollen vary from 4 percent (Solomon, 1967) to 10-20 percent (Payne, 1962).

A. artemisiifolia is also a common annual weed in many crops (Dickerson, 1968). Vengris (1953), sampling in the Connecticut River valley, found A. artemisiifolia in 69 percent of the corn fields, 50 percent of the potato fields, 24 percent of the onion fields and 18 percent of the tobacco fields. A. artemisiifolia has been observed in fields of parsley and carrots as well (Dickerson and Rhan, 1963; Ferrant and Bayer, 1966). It is also listed as one of the most widely spread taxa in corn and tomato fields in Ontario (Alex, 1964). Because of its weediness in cultivated crops and aeroallergenic properties of its pollen, A. artemisiifolia is classified as a secondary noxious weed under the Seed Act in Canada (Agriculture Canada, 1967).

(e) Control

A. artemisiifolia is susceptible to both cultural and chemical control methods. Cultivation in the field, mowing and hand pulling or hoeing in the home garden will control it (Watson and Hartmann, 1978). Foliar-applied herbicides such as 2,4-D, MCPA, fenoprop and mecoprop are recommended for A. artemisiifolia control (Ontario Herbicide Committee, 1978). However, in many situations, such as urban areas or national parks where chemical weed control is not usually considered and physical means of weed control would be too costly, there is a need for alternate methods of control, such as use of host specific biotic agents.

(f) Parasites

A. artemisiifolia has a large number of organisms associated with it in North America. Harris and Piper (1970) listed 217 taxa of insects as occurring on Ambrosia species, seven of which were listed as monophagous on A. artemisiifolia (Table 1). Goeden et al. (1974), in California, selected three arthropods parasitic on Ambrosia - Tarachidia candefacta Hubner (Lepidoptera : Noctuidae); Coleophora sp. near annulatilla Braun (Lepidoptera : Coleophoridae); and Eriophyes boycei Keifer (Acarina : Eriophyidae) to be tested in the U.S.S.R. as potential biological control agents.

A number of parasitic fungi have been found on A. artemisiifolia (Table 1, page 6).

2. Albugo tragopogi

(a) Description (Baker, 1955)

Parasitic and specific to Compositae. Mycelium intercellular, bearing simple globose haustoria. Hyphae 4-6 µm in diameter, hyaline, thin walled and much branched. Conidial sori subepidermal, later erumpent, forming white pustules on leaves. Conidiophores subclavate, hyaline, 20-48 x 12-24 µm, average 30 x 19 µm. Conidia joined by hyaline connecting links into chains; terminal conidia pallid yellow, 20-32 µm in diameter, average 24 µm, wall 4 µm equally thickened; other conidia hyaline to pallid yellow, thin walled except for annular thickened band, 3-4 µm thick, subcylindrical, 17-30 x 14-28 µm in diameter. Sexual organs include antheridia and oogonia borne on mycelial branches within host tissue. The oogonia are globose, dark brown to black, 40-72 µm in diameter; epispore 4-5 µm thick, finely meticulated with areolae 3-4 µm wide. Antheridia are small and club shaped.

(b) Taxonomy

Albugo is a cosmopolitan genus belonging to the order Peronosporales of the Oomycetes (Baker, 1955). There are 30 known species and 7 varieties in the genus (Biga, 1955). All members are obligate parasites of flowering plants, with many physiological forms identifiable only according to the respective hosts they attack.

TABLE 1. Partial List of Parasites Recorded on A. artemisiifolia

Insecta	
Homeoptera	
Aphidiae	
Pseudococcus salenopsis Tinsley	McKenzie, 1967.
Coleoptera	
Curculionidae	
Simcoronyx perpusillus Casey	Anderson, 1962.
Lepidoptera	
Pterophoridae	
Adania ambrosiae Murt	Barnes and Lindsey, 1921.
Gelechiidae	
Trichotaphe chamberselia Murt	Forbes, 1923.
Trichotaphe washingtoriella Busch	Forbes, 1923.
Diptera	
Tephritidae	
Callachna gibba (Loew)	Phillips, 1946.
Euaresta bella (Loew)	Foote, 1965.
Fungi	
Basidiomycetes	
Puccinia xanthii Schw.	Conners, 1967.
Entyloma compositarum Farl	Conners, 1967.
Entyloma polysporum (Pk) Farl	Conners, 1967.
Ascomycetes	
Erysiphe cichoracearum DC ex Mérat	Conners, 1967.
Oomycetes	
Albugo tragopogi (Pers) S. F. Gray	Conners, 1967.
Plasmopara halstedii (Farl) Berl & de Toni	Conners, 1967.
Deuteromycetes	
Septoria bacillugera Wint.	Conners, 1967.

Considerable controversy exists in the literature regarding the Albugo complex. Early descriptions were provided by Tozzetti (1776). Persoon in 1801 included Albugo as a section of the genus Uredo, characterized by white spores (Baker, 1955). Later, Gray (1821) recognized the affinities of Albugo with the Phycomycetes and he redefined Albugo as a separate genus. At that time, three species were included in the genus: A. cruciferarum, A. tragopogi and A. petroselini. None of these taxa were mentioned by Fries in his "Systema mycologicum" (Baker, 1955). Léveillé (1847) did not accept Albugo and founded the genus Cystopus; the sexual stage was described by de Bary (1863). Ramsbottom (1916) adopted Cystopus on the grounds that the original three species named by Persoon were not included in Fries' "Systema mycologicum" and, therefore, Léveillé's Cystopus should stand. Wakefield (1927) also accepted Cystopus de Bary over Albugo in accordance with the priority rules of botanical nomenclature stating that the first name given to the "perfect" stage (antheridium and oogonium in Oomycetes is valid. This, however, has been changed according to Article 20(f) of the revised International Botanical Nomenclature made at the 7th International Botanical Congress, Stockholm:

> ". . . fungus binomials set up by S. F. Gray, Schweinitz, Persoon and others between January 1, 1821 and December 31, 1832 may be legitimate if not included by Fries."
>
> (Stevenson and Rogers, 1950)

In addition to the ruling by the international body, the fact that Albugo is so descriptive and widely accepted by mycologists underlines the need to conserve Albugo. Nevertheless, the controversy and confusion still continues. Recently, Novotel'nova (1966) published a paper on the biology of a pathogen clearly belonging to Albugo. She designated this organism Cystopus tragopogonis (Pers) Schroct. In view of the reasons

Figure 1. Life cycle of Albugo tragopogi (Pers)S.F.Gray

1) Conidia and conidiophores (after Barnes, 1968) (x 400)

2A) Zoosporangium (x 400)

2B) Zoosporangium with papillum (x 400)

2C) Zoosporangium with vesicle partly released (x 400)

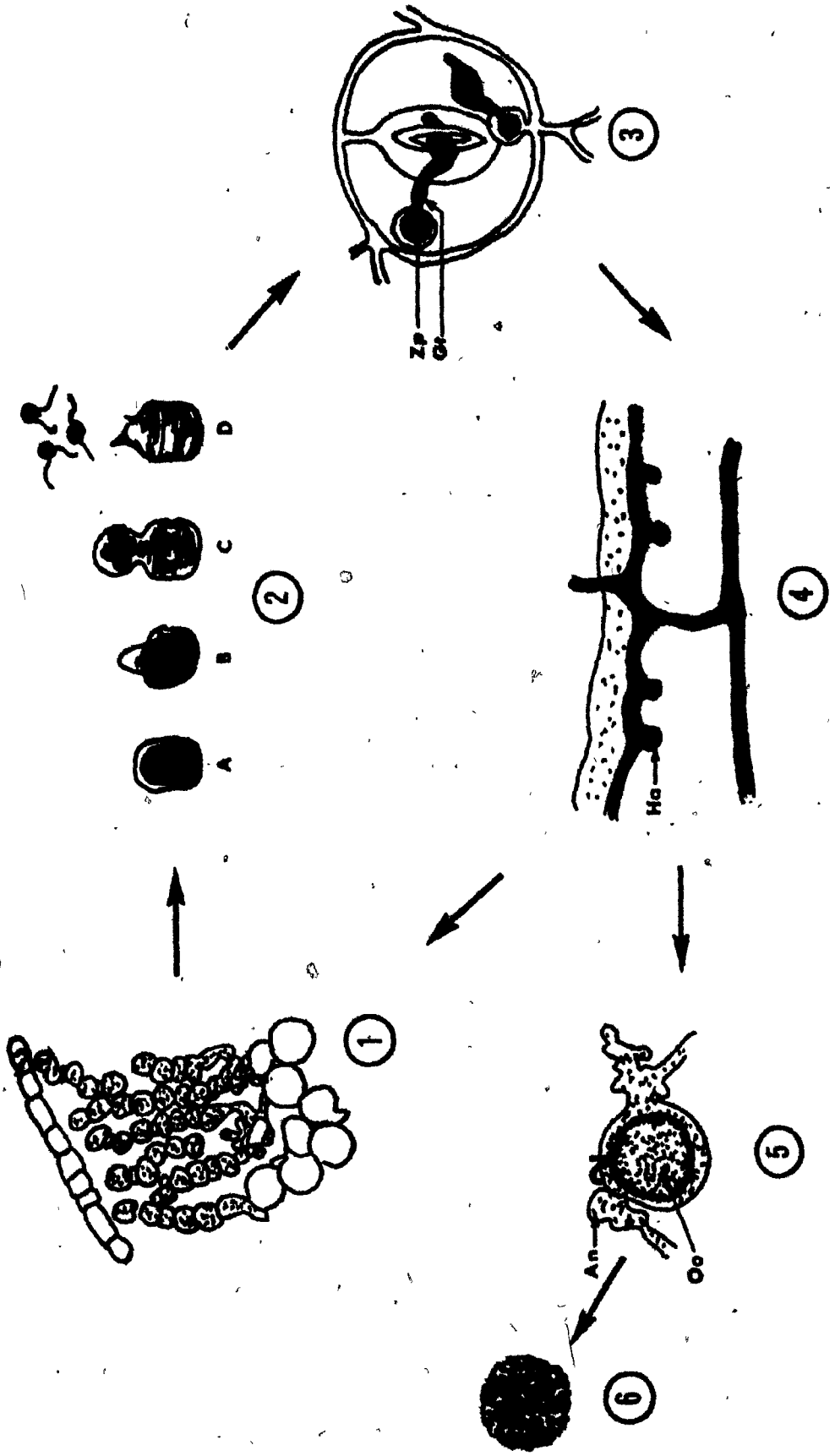
2D) Zoosporangium releasing zoospores

3) Zoospore germinates and penetrates host (x 1000)
Zp - zoospore; Gt - germ tube

4) Haustoria in host tissue (after Fraymouth, 1956)
Ha - Haustoria

5) Oogonium and antheridium (after De Bary, 1863) (x 250)
Oo - oogonium; An - antheridium

6) Oospore (x 250)


3
Ns
Ge
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C
B
A
2
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Ha
1
5
An
Oo
6

given above, this organism should be designated Albugo tragopogi (Pers) S. F. Gray and this designation will be used throughout this thesis.

Perhaps no other single aspect of the scientific literature reveals, more appropriately, the mass confusion that exists with regard to the taxonomy of A. tragopogi, than the number of synonyms applied to it. A partial list is given below (Biga, 1955):

1801. Uredo candida B. tragopogi Pers Syn. Fung, p. 223.
1809. Uredo obtusata Link, Mg Naturf Freunde Berlin, observ. 1, 4.
1810. Uredo cubica strauss, Wetteranisch Ges. f. Naturk, 2, 86.
1815. Uredo tragopogi DC, Fl, France, 2, 238.
1815. Uredo candida B. tragopogonum DC apud DC et lemark, Fl, France, 6, 88.
1817. Caeoma tragopogonis Nees, Syst, p. 15.
1817. Caeoma candidum Nees, Ibid.
1818-1819. Uredo tragopogonis Opiz, Crypt, p. 150.
1821. Albugo tragopogonis S. F. Gray, Nat. Arr. Brit. Pl. 1, 540.
1821-1823. Uredo candida b cnici Schub apun Ficin; Fl der beg um Dreseden 2, 237.
1824. Caeoma candidum b compositarum Schlecht, Fl, Berol 2, 2, 117.
1825. Caeoma centaureas Ehremberg fide link, Sp pl 6, p. 2.
1833. Uredo albugo, Unger, Exanth, p. 192.
1847. Cystopus cubicus Lév, Ann Sci Nat, Ser 3, 8, 371.
1862. Cystopus spinulosus de Bary, Ann Sci Nat, Ser 4, 20, 133.
1866. Cystopus tragopogonis Schr. kryptfl 3, pl 234.
1889. Cystopus brasiliensis Speg, Bol Ac Nac Cl, 2, 481.
1897. Cystopus tragopogonis spinulosus Davis, Trans Wiscon Acad, 2, 165.
1902. Cystopus mikaniae Sped Myc Agr Ser 2, p. 67.

(c) Life Cycle

(i) Asexual cycle (after Novotel'nova and Minasyan, 1970; Kajornchaiyakul and Brown, 1976)

Upon coming in contact with free moisture, a mature zoosporangium of A. tragopogi will produce a papillum. This papillum ruptures, allowing the release of a thin vesicle containing 7-11 zoospores (see Figure 1, 2 A-D). The zoospores separate and disperse from the vesicle. These motile

zoospores come to rest and encyst. If the zoospore encysts in a substomatal cavity on the leaf of a suitable host, it germinates, forming a germ tube which penetrates through the stoma (Figure 1, 3). Within hours, highly branched, intercellular hyphae are produced (Figure 1, 4). These hyphae produce conidiophores which in turn form conidia in a basipetal manner (Figure 1, 1). The conidia are produced subepidermally, forming irregularly shaped small pustules, usually on the abaxial side of the leaf. When sufficient number of conidia have been produced in a sorus, the pressure on the leaf epidermis will cause the pustules to burst open, releasing the zoosporangia which are dispersed primarily by wind (Populer, 1966).

(ii) Sexual cycle (after Stevens, 1901; Fitzpatrick, 1930)

Sexual organs are formed in the intercellular spaces of the host (Wilson, 1907). They consist of oogonium and antheridium (Figure 1 5,6) (de Bary, 1863). When ready to undergo gametogenesis, the contents of the oogonium separate into two distinct zones: the outer periplasm and the inner ooplasm. This process was termed "zonation" by Stevens (1901). While zonation is underway, both the antheridium and the oogonium nuclei undergo mitotic-like division. Some of the resultant daughter nuclei in the oogonium enter the ooplasm, resulting in a multinucleated oosphere. At this point, a fertilization tube from the antheridium penetrates into the oosphere, and ruptures, releasing numerous nuclei. A second mitosis takes place, and all but two resultant daughter nuclei disintegrate - one from the oogonium and another originating from the antheridium. These two promptly fuse and the fusion nucleus undergoes numerous divisions. The oogonium now has become an oospore and, in this multinucleate stage, it overwinters. It will germinate by releasing zoospores to initiate infection once more (Verma and Petrie, 1975). Palm (1932) reported A. tragopogi oospores inside the achenes of Tragopogon pratense. Oospores are also likely to be seed borne in A. artemisiifolia.

(d) Specialization

A survey of the literature indicates that A. tragopogi (Pers)S.F.Gray is restricted in its host range to Compositae (Wakefield, 1927, Baker, 1955;

Biga, 1955). Further specialization within the species has been indicated by the work of Pfister (1927). He listed five isolates, each specific to the genus from which it was isolated: on Tragopogon species, Centaurea species, Cirsium species, Xerantherum species and Chrysanthemum species. Pfister has not erected any new taxa though. Biga (1955) listed five forma specialis of A. tragopogi distinguished on basis of hosts and size of conidia.

These were: 1. A. tragopogi f. tragopogi Cif. et Biga
2. A. tragopogi f. pyrethri Cif. et Biga
3. A. tragopogi f. xeranthemi annui Sav. & Ryss.
4. A. tragopogi f. inulae Cif. et Biga
5. A. tragopogi f. cirsii Cif. et Biga

Each of the above strains has restricted host ranges with Compositae (see Table 2). Novotel'nova (1966) added Cystopus tragopogonis f. helianthi, a strain parasitizing Helianthus annus L. There is no agreement among mycologists regarding the taxonomy of Albugo, especially at the subspecies level (Waterhouse, 1973). However, it is clear, from the work of various researchers, that there is a great deal of specialization within A. tragopogi (Pfister, 1927; Savalescu and Rayss, 1946; Biga, 1955). Therefore the isolate from A. artemisiifolia is likely host specific and thus worthy of investigation as a biological control agent of this noxious weed.

TABLE 2. Host Specificity of Albugo tragopogi (Pers)S.F.Gray Types at Tribe Level

Tribe \ Taxon[a]	I	II	III	IV	V	Type unknown No. of genera
Helianteae	X	X				1
Anthemideae	X					
Inuleae	X		X	X	X	
Eupatorieae	X					
Cichorieae	X		X			1
Ambrosiae*	X					1
Total number of genera attacked	19	2	2	1	2	Total = 3

*Ambrosia and Franseria combined.

I Albugo tragopogi (Pers)S.F.Gray tragopogi Clf. et Biga
II Albugo tragopogi (Pers)S.F.Gray pyrethri Clf. et Biga
III Albugo tragopogi (Pers)S.F.Gray xeranthemi annui Sav. et Rayss.
IV Albugo tragopogi (Pers)S.F.Gray inulae Clf. et Biga, var. Nova
V Albugo tragopogi (Pers)S.F.Gray cirsii Clf. et Biga

[a] After . . . (Biga, 1955)

CHAPTER II

EXPERIMENTS

1. Zoospore Release

(a) Introduction

Zoospore release is the first stage in the infection process, leading to disease. In order to understand the host-parasite relationships between A. tragopogi and Ambrosia artemisiifolia, it is necessary to determine the exact environmental conditions required for zoospore release. Once obtained, this information can be used to develop an inoculation technique in the laboratory. Previous studies on zoospore release by A. tragopogi zoosporangia were carried out on A. tragopogi isolated from Helianthus annus (Novotel'nova and Minasyan, 1970; Kajornchaiyakul and Brown, 1976). The isolate from A. artemisiifolia is likely to be a different forma specialis, and may have slightly different environmental requirements than those studied in Russia and Australia. Tests were carried out to determine the optimum conditions required by the zoosporangia for zoospore release (Plate II, 7, 8).

(b) Materials and Methods

Zoosporangia were collected by means of an aspirator (Plate I, 6) from 5 to 7 day old pustules on plants maintained in ~~environmentally controlled~~ "controlled environment" chambers set at 22°C, 14-hour day and 19°C, 10-hour night.

The zoosporangia were added to distilled water to form a suspension and adjusted to 150,000 zoosporangia per milliliter. One hundred and twenty milliliters of the suspension were transferred into each of 36 one hundred and fifty milliliter beakers. Three beakers with suspension were placed in each of the following conditions: 5°C, 10°C, 15°C, 20°C, 25°C, and 30°C, illuminated with fluorescent tubes and incandescent bulbs, giving a total of 24,000 lx, and the same temperatures (5, 10, 15, 20, 25 and 30°C) but without light. All treatments were started at the same time in different environmentally controlled cabinets set at 60 percent relative humidity.

Aliquots of 3 ml were taken from each beaker at one-hour intervals for 12 hours; two drops of 0.05 percent lactophenol cottonblue were added to each sample to stain the zoosporangia and prevent further zoospore release.

Percent of zoosporangial release was observed by counting random microscope fields in each sample until 300 zoosporangia had been observed. Zoosporangia that only released some of their zoospores were not counted. Percentage data were transformed to arcsine (percentage), analyzed by analysis of variance and means were compared by Duncan's Multiple Range Test.

(c) Results and Discussion

Zoospore release occurred from 5°C to 30°C with the optimum occurring at 10 and 15°C. At 5°C, zoospore release rate increased linearly, reaching 10 percent at the end of 12 hours (Figure 2). At 10°C, zoosporangia released their zoospores at a high rate for the first 6 hours, levelling off at 48 percent (Figure 3). Zoospore release at 15°C increased linearly, reaching 65 percent after 12 hours (Figure 4). Release rate at 20°C was similar to that at 5°C with a linear increase, reaching 15 percent with no signs of levelling off at 12 hours (Figure 5). At 25°C, germination was low, 1-2 percent, and remained at that level throughout the experiment (Figure 6). At 30°C, results were similar to those obtained at 25°C with germination rate steady at one percent (Figure 7).

Release of zoospores at all temperatures occurred in both light and dark. At 5°C and 25°C, significantly ($P \leq 0.05$) more zoospores were released in the dark, while, at 15°C, significantly more ($P \leq 0.05$) were released in light. At 10°C, 20°C and 30°C, no significant differences were found in the levels of zoospores released (Figure 8).

The results from this study are in general agreement with previous studies on *A. tragopogi* and related species such as *A. cruciferarum* (= *A. candida*), *A. blitt*, *A. occidentalis* (Melhus, 1911; Napper, 1933; Hougas *et al.*, 1952, Raabe and Pound, 1952; Balasubramanian, 1967; Novotl'nova and Minasyan, 1970; Kajornchaiyakul and Brown, 1976).

In all these cases, zoospore release occurred over a relatively wide range of temperatures (5-25°C) with the optimum between 10°C and 15°C and

Figure 2. Zoosporangial release at 5°C, in light and dark over 12 hours.

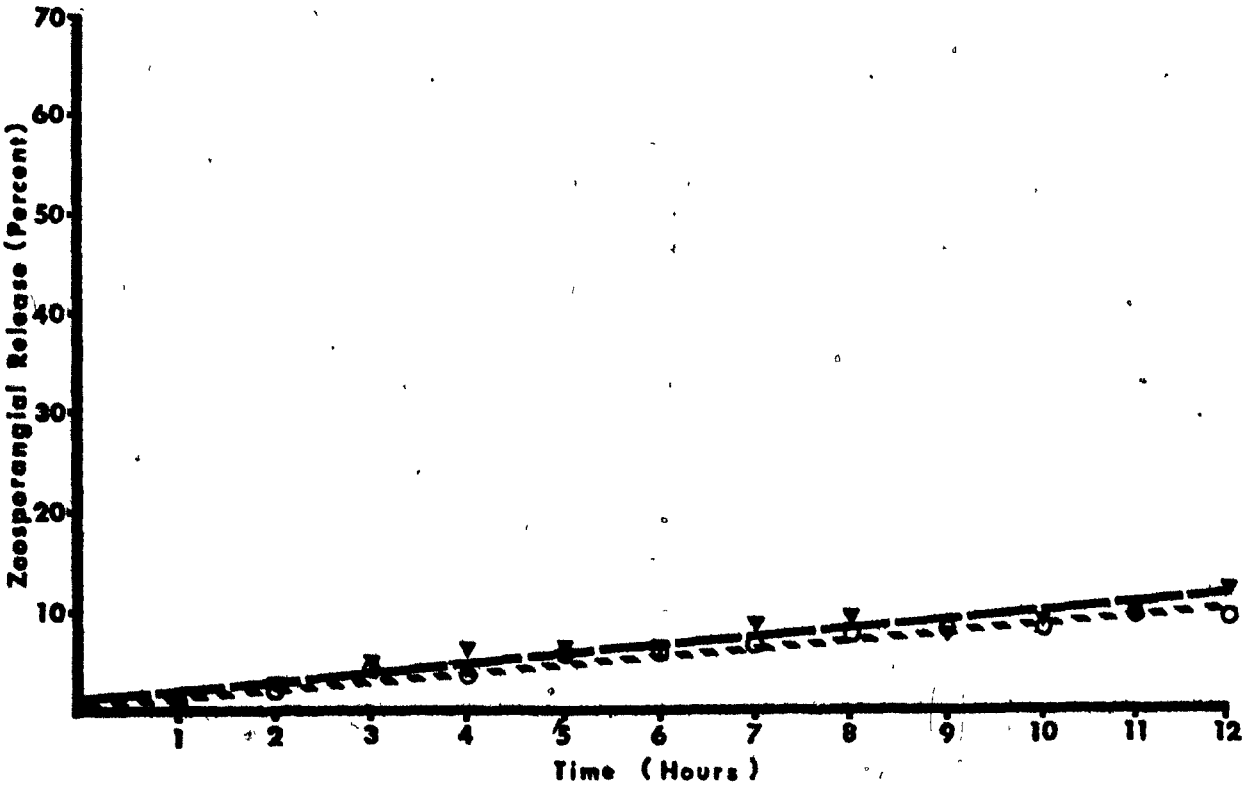
For light, $y = 0.76X + 0.54$
and dark, $y = 0.84X + 1.09$

o- - - - light
v——— dark

Figure 3. Zoosporangial release at 10°C, in light and dark over 12 hours.

For light, $y = 9.91X - 7.22$, with $0 < X \leq 6$
$y = 0.274X + 46.20$, with $6 < X \leq 12$
and dark, $y = 9.22X - 5.19$, with $0 < X \leq 6$
$y = 0.57X + 46.56$, with $6 < X \leq 12$

o- - - - light
v——— dark


Zoosporangial Release (Percent)
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
Time (Hours)

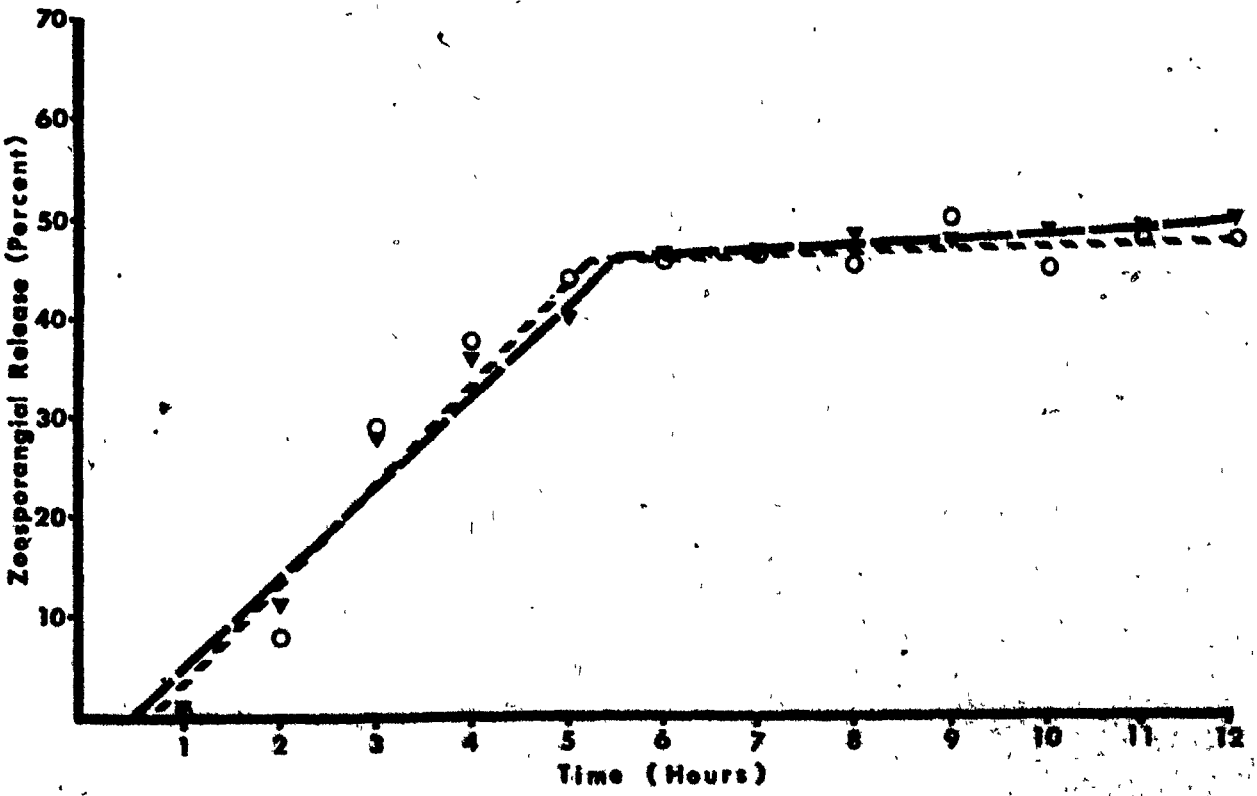

Zoosporangial Release (Percent)
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
Time (Hours)

Figure 4. Zoosporangial release at 15°C in light and dark over 12 hours.

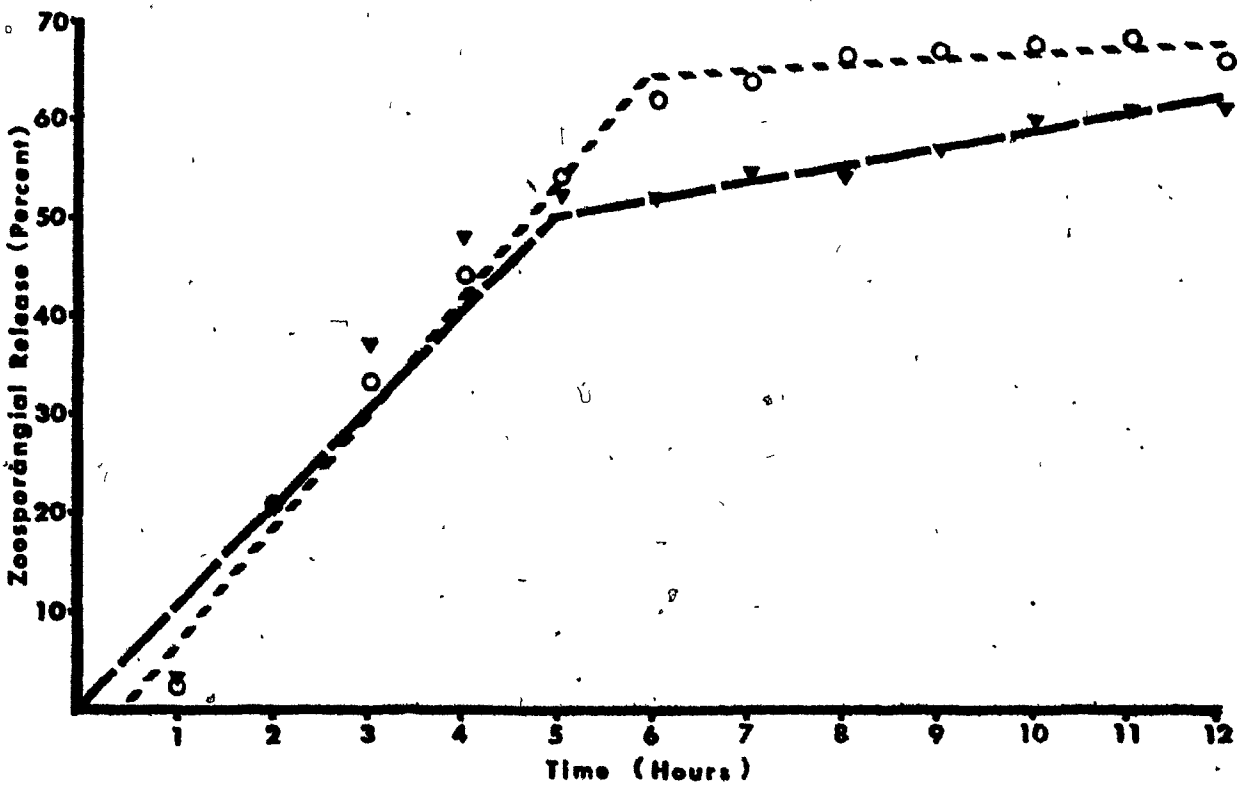
For light, $y = 11.82X - 5.277$ with $0 < X \leq 6$
$y = 0.46X + 65.17$ with $6 < X \leq 12$
and dark, $y = 10.03X + 0.24$ with $0 < X \leq 6$
$y = 1.66X + 52.05$ with $6 < X \leq 12$

o- - - -light
▼——dark

Figure 5. Zoosporangial release at 20°C in light and dark over 12 hours.

For light, $y = 1.17X - 0.18$
and dark, $y = 1.61X - 2.78$

o- - - -light
▼——dark


Zoosporangial Release (Percent)
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
Time (Hours)

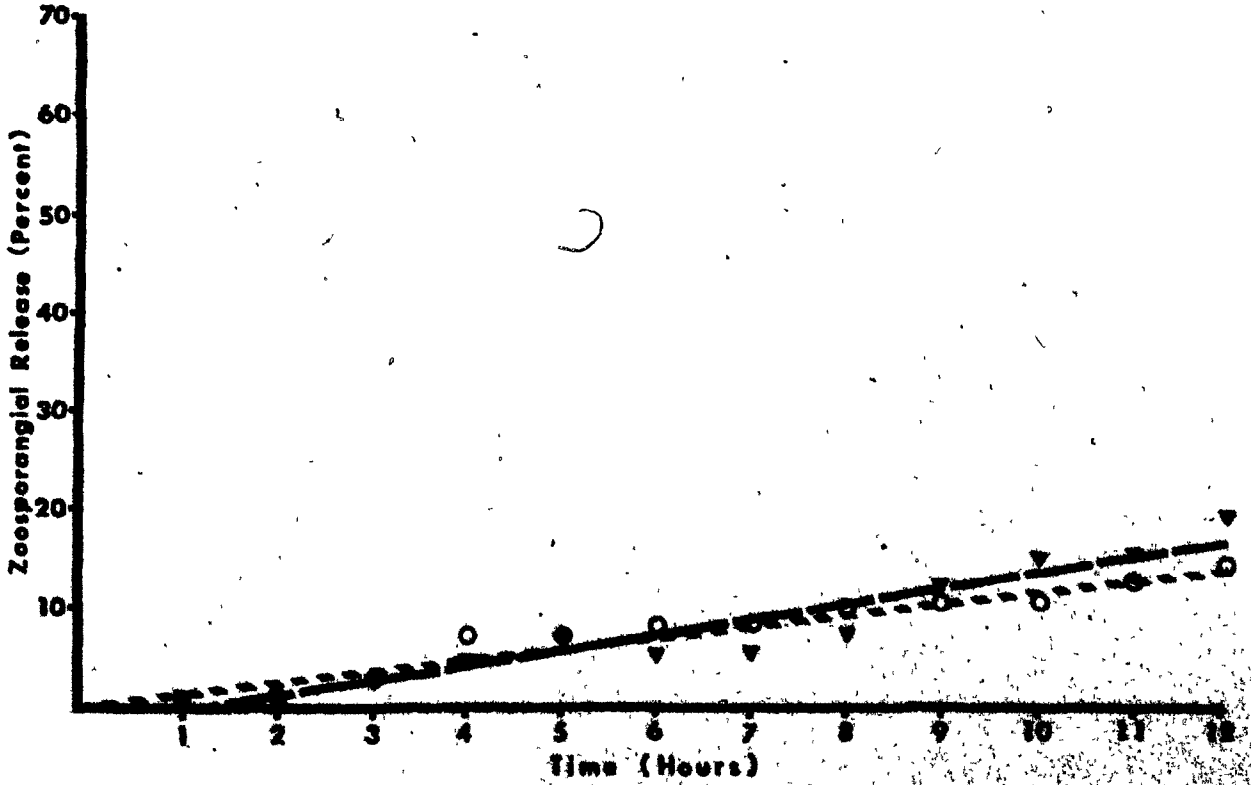

Zoosporangial Release (Percent)
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
Time (Hours)

Figure 6. Zoosporangial release at 25°C in light and dark over 12 hours.

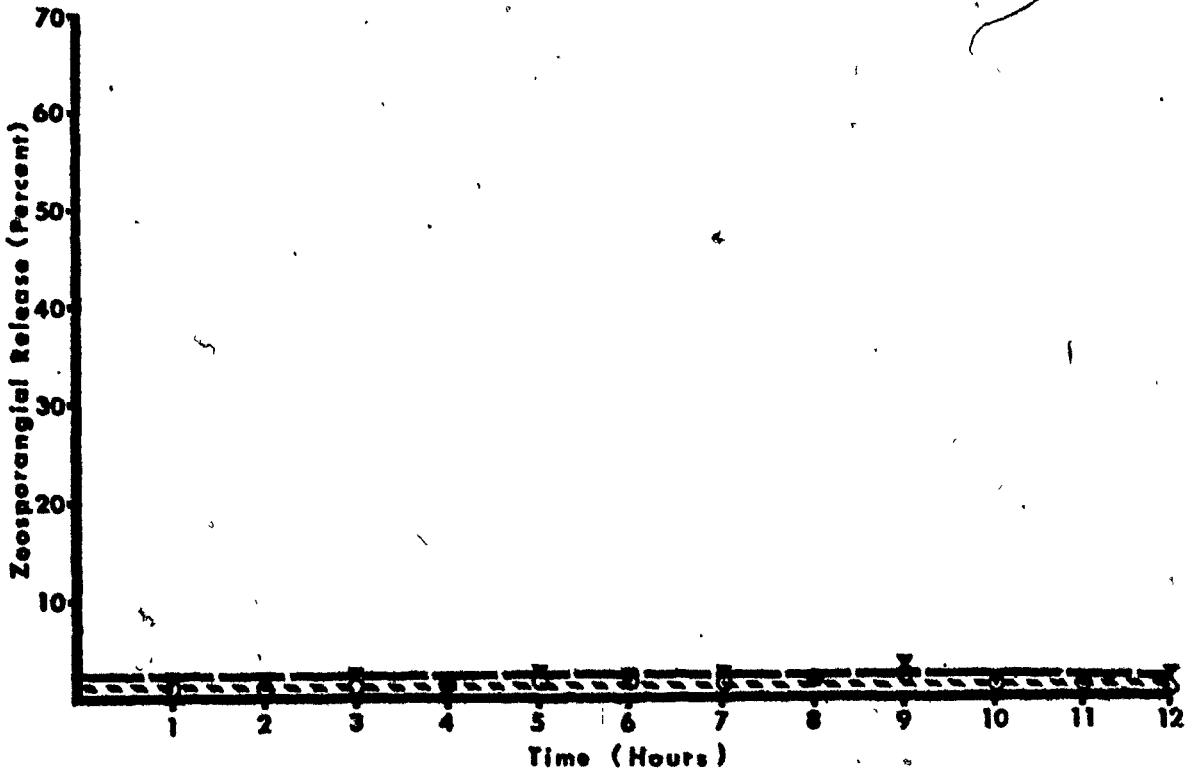
For light, $y = 0.006X + 0.88$
and dark, $y = -0.015X + 1.88$

o- - - -light
v———dark

Figure 7. Zoosporangial release at 30°C in light and dark over 12 hours.

For light, $y = 0.024X + 0.54$
and dark, $y = -0.009X + 0.64$

o- - - -light
v———dark


Zoosporangial Release (Percent)
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
Time (Hours)

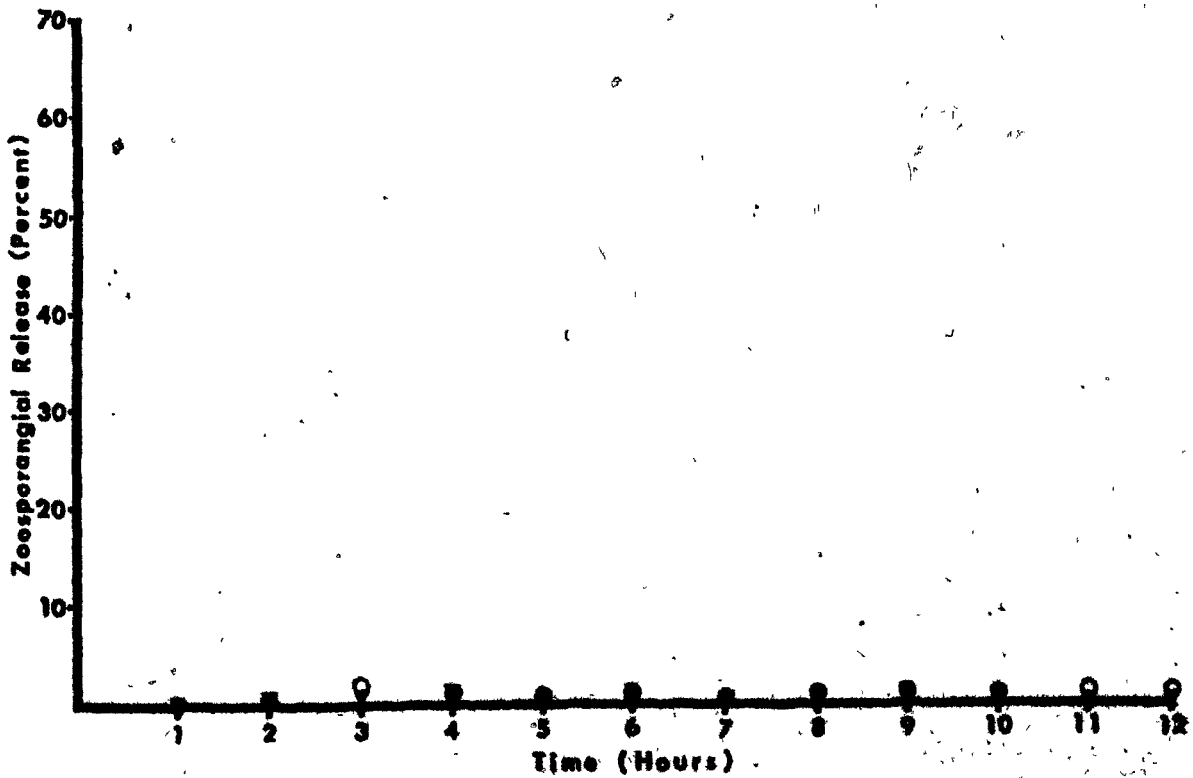
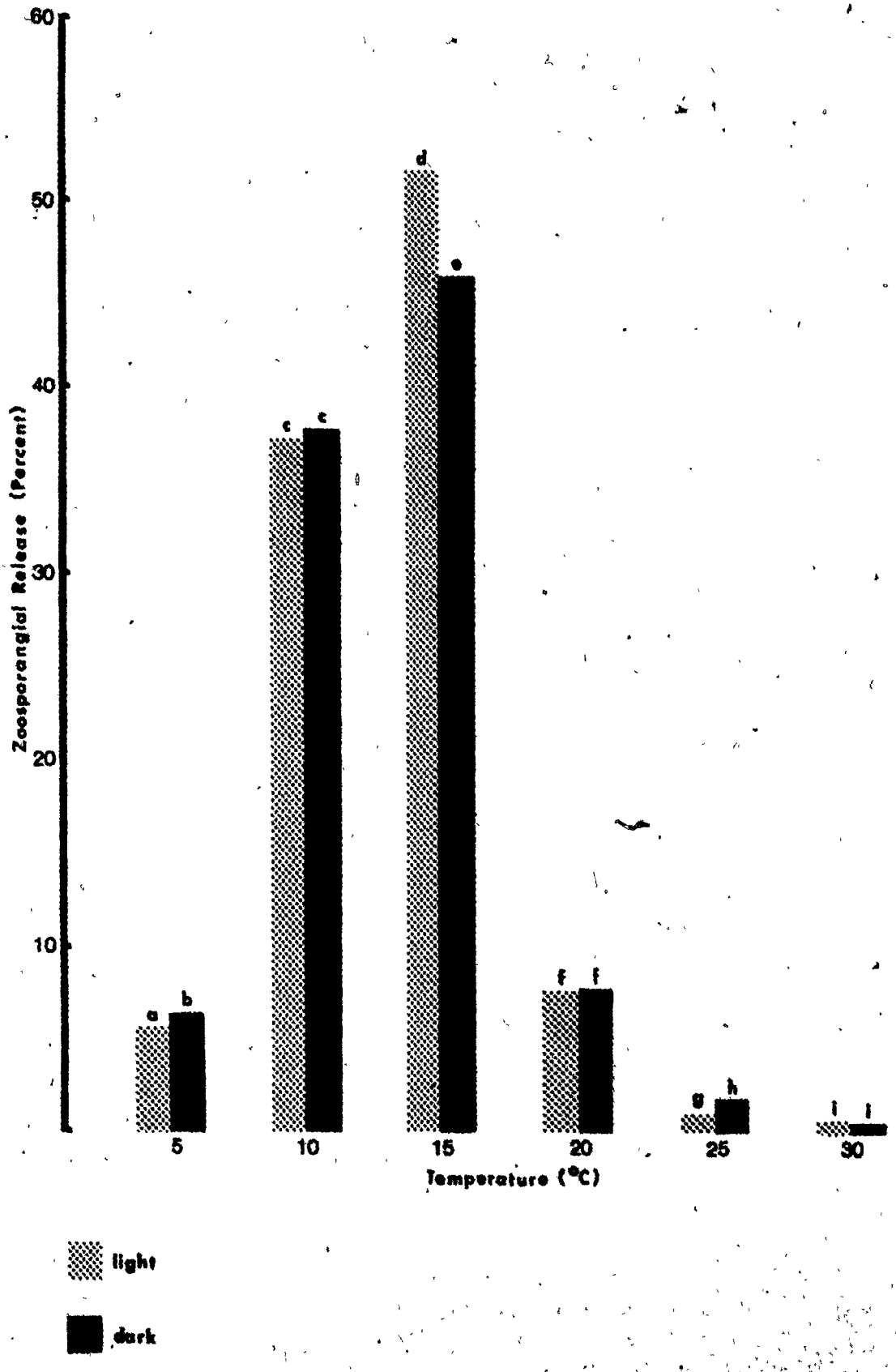

Zoosporangial Release (Percent)
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
Time (Hours)

Figure 8. Zoosporangial release at various temperatures in light and dark.

Figures with the same letters are not significantly different at $P < 0.05$.


60
50
40
30
20
10
Zoosporangial Release (Percent)
a
b
c
c
d
e
f
f
g
h
i
i
5
10
15
20
25
30
Temperature (°C)
light
dark

a sharp decline in zoospore release at higher temperatures. Light does not appear to have a profound effect on zoospore release even though differences were found between some temperatures in the study (Figure 8).

2. Host Damage

(a) Introduction

A. tragopogi has been observed to cause considerable damage to host plants. Williams (1892) reported heavily infected A. artemisiifolia were so damaged as to set no seed. Others have reported the pathogen parasitizing and damaging a variety of plants, including sunflower (Helianthus annus L.) and salsify (Tragopogon porrifolius L.) (Sackston, 1957; Bertossi, 1962; Novotel'nova, 1962; Middelton, 1971).

The existance of various forma specialis within Albugo tragopogi have been indicated by several authors (Pfister, 1927; Biga, 1955; Novotel'nova and Minasyan, 1971). These strains may well vary in their degree of pathogenicity towards their respective hosts. Furthermore, Daniel et al. (1973) have demonstrated that it is possible to manipulate pathogens with relative ease so as to increase their impact on a weed population. Thus a host specific strain that has not caused any significant reduction in its host population under normal conditions may, nonetheless, be considered as a possible biological control agent.

The potential of the Albugo tragopogi isolate from A. artemisiifolia to damage its host was tested under controlled environmental conditions.

(b) Materials and Methods

Fifty seedlings at the two-leaf stage were inoculated using zoosporangia collected from 5 to 7 day old sorii on A. artemisiifolia. The ragweed (A. artemisiifolia) plants used as source of inoculum were maintained at 20°C in environmentally controlled chambers.

Zoosporangia were collected with an aspirator (Plate I, 6) and transferred into precooled (15°C) distilled water to form a suspension. The concentration was adjusted to 150,000 spores per ml with the aid of a Leavy hemocytometer. One hundred and twenty milliliters of the suspension were

placed into flat glass dishes (2 x 8 x 20 cm) (Plate I, 1) which were then placed in environmentally controlled chambers set at 15°C for 3 hours to allow for zoosporangial release.

In order to prevent the roots of the seedlings from dessicating, they were placed together with some moist soil between two small (4 x 4 cm) glass squares, fastened together with tape (Plate I, 2, 3). The plants were inserted in an inverted position in a metal rack so that the leaves protruded below it (Plate I, 4). The racks were placed over the dishes containing the inoculum (so that the leaves were well immersed in the suspension) (see Plate I, 5) and left in the environmentally controlled chamber for 8 hours in dark at 15°C. Following inoculation, the plants were removed and planted in 10 cm diameter pots in a greenhouse soil mix of clay loam, sand and peatmoss (3:3:1).

Control consisted of 50 A. artemisiifolia plants (at 2-leaf stage) that were treated similarly to those above, but immersed in distilled water only. All 100 pots were placed inside an environmentally controlled chamber with 24,000 lux light provided by cool white fluorescent tubes. Photoperiod was 15 hours light and 9 hours dark for the initial 21 days. Daylength was subsequently reduced by 20 minutes every 7 days. When 13-hour daylength and 11-hour dark was reached, the photoperiod was kept constant for the rest of the experiment. Temperature was maintained at 21°C ± 0.5°C, day, and 19°C ± 0.5°C during the night with relative humidity at 40 percent.

Plants were examined frequently and numbers of pustules per leaf were recorded every 7 days. Upon the onset of anthesis, clear dializer tubes (28 mm wide) were placed over and sealed above and below the male spikes to trap all pollen shed. Five control and five inoculated plants were selected randomly to provide sources of pollen for pollination; therefore, their spikes were not covered. One hundred and thirty days after inoculation, the experiment was concluded. Pollen from each plant was collected, suspended in 200 ml of water and 3 samples from each plant were counted with a Leavy hemocytometer. The number of male flowers and seed set per plant was also recorded. Plants were harvested and oven dried at 40°C for 24 hours for dry weight determination. Weights of roots were tabulated separately from the rest of the plant. The data obtained for each parameter was subjected to a computerized t test (Bar et al., 1976).

(c) Results and Discussion

Within 21 days of inoculation, 37 of the 50 plants inoculated (74 percent) had shown some symptoms of disease, mostly on the leaves actually inoculated. The symptoms consisted of white pustules, 1 to 3 mm in size, mostly on the lower side of the leaves and chlorosis on the upper part in the general area of the pustules (Plate II, 9, 10). Within 5 weeks of inoculation, 19 of the plants (61 percent) having initially 51 or more pustules per plant lost their first two leaves and, with them, most disease symptoms (Figure 9). New leaves growing on these plants had no pustules or chlorosis. At this stage, two distinct categories of plants emerged: (i) plants with localized symptoms - pustules on the first 4 leaves, but none on new leaves; and (ii) systemically infected plants, characterized by numerous (50 or more) pustules emerging simultaneously on each leaf. These systemically infected plants also frequently had pustules along the petioles of the leaves. By the 56th day after inoculation, only 7 plants showing systemic infection symptoms continued to produce pustules.

Male flower head numbers per A. artemisiifolia were found to range from 0 to 2,000. When the mean number of male head on inoculated plants ($n = 50$) were compared to those produced on control plants ($n = 50$), no significant differences ($P \leq 0.05$) were found (Table 3). However, when considering inoculated plants according to the severity of symptoms, plants systemically infected ($n = 7$) produced significantly less ($P \leq 0.01$) male heads than control ($n = 50$) (Table 3a). Plants that have shown no further disease symptoms after the 8th week post inoculation ($n = 31$) produced significantly ($P \leq 0.01$) more male heads than control with $n = 12$ (Table 3b). Plants that did not exhibit any disease symptoms produced comparable numbers (Table 3c) of male heads to those of control plants ($n = 50$).

A. artemisiifolia has an indeterminate type inflorescence; thus, some male flower heads and flowers mature earlier than others (Jones, 1936). No two spikes on A. artemisiifolia plants mature simultaneously (Bassett et al., 1961). The amount of pollen shed by individual plants depends on the number of mature flowers per spike and other factors such as relative humidity, temperature, photoperiod and genetics (Bianchi et al., 1959).

A. artemisiifolia plants vary in size from 5 to 200 cm with few to numerous leaves (Bassett and Crompton, 1975) (see Plate III, 2). Payne (1963) has distinguished no less than 16 distinct forms. The observed dry weights of tops reflected this and ranged from 0.5 g to 20.2 g in the present experiment. When the mean dry weight of the inoculated (n = 50) plants and non-inoculated plants (n = 50) were compared, no significant difference ($P \leq 0.05$) was found (Table 3). However, when the mean heights of plants showing systemic disease symptoms (n = 7) were compared to that of the control plants (n = 50), a significant reduction ($P \leq 0.01$) was found (Table 3a). Mean top weights of plants on which disease symptoms were not observed after the 8th week (n = 31) and those that have shown no disease symptoms (n = 13) were not significantly different ($P \leq 0.05$) from those observed for control plants (n = 50) (see Tables 3b, 3c).

Root weights for inoculated plants (n = 50) were not significantly different ($P \leq 0.05$) from those recorded for control plants (Table 3). However, when weight of roots of plants showing systemic disease symptoms (n = 7) were compared to those of the control plants (n = 50), a significant reduction ($P \leq 0.01$) of root weight was observed for the former group (Table 3a). Mean root weight for plants on which disease symptoms were no longer observed after 8 weeks post inoculation was not significantly different ($P \leq 0.05$) than that of the control (Table 3b). Similarly, for plants that showed no disease symptoms during the experiment, no significant ($P \leq 0.05$) reduction was observed from that of control (Table 3c).

It should be noted that even though results obtained for plants exhibiting systemic disease symptoms when compared to those observed for control plants were significantly different, these results should be viewed with caution, due to the small sample size. It is evident that A. artemisiifolia, a successful weed, is very adaptable and plastic in most aspects of its biology. This variability within A. artemisiifolia populations necessitates a very large sample size when testing effects of disease on the plants. To obtain such a sample would be very difficult within controlled environmental conditions. Perhaps the most reasonable solution would be to test the effect of the pathogen on its weed host in large field tests.

These facts would suggest wide variations in quantities of pollen shed by individual A. artemisiifolia plants. Indeed, quantities observed in this experiment ranged from 0 to 294 million pollen grains per plant.

When inoculated plants (n = 45) were compared to control plants (n = 45), no significant differences ($P \leq 0.05$) were found in the mean number of pollen shed (Table 3). However, when the quantity of pollen shed by plants showing systemic infection symptoms (n = 7) was compared to that shed by control plants (n = 45), a significant reduction ($P \leq 0.01$) was observed in the former group (Table 3). When comparing pollen quantities shed by plants that showed no disease symptoms after the 8th week post inoculation (n = 25) to those of control plants, no significant differences ($P \leq 0.05$) were found (Table 3). Similarly, there was no significant difference ($P \leq 0.05$) found between mean number of pollen shed by plants that never developed disease symptoms (n = 13) and that of the control plants (n = 45) (Table 3).

Seed production by A. artemisiifolia is also highly variable. A single plant may produce between 3,000 and 62,000 seeds; some plants, however, tend to be predominately pistillate (Gebben, 1965; Dickerson and Sweet, 1971). Total numbers of seed produced per plant depend on environmental factors, such as temperature, photoperiod and length of growing season (Dickerson, 1968). Seed production is also highly correlated with fresh weight of plants (Bassett and Cromptom, 1975). Thus it is safe to assume that larger plants will produce more seeds.

In this experiment, seed numbers observed ranged from 0 to 293 per plant. When the mean number of seeds produced by inoculated plants (n = 50) was compared to those produced by non-inoculated plants, no significant differences were found at $P \leq 0.05$ (Table 3). However, when the mean number of seeds produced by plants exhibiting systemic disease symptoms (n = 7) was compared to that of the control plants, a highly significant reduction ($P \leq 0.01$) was found in the former (Table 3). Mean number of seeds produced by plants in which disease symptoms were no longer observed after the 8th week post inoculation did not differ significantly ($P \leq 0.05$) from that of the control plants (n = 50) (see Table 3). The same was true of plants that, although inoculated, did not show disease symptoms (Table 3).

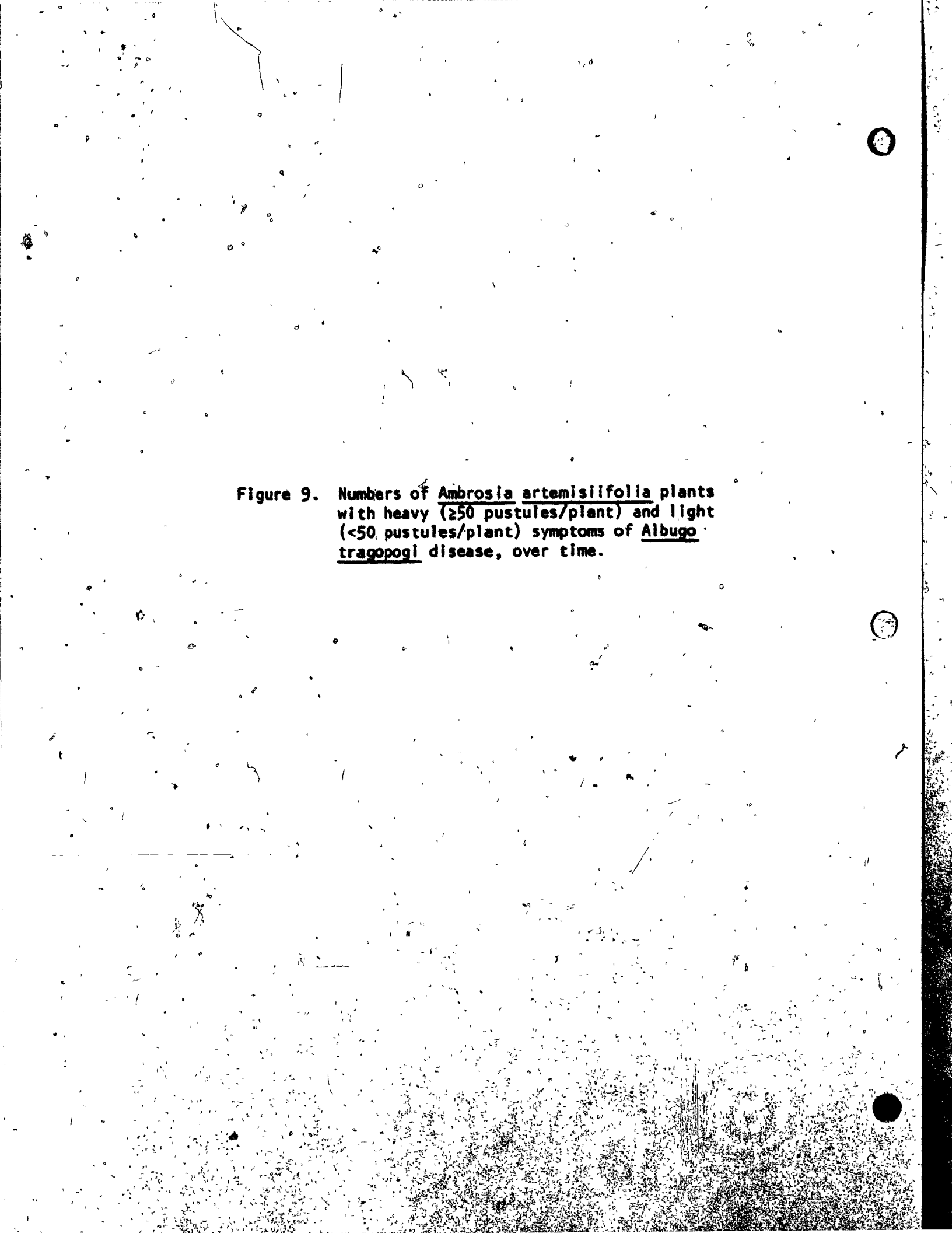


Figure 9. Numbers of Ambrosia artemisiifolia plants with heavy (≥50 pustules/plant) and light (<50 pustules/plant) symptoms of Albugo tragopogi disease, over time.

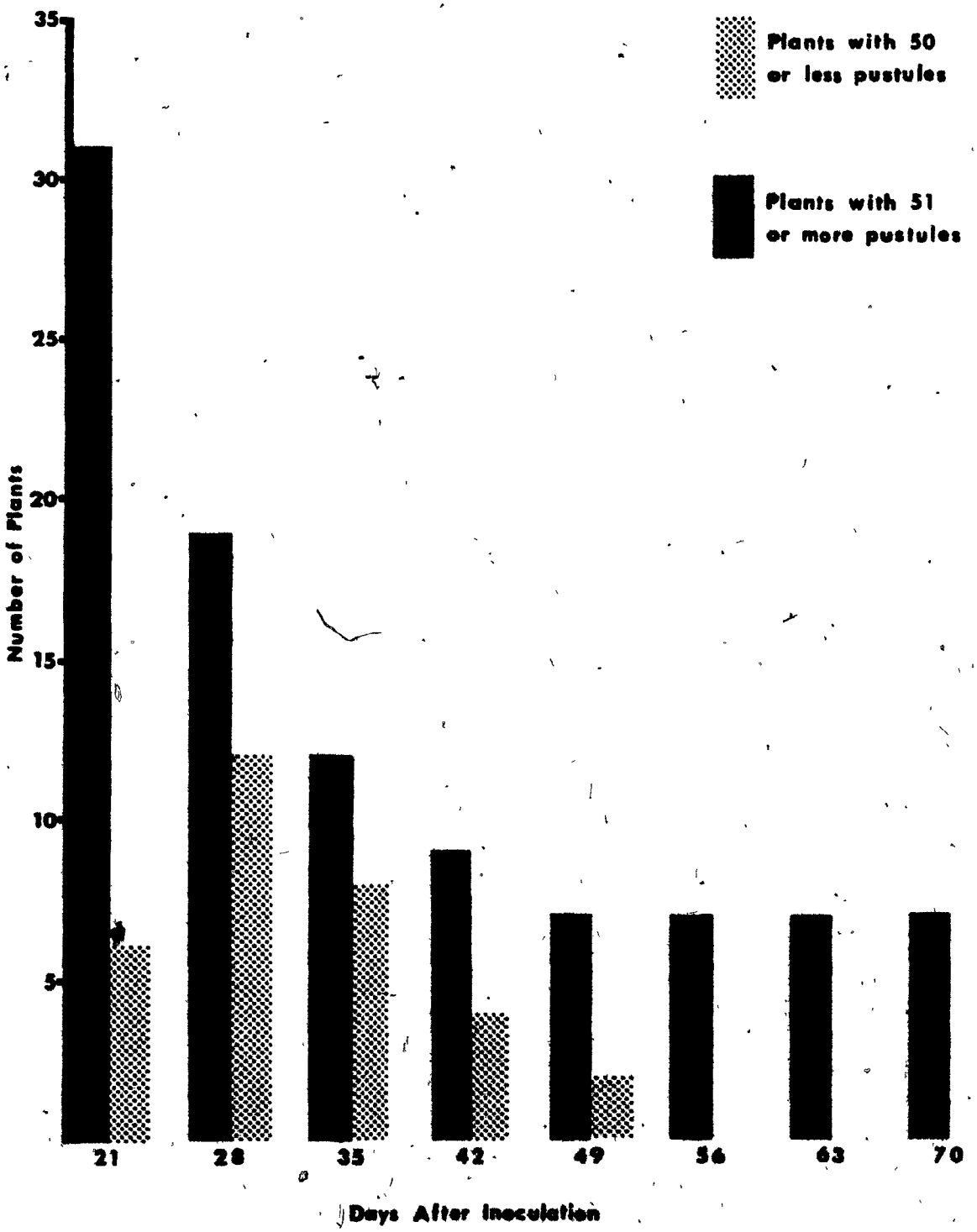

Plants with 50 or less pustules
Plants with 51 or more pustules
Number of Plants
35
30
25
20
15
10
5
21
28
35
42
49
56
63
70
Days After Inoculation

TABLE 3. Effects of A. tragopogi on flowering, pollen shed, seed production and biomass weight of A. artemisiifolia L.

Parameters	Infected				Control
	Total	Symptomless	Localized	Systemic	
	(n = 50[1])	(n = 12)	(n = 31[3])	(n = 7)	(n = 50[1])
Pollen shed[2]	30.6 ± 50.4[a]	46.9 ± 80.2[a]	30.6 ± 32.0[a]	0.02 ± 0.06[b]	22.0 ± 21.0[a]
Male flower heads	783.7 ± 517.1[b]	712.2 ± 551.1[b]	950.5 ± 423.3[c]	26.4 ± 60.4[d]	691.5 ± 385.8[b]
Seeds set	73.5 ± 60.2[e]	75.8 ± 55.2[e]	91.1 ± 56.4[e]	1.7 ± 4.5[f]	98.7 ± 71.8[e]
Top weight (g)	10.5 ± 5.1[h]	12.1 ± 4.3	11.2 ± 4.3[h]	2.4 ± 2.2[i]	11.6 ± 3.9[h]
Root weight (g)	3.8 ± 2.0[k]	4.8 ± 2.7[k]	3.7 ± 1.5[k]	1.9 ± 1.2[l]	4.2 ± 1.8[k]

Notes for Tables 3

[1] n = 45 for pollen shed

[2] numbers representing millions of pollen grains shed

[3] n = 25 for pollen shed

Means with the same letter in the same row are not significantly different at $P \leq 0.05$ (computerized t test; Bar et al., 1976).

3. Host Range

(a) Introduction

If an organism is to be useful as a biological control agent, it must have a narrow host range. Various methods have been suggested for the selection of plants to be used to determine host specificity, among them crop testing, which involves exposing large numbers of crop plants to the organism (Wapshere, 1974), and the biologically relevant method (Harris and Zwolfer, 1968) which involves the examination of the biology and evolutionary relationships between the control agents and host. A third approach, incorporating features from both of the above two methods, was used in the present study. This method, the centrifugal phylogenetic approach (Wapshere, 1974), requires extensive testing of plants closely related to the target weed as well as various crop plants.

Reports from the literature indicate that various A. tragopogi isolates have restricted and mutually exclusive host ranges (Biga, 1955). This was tested by cross inoculating Helianthus annus L., Ambrosia artemisiifolia L. and Tragopogon pratensis L. with A. tragopogi isolates from each of the above plants.

(b) Materials and Methods

Sixty species in 47 genera were tested. Out of these, 36 species in 27 genera belong to Compositae. Four collections of A. artemisiifolia and 8 varieties of Helianthus annus L. were tested. The remaining 24 species were selected from 13 families having members with economic importance.

(i) Host specificity

Plants were grown from seed in a soil mixture of clay loam, sand and peat moss (3:3:1). Seedling at the 2-leaf stage were inoculated with a zoosporangial suspension in distilled water as outlined previously (Chapter II, 2b). After inoculation, the seedlings were transplanted into 10 cm diameter pots containing greenhouse soil mixture (clay loam, sand, peat moss, 3:3:1). Initially, inoculated plants were placed in the greenhouse in a complete randomized design with control plants of A. artemisiifolia inoculated in a similar manner. Conditions were as follows: day temperature 20 $\pm$ 4°C; night

temperature 18 ± 4°C; natural daylength was extended to 14 hours by means of 20 flood lights (150 watts each) having 11,000 lux intensity. These conditions proved to be less than optimum for disease development. After 20 days in the greenhouse, the plants were removed and re-inoculated together with 44 healthy A. artemisiifolia plants as outlined above. These plants were then placed in environmentally controlled chambers set at the following condition: day temperature 21 ± 0.5°C; night temperature 19 ± 0.5°C; light intensity 24,000 lux; relative humidity 60 percent. Subsequent host specificity tests were all conducted in environmentally controlled chambers. Plants were examined frequently and the numbers of leaves and pustules were tabulated every five days.

(ii) Cross inoculation

- Twenty sunflower plants (variety Sundak) and 20 A. artemisiifolia plants inoculated with an isolate of A. tragopogi from H. annus obtained from Argentina, South America.
- Twenty Tragopogon pratensis plants with 20 A. artemisiifolia plants were inoculated with A. tragopogi isolates from T. pratensis.
- Twenty Tragopogon pratensis plants and 20 A. artemisiifolia plants were inoculated with A. tragopogi isolate from A. artemisiifolia. All plants were inoculated at the 2-leaf stage using the same procedure as outlined above (Chapter II, 2b)

(c) Results and Discussion

(i) Host specificity

A. tragopogi isolated from A. artemisiifolia has a very restricted host range. In all tests conducted, only A. artemisiifolia and Helianthus annus developed symptoms (Table 4).

A. artemisiifolia plants were clearly susceptible to the disease - 34 out of 44 inoculated developed symptoms (Table 4). Helianthus annus, however, is an incompatible host for the ragweed isolate of A. tragopogi. Pustules in all varieties tested remained small (0.5-1.5 mm) as compared to those on A. artemisiifolia (1.0-3.0 mm). Although chlorosis appeared on H. annus leaves in the general area of the pustules, the symptoms were restricted to those leaves that were actually inoculated and the disease never became systemic. See Table 5.

TABLE 4. Plants tested for susceptibility to *Albugo tragopogi* (Pers) S.F. Gray

PLANTS TESTED:	INFECTION LEVEL
Compositae	
Tubuliflorae	
Helianthae	
Ambrosia artemisiifolia (Québec)	++++
Ambrosia artemisiifolia (Ontario)	++++
Ambrosia artemisiifolia (U.S.A.)	++++
Ambrosia artemisiifolia (U.S.S.R.)	++++
Bidens frandosa L.	-
Carthamus tinctoris L.	-
Galinsoga ciliata (Raf.) Blake	-
Helianthus annus L. var. Sundak	+
Helianthus annus L. var. Peredovick	++
Helianthus annus L. var. Krasnodarets	+
Helianthus annus L. var. Voschod	+
Helianthus annus L. var. Commander	+
Helianthus annus L. var. Saluit	+
Helianthus annus L. var. Corona	+
Helianthus annus L. var. Latuin	+
Lactuca sativa L. var. Buttercrunch	-
Lactuca serriola Michex	-
Silphium integrifolium L.	-
Anthemidae	
Artemisia vulgaris L.	-
Chrysanthemum leucanthemum L.	-
Chrysanthemum parthenium (L.) Bench	-
Matricaria chamomila L.	-
Tanacetum vulgare L.	-
Senecioneae	
Senecio vulgare L.	-
Asterae	
Aster brachyates Blake	-
Aster vimineus Lam.	-
Erigeron acris L.	-
Erigeron philadelphicus L.	-
Inuleae	
Inula helenium L.	-
Cynareae	
Arctium minus Schk.	-
Arctium tomentosum Mill	-
Carduus acanthoides L.	-
Centaurea montana L.	-

TABLE 4. (continued)

PLANTS TESTED:	INFECTION LEVEL
Compositae (continued)	
Tubuliflorae (continued)	
Cynareae (continued)	
Centaurea jacea L.	-
Cirsium arvense (L.) Scop	-
Cirsium discolor (Muhl) Spreng.	-
Cirsium vulgare (Savi) Ten.	-
Cynara scolymus L.	-
Echinops spherocephalis L.	-
Liguliflorae	
Cichorieae	
Cichorium intybus L.	-
Crepis tectorum L.	-
Hieracium aurantiacum L.	-
Leontondon autumnalis L.	-
Sonchus asper (L.) Hill	-
Sonchus oleraceous L.	-
Taraxacum officinale Weber	-
Tragopogon pratensis L.	-
Caryophyllaceae	
Dianthus caryophyllus L., cv. Enfant de Nice	-
Chenopodiaceae	
Beta vulgaris L., cv. Stokes Special	-
Convolvulaceae	
Ipomea purpurae Lam., cv. Imperial Japanese mixed	-
Cruciferae	
Brassica oleracea f. *italica* L., cv. Rapine (spring Raab)	-
Brassica oleracea f. *botrytis* Mill, cv. Snow Crown	-
Brassica oleracea f. *gemmifera* L., cv. Long Island Improved	-
Brassica oleracea f. *capitata* L., cv. Emerald Green	-
Raphanus sativus L., cv. Scarlet Globe special	-
Cucurbitaceae	
Cucumis melo L., cv. Sugar Salomon	-
Cucumis sativus L., cv. Victory	-
Fabaceae	
Lotus corniculatus L., cv. Mirabel	-
Gramineae	
Triticum aestivum L., cv. Glenlea	-

TABLE 4. (continued)

PLANTS TESTED:	INFECTION LEVEL
Mentha piperita L.	-
Liliaceae	
Allium cepa L., cv. Southport Red Globe	-
Malvaceae	
Abelmoschus esculantus L., cv. Perkins Mammoth Long Pod	-
Althea rosea Cav., cv. Summer Carnival	-
Rosaceae	
Fragaria virginiana L., cv. Baron Salemancher	-
Solanaceae	
Capsicum annum L., cv. Earliest Red Sweet	-
Lycopersicum esculantum var. *Commune* Mill, cv. Beafeater VFN	-
Petunia hybrida Vilm, cv. Red Devil	-
Solanum melongena var. *esculantum* Nees., cv. Imperial Black Beauty	
Umbelliferae	
Daucus carota L., cv. Babyfinger Nantes	-

++++ 100 pustules or more per plant
+++ 50-99 pustules per plant
++ 11-49 pustules per plant
+ 1-10 pustules per plant
- no pustules or chlorosis

TABLE 5. Average number of pustules per plant of the different sunflower varieties and A. artemisiifolia inoculated with A. tragopogi.

Host	Days After Inoculation 20	25	30	35	40
Sunflower					
Sundak	1.4	1.8	1.8	0.4	0
Peredovic	29.4	28.2	21.6	13.0	0
Krasnodovitos	2.0	4.2	2.6	2.0	0
Voschod	6.6	6.0	1.6	0.2	0
Commander	4.0	3.0	4.0	0	0
Saluit	0	2.2	4.0	2.6	0
Corona	0	0.4	0.4	0.4	0
Latuin	3.4	4.4	4.2	3.4	0
A. artemisiifolia	15.0	25.59	48.52	102.71	208.1

(ii) Cross inoculation

The Albugo tragopogi isolate on H. annus from Argentina failed to produce any disease symptoms on A. artemisiifolia. However, on H. annus, this isolate produced large pustules (2-4 mm diameter) and chlorotic areas which later became necrotic around the pustules. The disease was clearly more damaging and extensive than that produced by A. tragopogi isolate from A. artemisiifolia on the same host variety. Inoculations of T. pratensis with isolate from A. artemisiifolia failed to produce any symptoms, while, on T. pratensis, pustules appeared within 10 days of inoculation. No symptoms were observed on A. artemisiifolia inoculated with A. tragopogi isolated from T. pratensis, but, on T. pratensis, characteristics pustules appeared within 7 days (Table 6).

TABLE 6. Results of cross inoculations involving three plant hosts and three respective isolates of Albugo tragopogi.

Source of Inoculum	Hosts		
	Ragweed	Tragopogon	Sunflower ✓
Ragweed	++++	----	+
Tragopogon	----	++++	*
Sunflower (Argentina)	----	*	++++

✓ variety Sundak

++++ 100 pustules or more per plant
+++ 50-99 pustules per plant
++ 11-49 pustules per plant
+ 1-10 pustules per plant
---- no pustules nor any other disease symptoms
* not tested

Plate I.

1. Inoculation apparatus.
   (a) Glass plates to hold roots of plants.
   (b) Metal rack used in inoculation.
   (c) Glass dish used for zoosporangial suspensions.

2,3. A. artemisiifolia plants being prepared for inoculation. Roots between glass plates to avoid dessication.

4. Plants inserted into metal rack, ready to be inoculated.

5. Plant leaves immersed in zoosporangial suspension.

6. Aspirator for zoosporangial collection.
   (a) Collecting tube.
   (b) Spore trap, made of filter paper.
   (c) Outlet for vacuum pump.

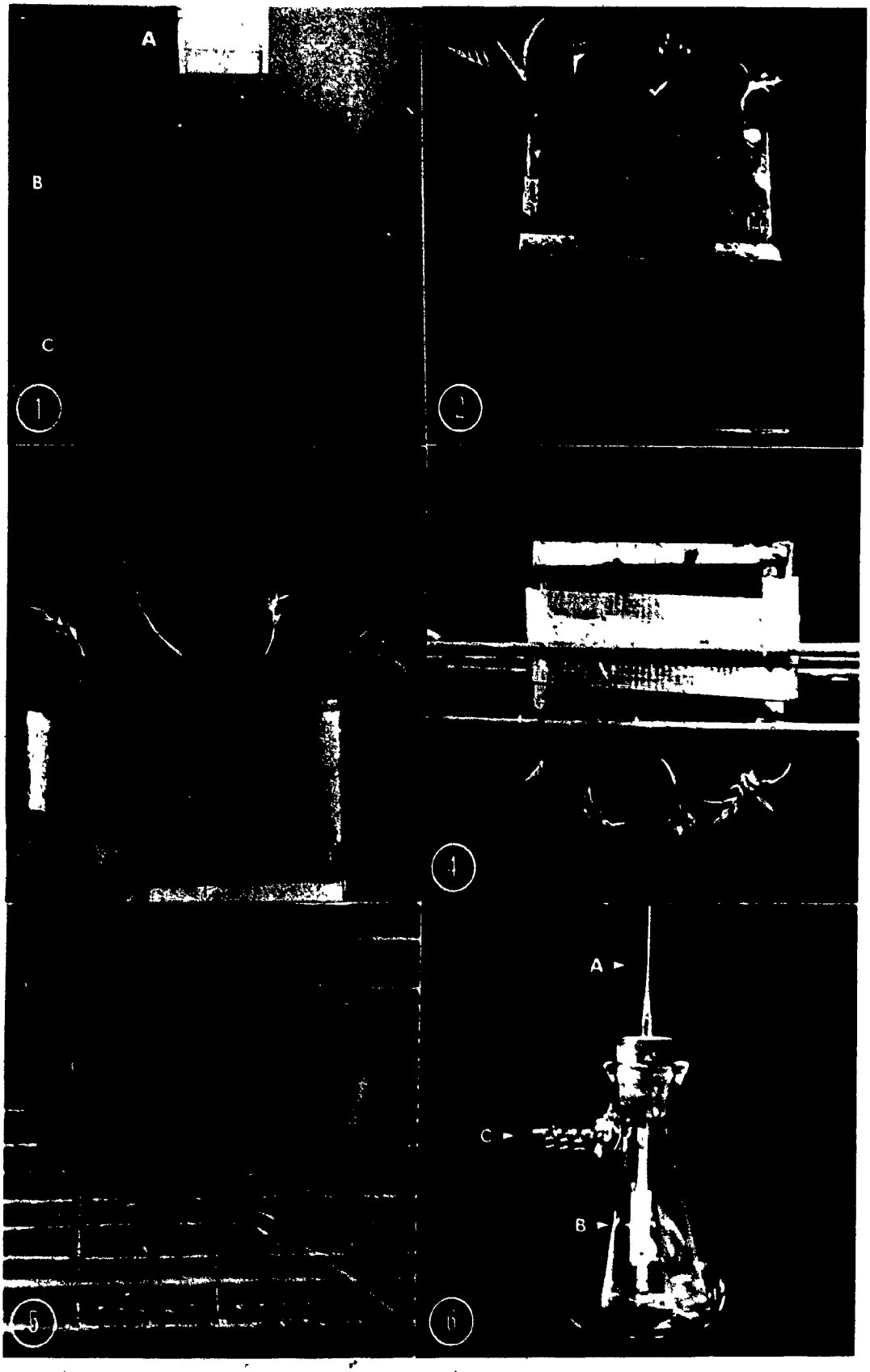

A
B
C
1
2
4
5
A
C
B
6

Plate II.

7. Zoosporangia releasing zoospores. Arrow - partially released zoosporangium (x 2700).

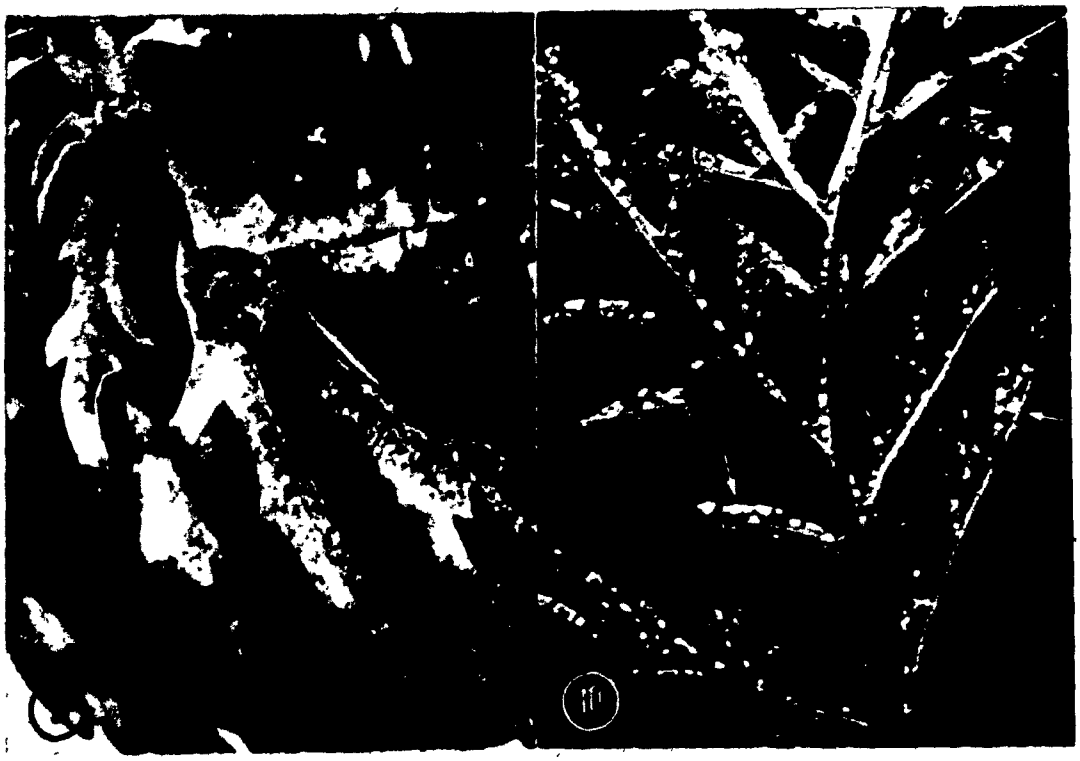
8. Zoosporangia.
   (a) Empty zoosporangium, after having released all zoospores (x 2700)

9. Chlorosis of Ambrosia artemisiifolia leaves due to infection by Albugo tragopogi.

10. Pustules formed by A. tragopogi on Ambrosia artemisiifolia leaves.

(7)

(8)



(10)

Plate III.

11. Variability in size found in *Ambrosia artemisiifolia* plants.

12,13. Infected and healthy *Ambrosia artemisiifolia* plants.
(a) Infected.
(b) Healthy.

14. Flower spike of *Ambrosia artemisiifolia*.
(a) Immature male flower heads.
(b) Mature male flower heads, shedding pollen.


30
15
11
30
CM
25
20
15
10
5
0
12
A
B
CM
30
25
20
15
10
5
0
A
B
13
A
B
14

CHAPTER III

SUMMARY AND CONCLUSIONS

1) A. artemisiifolia is a variable, widespread weed that is a problem both as a major source of aeroallergenic pollen and as a competitor with agricultural crops. Chemical control, especially in urban areas, has proven difficult. Research into alternate methods involving biocontrol are required.

2) Albugo tragopogi (Pers)S.F.Gray, the white rust of Compositae, has been reported in many areas of the world under various synonyms. However, the above name is both descriptive and has priority according to the rules of nomenclature over any synonym.

3) A. tragopogi is a member of the Oomycetes. In its ecological requirements, it closely resembles other species of Albugo. Zoospore release occurs at temperatures ranging from 5°C to 30°C with the optimum between 10°C and 15°C. This will occur both in the presence and the absence of light.

4) An inoculation technique was developed to maintain the pathogen in the laboratory. This consisted of immersing leaves of A. artemisiifolia in a suspension of zoosporangia in distilled water for eight hours at 15°C in darkness. Immersion resulted in heavy disease symptoms 5 to 10 days later. Further studies are required to delineate the effects of light, dark and temperature on the infection process and disease development.

5) Reaction of inoculated A. artemisiifolia plants varied. Some inoculated plants developed systemic infection, others had localized symptoms which did not reach beyond areas actually inoculated, and some plants apparently never developed disease symptoms. There are resistance factors

involved within the A. artemisiifolia population which are probably genetically based since the disease in some plants was arrested and did not develop in others. Further studies are required to determine the nature of this resistance.

6) Marked suppression of both vegetative (biomass weight) and reproductive (flowers, pollen, seed) phases of plants showing systemic infection symptoms was observed. However, in plants devoid of disease symptoms and those with localized symptoms, no significant ($P \leq 0.05$) impact on the biomass or flowering phases was observed. It must be pointed out that no conclusive results could be reached with the restrictions on sample size imposed by controlled environment experiments. However, the pathogen did not seem to have a major impact on the plant population tested. Histological studies should be carried out, especially on plants shown non-systemic symptoms. These studies would further clarify the nature of the host-parasite relationship.

7) Host specificity studies have clearly shown further specialization within A. tragopogi (Pers)S.F.Gray; the pathogen isolated from A. artemisiifolia should be termed a forma specialis and distinct from those occurring on Tragopogon pratensis L. and Helianthus annus L.

8) Daniel et al. (1973) have set out three requirements that must be met if an endemic pathogen is to be effective as a biological control agent: (a) must be able to reproduce abundantly in culture and produce durable inoculum; (b) must be genetically stable and specific for the target plant; (c) able to infect and kill the weed under a relatively wide set of conditions. Albugo tragopogi does meet two out of the three requirements only - it can be cultured in the laboratory, even though, being an obligate parasite, it will require more resources than a pathogen able to grow on readily-available artificial media; Albugo tragopogi isolated from A. artemisiifolia is specific to its target; however, it has strict environmental requirements for infection and disease development. Furthermore, it does not seem very damaging to most plants inoculated.

9) A major difficulty in using a biological control agent against A. artemisiifolia is its tremendous variability. A species that is present in many parts of the world, adapted to many environmental habitats, cross pollinated and hybridizing with other species of the genus, has a potentially formidable gene pool available for the target plant to develop resistance to the organism attempting to control it.

10) Although studies have not been conducted under field conditions, these preliminary studies demonstrate that the white rust, A. tragopogi, is not sufficiently damaging to its host and therefore is not promising as a biological control agent of A. artemisiifolia L.

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